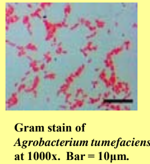
Crown Gall Due to *Agrobacterium tumefaciens*: A Model System for Cellular Biology

Brandon C. Banks 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

Gall formation in plants can be used as a learning model for a cellular biology class. Cellular organization, division, and DNA expression can be compared between normal and tumorous tissue. *Agrobacterium tumefaciens* was used to generate the tumors. When a plant becomes wounded and subsequently infected, a Ti plasmid is transferred from *Agrobacterium sp.* into the plant cell's genome where changes in protein production take place. In this study, we grew two dicots, tomatoes (*Solanum lycopersicum*) and sunflowers (*Helianthus annuus*), for two weeks and inoculated the stems with *Agrobacterium tumefaciens*. Plants were placed into three experimental groups: wounded with a non-tumor inducing *Agrobacterium sp.*, wounded with gall-forming *Agrobacterium sp.*, and wounded with a toothpick or sterile inoculating needle. After another two weeks of growth, we sectioned the stems and are currently extracting the proteins to determine expression. Different ways of introducing the bacteria into the wounded stem produced distinct external tumor organization. The results confirmed that wildtype bacteria induce changes in the stems. Future studies will focus on the motility of the bacteria inside the plant, neoplastic vascular organization, and the speed of aging in the neoplasm. Supported by HHMI.



Gram stain of *Agrobacterium tumefaciens* at 1000x. Bar = 10µm.

Background

Gall formation within a plant occurs through the induction of *Agrobacterium tumefaciens,* specifically through the transmission of a plasmid. In order for the bacteria to transmit the plasmid, the plant requires wounding, which creates an entry for the bacteria, a suitable environment for bacterial growth, and access to target receptor sites on the cell wall (1). Cells around the wound release phenolic compounds that signal division (2). The Ti plasmid from *Agrobacterium tumefaciens* consists of a T-DNA region that becomes inserted into the plant genome and a vir region that generates the protein machinery needed to transfer DNA. The T-DNA from the plasmid is transferred into the plant cell through the T-Pilus (3). *Agrobacterium* is chemoautotrophic for auxin but also induces plant production of hormones. The plasmid expresses genes that lead to the plant production of auxin and cytokinin, transforming normal plant cells into tumor cells. Auxin is a growth regulator required for cell elongation and differentiation, and cytokinin may be responsible for the uncontrolled synthesis of auxin (2). It has also been determined that in order for the tumor to grow, new vascular tissue is made inside the neoplasm (4).

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Goals for Using a Plant Model System

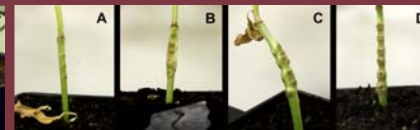
- Optimize production of gall formation in *Helianthus annuus* and *Solanum lycopersicum* for use in a classroom setting (Fig. 1A and Fig 1B).
- Analyze the structural differences between gall and normal tissue (Fig 2).
- Determine if the structural differences could be comparable and useful as a tool for a cancer model.
- Determine if any molecular changes have occurred that would be applicable to the study of cancer (Fig 3).
- Determine if any possible differences have occurred within the plant outside of the neoplasm.

Results

Figure 1 A

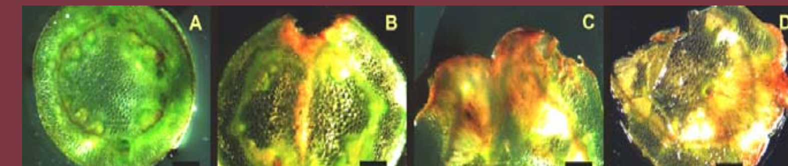
Differences in Tomato Tissues. (A) Stem inoculated with A136 strain. (B) and (C) Stem inoculated with wildtype *Agrobacterium.*

Figure 1 B



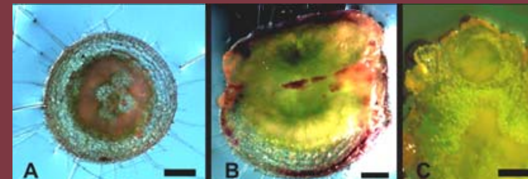
Methods of Wounding Sunflowers. (A) A136 toothpick poke with no gall formation. (B) Wildtype toothpick scratch with gall forming down the wound. (C) Wildtype toothpick poke on stem covered with wild type *Agrobacterium* before wounding. (D) Wildtype toothpick poke with toothpick covered with *Agrobacterium* during wounding.

