Impacts of antibiotic reagents on morphology and differentiation in *Phaseolus vulgaris* callus tissue

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Introduction & Objective

With the global population expected to reach **9.8 billion by 2050 (UN)**, there is a pressing need to increase crop yields and food production, while also addressing the challenges of climate change and environmental degradation. Green bean callus has long been a crucial area of study in plant biology, as it provides a **unique model system** to study the genetic and epigenetic mechanisms underlying plant growth and development in response to environmental stresses. This research project aims to evaluate the **feasibility of using *Phaseolus vulgaris* (common bean) as a model organism for studying callus formation in vitro** (Figure 1). While *Arabidopsis thaliana* has long been regarded as the standard model organism for plant biology, its extensive evaluation warrants the investigation of alternative model organisms. By studying the regeneration of common bean callus in vitro, this project seeks to shed light on the **epigenetic pathways** that govern callus formation and optimize its growth conditions. One aspect of involves testing the **efficacy of external antibiotics integrated within the callus growth medium to maximize callus growth** and **minimize infections**. This is an important consideration as callus growth can be impeded by common infections that prevent its growth, hindering accurate evaluation of its capabilities.

Materials & Procedure

Plant Media Preparation (~10 plates per 200 mL)
Experimental Group: DI water, sucrose, agar, carrot initiation media, potassium hydroxide, and respective amounts of cefotaxime, kanamycin, streptomycin
Control Group: DI water, sucrose, agar, carrot initiation media, potassium hydroxide

***Phaseolus vulgaris* Sterilization**

1. Sterilize beans with IPA and bleach solutions.
2. Extract callus from each bean and plate on poured plate.
3. Parafilm plates and store in a relatively cool/dry place.
4. Observe growth over ~2 - 4 weeks.

Data Analysis

- **Dry Mass of Callus (~2 weeks):** Dehydrate callus samples and compare data to see morphological differences between different media
- **Measuring Mitotic Index:** Stain nucleic acid (acetocarmine & N/10 HCl) inside cells, cut callus tip (plant & root). Observe under microscope to detail the steps of mitosis and quantify cell morphology.

Data Collection & Image Processing

1. Mitotic Index between each plant & root for each antibiotic → average MI
2. Comparison between rate of mitosis by antibiotic in root and plant areas
3. Thresholding of plant & root callus growth images via Otsu (binarization thresholding mechanism) and Sobel (edge detection thresholding)
4. P-value using Mann-Whitney U (nonparametric test)

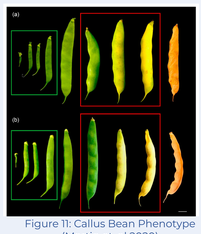
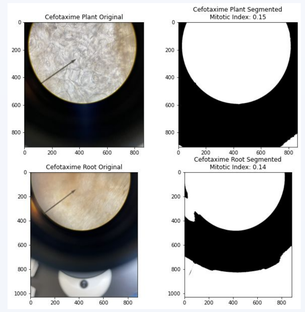
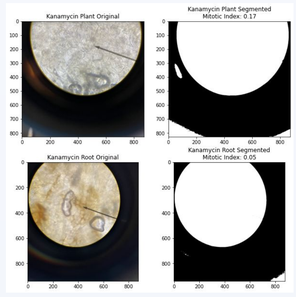


Figure 11: Callus Bean Phenotype (Martin et. al 2020)

