College of Agricultural Sciences

APPLICATION OF MEDIA COMPOSITION, TEMPERATURE ADJUSTMENTS, AND SILVER COMPOUNDS TO IMPROVE SOMATIC EMBRYOGENESIS IN CHINESE CHESTNUTS

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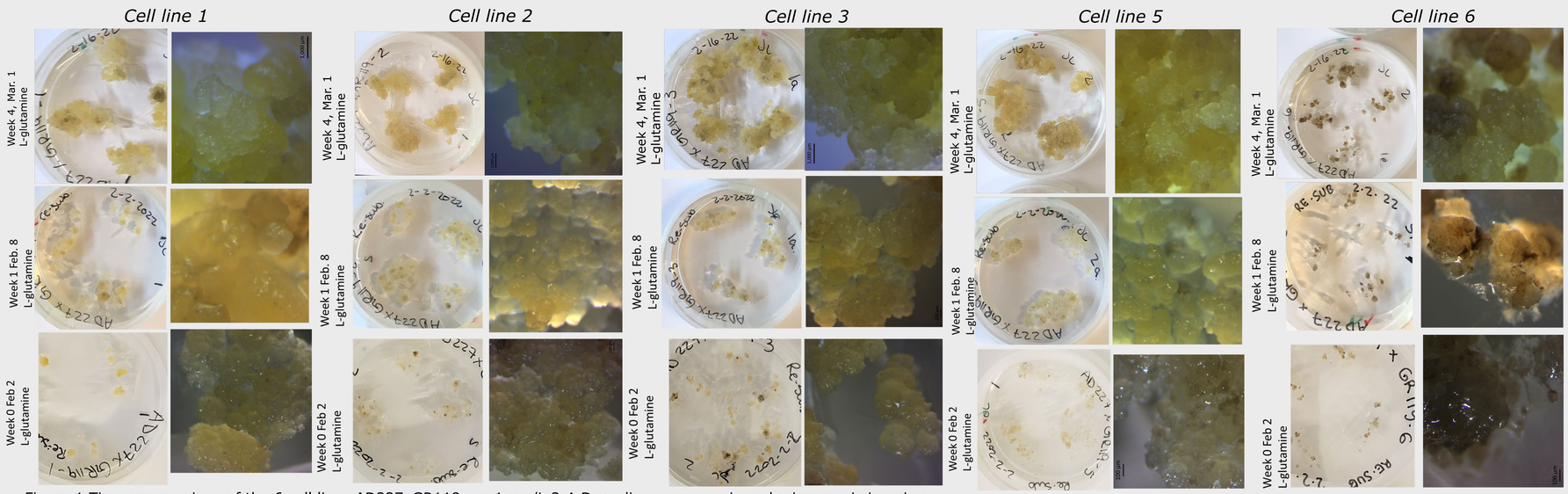

Figure 1 Time progressions of the 6 cell lines AD227xGR119, on 1 mg/L 2,4-D media, macroscopic and microscopic imaging

INTRODUCTION

The American Chestnut (*Castanea dentata*) was a dominant tree species in the Eastern US, until an invasive chestnut blight pathogen (*Cryphonectria parasitica*) came over from Asia hitchhiking on Chinese chestnut (*Castanea mollissima*) tree imports. The disease was first detected in the US in 1904, but the pathogen was likely introduced here earlier[1].Now the American chestnut is virtually extinct in the wild. Chinese chestnuts and European chestnuts (*Castanea sativa*) have more blight resistance than the American; crosses of these trees have been established to introduce the resistance genes from Chinese chestnut to American chestnut[4]. To expedite the resistance screening efforts in chestnuts, an efficient method to mass propagate the desirable genetic material is needed. Somatic embryogenesis (SE) is a tissue culture process which has the potential to allow the propagation of hundreds of tree seedlings with desirable genetic background for genetic study[2,3]. In SE, somatic plant cells are grown on artificial media with plant growth regulators to induce callus production, embryo initiation, and embryo maturation. After cold storage, the embryos are then germinated in tissue culture media to induce conversion to plantlets. However, SE application is currently limited by low conversion rates, especially with Chinese chestnuts[2]. This project aims to optimize SE for Chinese chestnuts by adjusting the composition of the tissue medium.