Figure 2A



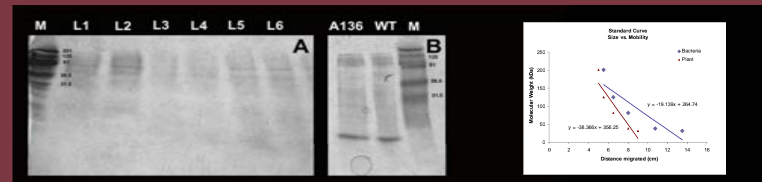
Structural Differences in Sunflower Tissue. (A) Normal stem section (B) Stab point of tissue inoculated with A136 strain (C) Stab point of tissue inoculated with wildtype strain showing neoplasm (D) Stem section of tissue inoculated with wildtype strain showing neoplasm. Bar = 1mm.

Figure 2B



Structural Differences in Tomato Tissue. (A) Normal stem section (B) Stab point of tissue inoculated with A136 strain (C) Stem section of tissue inoculated with wildtype strain showing neoplasm. Bar = 1mm.

Figure 3



Polyacrylamide Gels for Protein Analysis. Gel A: Tomato Plants. (M) Marker lane (L2) Normal tissue at 1:19 (L4) A136 Tissue at 1:19 (L6) Gall tissue at 1:19 Gel B : Bacterial strains A136 and wildtype *Agrobacterium tumefaciens.*

Standard Curve for Protein. Comparison of standard sizes to distance migrated to correlate weights of unknown protein bands by distance traveled.
Plant: Molecular weight vs. distance migrated in lane M on Gel A
Bacteria: Molecular weight and distance migrated in Lane M on Gel B

Methods and Materials

The seeds used for our experiments include *Helianthus annuus* (sunflower) and *Solanum lycopersicum* (tomato) bought at the local nursery. The plants were grown in potting soil and kept in a controlled environment with a 16 hour light cycle and a temperature range of 21-29 C°. They were watered every 2-3 days and allowed to grow undisturbed for 2-3 weeks prior to wounding. The sunflowers were 3-4 inches in height with 3-4 sets of leaves on the day of wounding. The tomatoes measured 1.5-3 inches in height.

Wildtype *Agrobacterium tumefaciens* and a nongall-forming A136 strain were cultured in LB broth and frozen in a -80 F° freezer in 50% broth/ glycerol solution. Two to three days prior to inoculating the plants, bacteria were streaked onto LB agar plates, and incubated at 30 C°.

Tomatoes were wounded with a sterile inoculating needle, while toothpicks were used to wound the sunflowers. Wounding sites were kept at a higher humidity within the growth chamber for two days. Plants were grown another two weeks post wounding.

Freshly hand sectioned plant tissue was viewed using either a compound microscope or a dissecting microscope. Images were captured with Pixel-Link camera. Protein extractions were done using a Plant Total Protein Extraction Kit from Sigma. Subsequent SDS-polyacrylamide gel electrophoresis was done using 4-15% polyacrylamide gel and run at 200 volts for 25 minutes. The gel was stained with Commassie and destained with deionized water.

Benefits of Using a Plant System

- Economical
- Equipment available
- Humane
- Portable
- Easily controlled & manipulated
- Results in 4-5 weeks

Future Objectives

- Determine protein expression differences.
- Describe the motility of bacteria within the plant tissue.
- Determine ultrastructural differences between gall tissue and normal tissue.
- Investigate neoplastic vascular organization and neoplastic aging.

References and Acknowledgments

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