Figure 12: Callus Bean grown





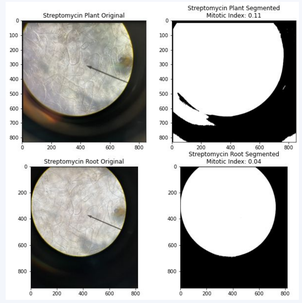


Figure 1-4. Calculated mitotic index for each antibiotic group

Standard Sample Size (by group): 5

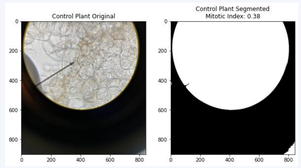


Figure 8. Comparison with standard error values

Figure 5. Otsu, Sobel, HSV Thresholding on Kanamycin Group

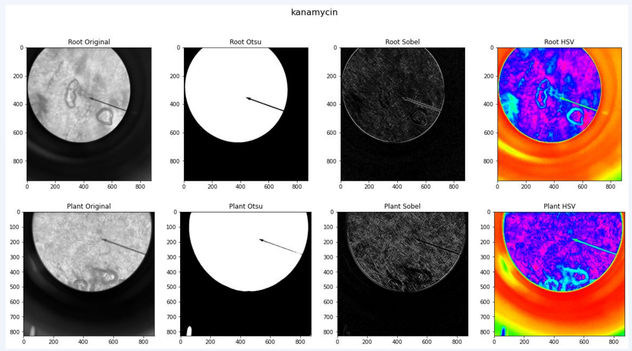


Figure 6. Otsu, Sobel, HSV Thresholding on Streptomycin Group

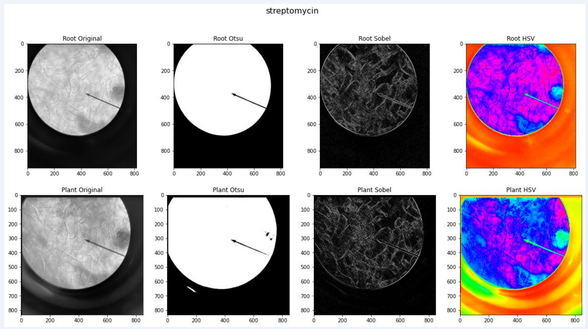
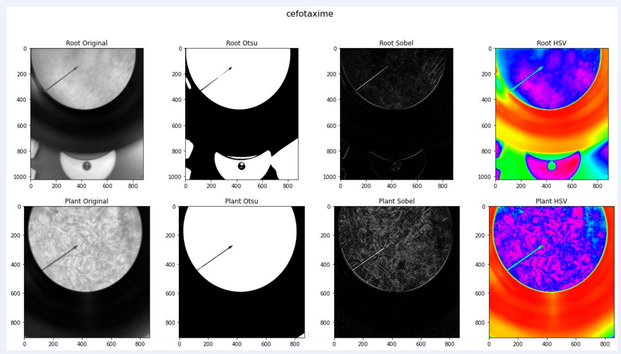


Figure 7. Otsu, Sobel, HSV Thresholding on Cefotaxime Group



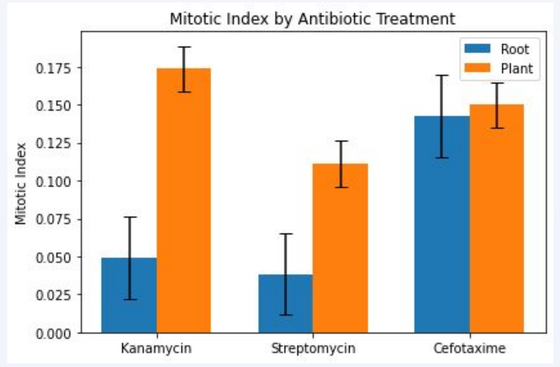


Figure 9. Plant data between antibiotic groups

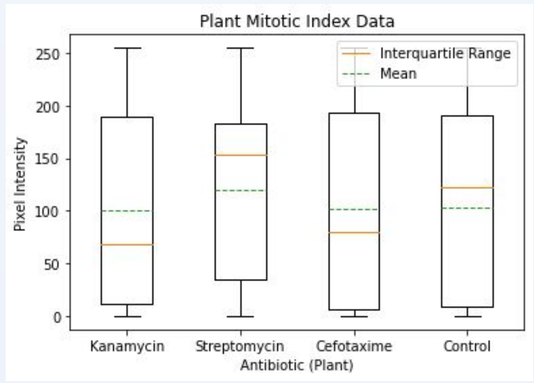
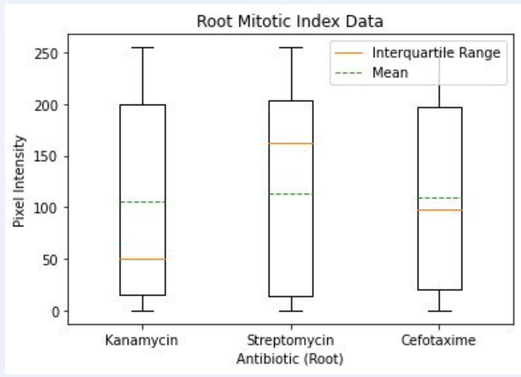


Figure 10. Root data between antibiotic groups



Data (Mann-Whitney U statistic: 22.0, p-value: ~0.03)

Results & Interpretation

The dry mass of the callus and mitotic index were measured to evaluate the effectiveness of the antibiotic reagents on callus health. Measurement of dry mass indicates changes in the morphological growth and accumulation of new tissue. **Higher dry mass indicates better growth and development of the callus tissue,** observed most consistently through the streptomycin medium which had the least number of infections within callus growth. The mitotic index measures the rate of cell division or the number of actively dividing cells in the callus tissue. A **higher mitotic index indicates a higher rate of cell division,** which means that the callus tissue is actively growing and proliferating, indicated most strongly through kanamycin. The Mann-Whitney U test was performed, which showed no statistically significant difference in dry mass between the experimental and control groups (U=11.0, p-value = 0.114). However, the mitotic index was higher in the kanamycin group compared to the control group (U = 3.5, p-value = 0.0385).

Conclusion & Applications to Biotechnology

Through its high mitotic index, the study suggests that **kanamycin** is most effective. It can be concluded that it is actively proliferating, indicating **positive chromosomal health** and **active growth**. .

1. **Selectivity -** Kanamycin makes for an effective selectable marker agent for screening modified cells. Cefotaxime and streptomycin are broad spectrum antibiotics that can kill both contaminated bacteria and plant cells that do not contain a resistance marker.
2. **Mode of action -** Kanamycin belongs to the aminoglycoside class of antibiotics, which target the bacterial ribosome and inhibit protein synthesis. Cefotaxime and streptomycin have different modes of action (inhibition of cell wall synthesis and protein synthesis) which may not be as effective for promoting callus growth.
3. **ROS Induction:** Kanamycin has been shown to induce the production of reactive oxygen species (ROS) in plant cells which play an important role in callus growth and development and may act as signaling molecules that trigger cell division and differentiation. Cefotaxime and streptomycin have not been shown to have this same stimulatory effect on ROS production in callus tissue.

Future applications of bacterial resistance can be studied with a **computational lens** to further study the effect kanamycin has on somatic embryogenesis via callus growth to inhibit bacterial growth, reducing the chance of infection in the callus, further **exploring the gene expression patterns** in callus tissue in response to the different antibiotics used in this study. This could be done through techniques such as **RNA sequencing** or **microarray analysis**, too identify which genes are upregulated or downregulated in response to the antibiotics.

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