Inducing Somatic Embryogenesis in Grape (*Vitis aestivalis* 'Norton/Cynthiana') Tissue Callus Derived from Ovary and Anther Explants

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Abstract

The grape plant *Vitis aestivalis* "Norton/Cynthiana" is known for its hearty nature and low maintenance. However, this grape is also known for its poor propagation. Plant tissue culture is one method that has been used to propagate other recalcitrant species. The first goal of this research was to propagate undifferentiated cell growth, or callus, from anther and ovary tissues of immature flower buds. Callus growth was successfully achieved using a Lloyd and McCown basal nutrient tissue culture media. Healthy callus tissue was quickly created from the ovary tissue. Whereas, callus was not immediately successful from the anther tissue. Anther explant tissues had to remain on the media for several weeks longer than expected before the callus began to grow. The second goal of this project is to successfully generate somatic embryogenesis from the callus. Both the anther and ovary callus are being placed on embryogenic tissue culture media in order to promote embryogenesis. As *Vitis aestivalis* is not easily propagated, the embryogenic tissue media must be carefully made and adjusted to find the exact mix of cytokinin and auxin concentrations that will generate embryogenesis. Somatic embryogenesis in *Vitis aestivalis* has been attempted using callus generated from leaf explant tissue, but to-date, has had no success. The use of floral tissues has shown some success in other grape species. Successful completion of these goals would allow for plantlet production, and ultimately, reintroduction of this species into vineyards as a fungal endophyte free plant.

Introduction

Vitis aestivalis 'Norton/Cynthiana' is a grape known for its poor propagation rates, with a success rate at about 10-20% (Uhls *et al*, 2018). Through the use of tissue culture media, a mass of undifferentiated cell growth, or callus, can be created. Tissue culture media can be useful in the success of embryogenesis, with different levels of auxin or cytokinin. With use of plant tissue culture technology, propagation rates in 'Norton/Cynthiana' can be improved if successful. The success of propagation has been unsuccessful through the use of the grape leaf and stems thus far in the research (Uhls *et al.*, 2018). Over time, undifferentiated cells (callus) will be produced from explant tissues of the anthers and ovaries, and subsequently, plantlets will be produced in the laboratory by way of embryogenesis.

Goals

The first goal of this project was to produce callus from both ovary and anther tissue of the 'Norton/Cynthiana' grape. Once this first goal is completed, the second goal is to accomplish somatic embryogenesis from the undifferentiated callus. The main goal of this project is to reach embryogenesis in *Vitis aestivalis* by the use of specialized media types which should promote callus growth and, eventually, plantlet production.

Materials and Methods

Flower buds were collected in the spring of 2018 and thoroughly sterilized in a 70% ethanol solution. After the ethanol bath, they are washed in a 10% bleach bath with 1% surfactant, and consecutively given three deionized water baths. Upon the sterilization, both the anthers and ovaries were dissected from the closed buds. Dissected ovaries and anthers were place on propagation media containing an anti-fungal chemical, Daconil, and incubated at 25ºC. To not overstress the tissue, they were transferred to propagation media without Daconil one week later. Once callus was successfully produced from the anthers and ovaries, it was trimmed from the ovary and anther tissue and placed on fresh growth media to continue to grow. The next steps include taking the grown callus from the propagation medium and placing it on semi-solid and liquid media with different levels of auxin and cytokinin. These hormones have been used to help generate embryogenesis at different levels in other genera of *Vitis* (Alavijeh *et al*., 2016).

Figure 1: Immature grape flower clusters on the vines

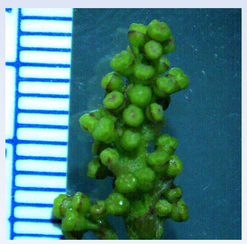


Figure 2: Immature grape flower clusters

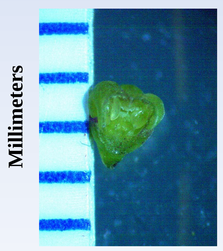


Figure 3: Immature grape flower

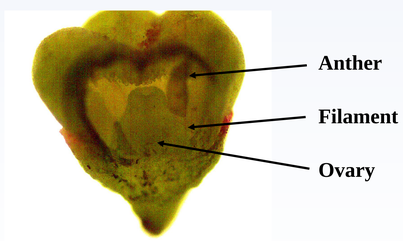


Figure 4: Individual grape flower (dissected)

Results & Conclusions

The successful creation of callus from the anther and ovary tissue has been achieved thus far in the experiment. Where the ovary tissue took roughly a month to produce callus on the primary callus propagation media, the anther callus took several roughly six months after the initial placement of the tissue on the initial propagation media to produce callus. Presently, the ovary callus and anther callus are thriving, producing white, fluffy, healthy callus tissue on semi-solid media.

In the liquid suspension media, we have observed healthy maintenance of the callus as well as bicellular growth by the ovary tissue.

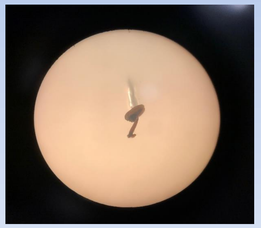
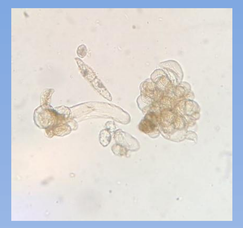


Figure 5: Grape anther on callus-inducing media

Figure 6: Grape anther explants and beginning of callus production

Figure 7: Grape ovary callus



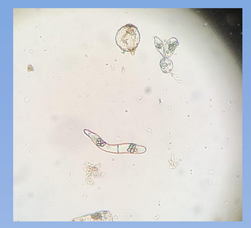


Figure 8: Callus growth from liquid suspension media displaying bicellular growth

Future Research

We will be utilizing various specialized media types with different amounts of Lloyd and McCown salt and auxin and cytokinin hormone to promote embryogenesis. Once we are successful in achieving embryogenesis, we will maintain the tissues on the successful media type to promote plantlet production.

References

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Uhls, A, Jolley, N, Johnston, T and DuBois, J. (2018) The Effect of Sample Date and Timing of Cuttings for Maximum Propagation Efficiency of the Grape, *Vitis aestivalis* "Norton/Cynthiana". Food and Nutrition Sciences, 9, 268-276. doi: 10.4236/fns.2018.93021.

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