CURRENTLY IN THE LAB

- 6 cell lines from the AD227xGR119 chestnut cross were acquired from S. Merkle and plated on media according to established SE protocols[2]. Prior to arrival to Keriö lab, S. Merkle[2] initiated cell cultures using immature chestnut burrs (Figure 2).
- Every three weeks, cell culture clusters– called calluses— have been observed, size noted, imaged, and then replated on fresh media according to protocol[2] (Figure 1).
- Once calluses grow to 1 cm[3] the cells will be ready for transfer to suspension culture, where their growth will be more rapid[2].
- Experiment prep.

PROJECT GOALS

The overall goal for this project is to test different media composition and temperatures on the SE of Chinese chestnuts once calluses have been established[3]. Chinese chestnuts have shown less success in cell initiation during SE than European or American chestnuts[2]. Since Chinese chestnuts have the most blight resistance of the three trees, it is important to establish a successful protocol for SE.

- How does temperature affect SE in Chinese chestnut hybrids[3]?
- What are the effects of using woody plant medium vs. Murashige-Skoog medium vs. Driver & Kuniyuki walnut medium[3]?
- Does the use of silver nanoparticles improve germination rate[3]?

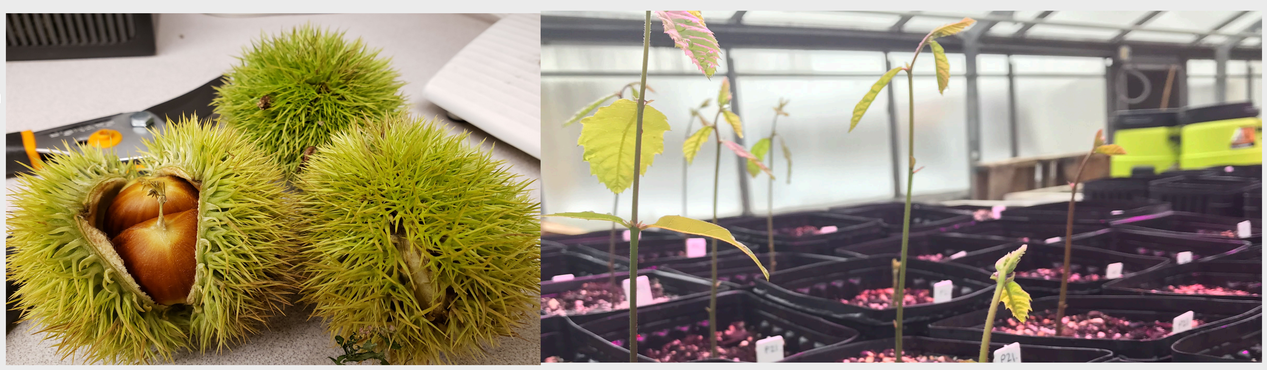

Figure 2 Figure 2 Chestnut seeds (left) and young chestnut seedlings (right) can be used as a cell source to initiate embryogenic cell lines. Photo of chestnut burs courtesy of S. Keriö (Right)

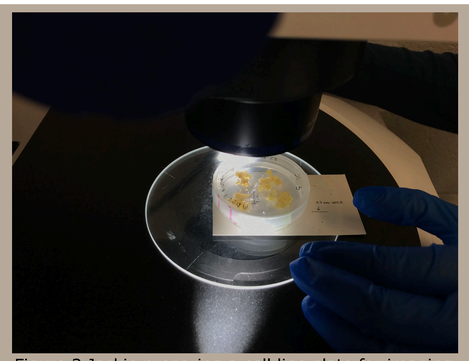

Figure 3 Jackie preparing a cell line plate for imaging, under a dissection microscope

MY ROLE AS AN INTERN

- Maintained cell lines by preparing fresh media and replating calluses every three weeks
- Monitored cell line growth and documented callus size using microscopic imaging (Zen software)
- Maintained a sterile environment to prevent cell line contamination, using a bio hood and aseptic technique
- Established a working protocol for imaging, and for transferring cell lines to new media

Figure 4 Jackie in the CAES greenhouse tagging newly-planted Chestnut seeds

ACKNOWLEDGEMENTS

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