

Optimizing *In vitro* Cultures of *Vitis vinifera* Varieties

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Introduction

The goal of this research was to develop an optimal media for growing standard grape varieties in tissue culture, and to develop a protocol for surface sterilization to reduce the amount of contamination of material from the greenhouse. *Vitis vinifera*, better known as European wine grape, originated from the southwestern region of Asia. Grapes are commonly grown for fresh or dried fruit consumption or juice production, but *V. vinifera* is primarily grown for wine production. In the United States, this grape is typically grown in California and Washington. The dwarf variety 'Pixie' was developed from the standard variety Pinot Meunier and represents a model for research with *Vitis vinifera*.¹

The following are objectives for this research:

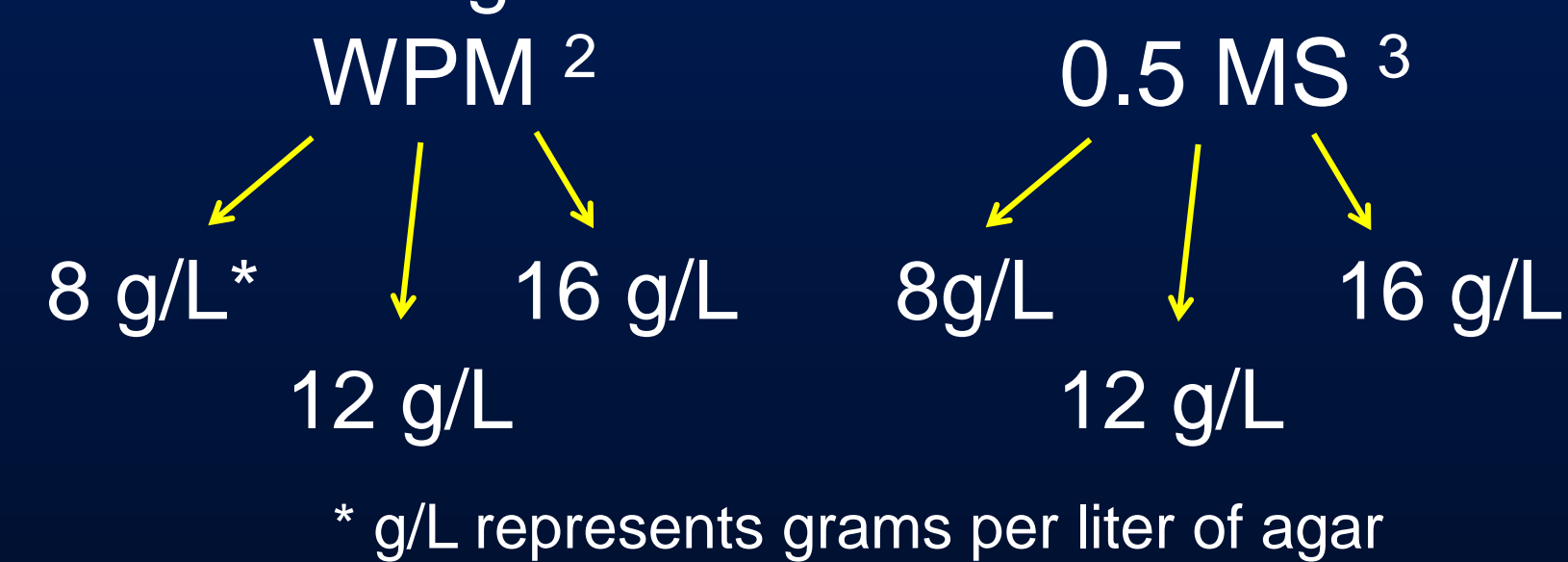
- ✓ Optimize the media to further research on grape regeneration
- ✓ Develop a surface sterilization protocol to minimize contamination, allowing a minimal amount of material to be maintained in greenhouse

Methods and Materials

Chardonnay, a standard variety, and the dwarf variety Pixie were used in this project. Plant material was collected from the WSU greenhouse.

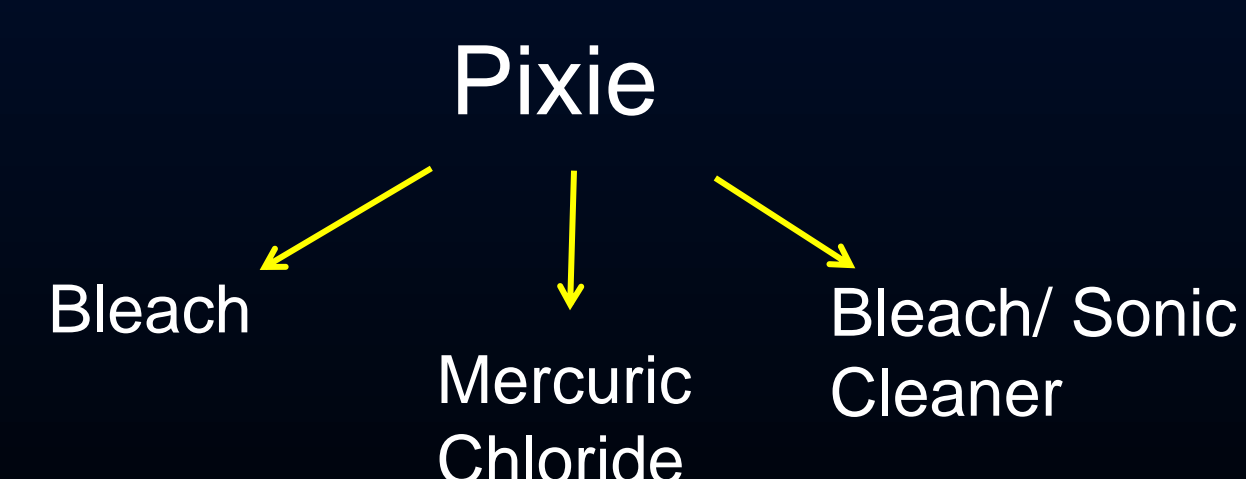
Media Optimization

Chardonnay and Pixie plant material was surface sterilized with bleach and placed in two different media with varying concentrations of agar.



Sterilization Protocol

Pixie plant material was surface sterilized by three methods.



Results

A significant amount of the media trial treatments were contaminated, making it difficult to assess the success of the media. Although the media trials experienced a high percentage of contamination, some valuable observations were made. Pixie rooted and grew well in WPM, but in 0.5 MS it exhibited a lot of callusing which prevented root formation. There were also some signs of stress, but not enough data to connect it with the agar concentrations.



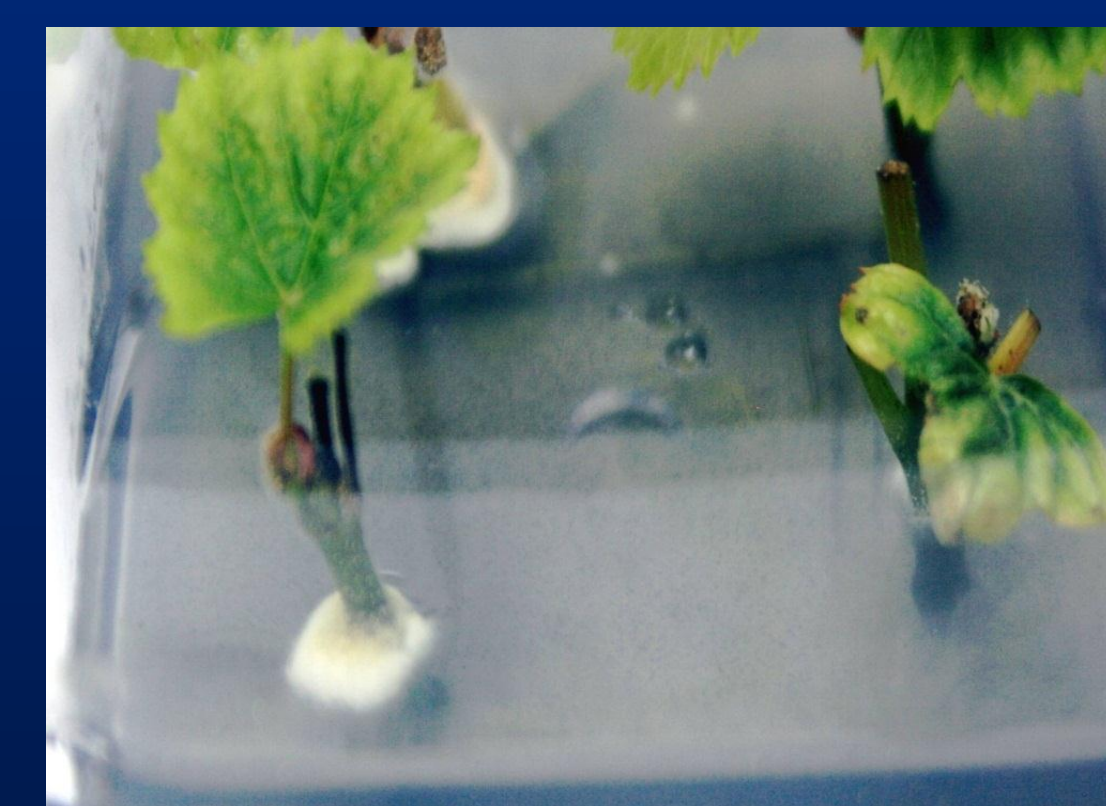
Pixie plant in greenhouse



The 0.5 MS 8 g/L Pixie is forming callus on the roots of the plants.



Pixie contaminated with mold four days after surface sterilization .



Chardonnay is contaminated twelve days after surface sterilization.

The results of the surface sterilization trials showed that the bleach and sonic cleaner treatment was the most successful at preventing contamination, although there was also more tissue death observed with this method. Percentages of contamination for each treatment is as follows:

- 1) bleach – 59% contamination
- 2) mercuric chloride – 24% contamination
- 3) bleach and sonicator – 17%. contamination

Conclusions

In conclusion, due to major contamination, the results of the agar concentration test were inconclusive. As a result, the research project took two different pathways. Since the optimal media and agar concentration experiment was compromised by contamination, the project investigated three different methods for surface sterilization. These two processes are the first essential steps for further research with grape regeneration and gene function studies.

Future Work

The results of the surface sterilization trials were very promising. Since the sonic cleaner may have caused some tissue death, further experimentation could include exposing the plant material to the sonic cleaner for a shorter period of time, or place the plant material in sterilized water for the sonic cleaner treatment and follow that with the bleach treatment



Chardonnay grapes in greenhouse

References

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