Potato Lab

Tissue Culture Upgrades

Doing more to protect your investment

Specialty Crop Block Grant

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In 2021, the potato lab submitted and was successfully granted funds from the State of Montana and the USDA for upgrading the Montana State Potato Certification tissue culture program. 7 potato varieties were chosen to represent the room. 5 widely grown Russets and 2 specialty varieties.

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The grant proposal identifies rick factors that have the potential to disrupt clean and optimal growing conditions for the plantlets. This is your mother source for all the potatoes grown in the state. The objectives of the proposal seek to reduce the possibility of contamination events through 3 sub-objectives, and to reduce the cost of temperature control by replacing the current lighting with cooler LED lights.

Project Objectives

Objective 1: Improve efficiency and reduce risk in the tissue culture program by updating SOP

How we are reducing required manhours & the possibility of contamination

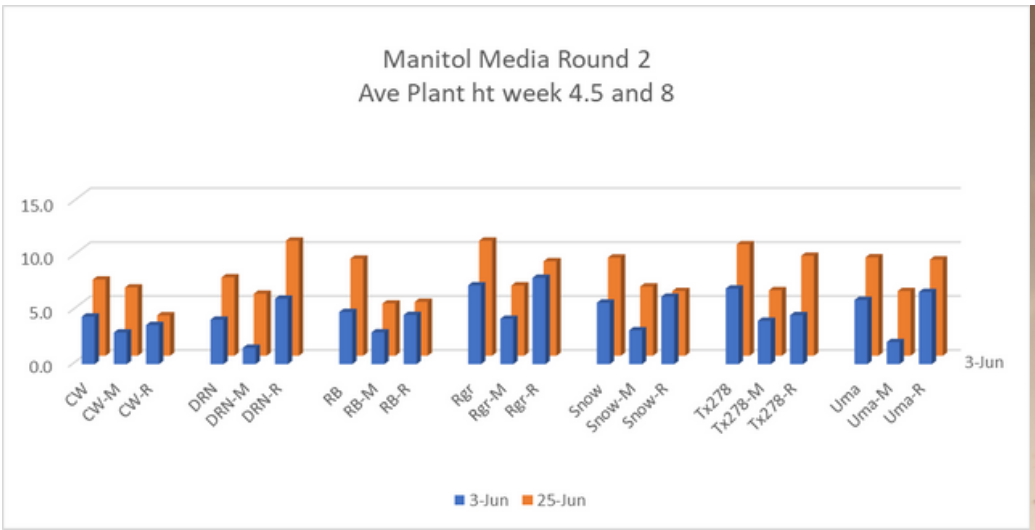
Current maintenance practices require the entirety of the Mother stock to be cut every 4-6 weeks. This equates to ~90 hours a month of highly detailed attention for quality. That time does not include the time needed to refresh the media stock, or the time required to monitor and assess growth quality and catch contamination in its early stages. Contamination can occur with every cutting, even with preventative measures, sanitary practices and aseptic techniques.

>>> Sub-objective A: Increase intervals between maintenance propagation

The goal of this objective is to extend the time needed between maintenance cuttings from 4-6 weeks to 12 or more weeks, with the ability to revive normal growth when replanted in current SOP media for order increases.

The method chosen for this experiment to extend time between cuttings we added mannitol to the media. Mannitol is an osmotic inhibitor, slowing down cell division, which slows down the plantlet growth. While the mannitol media performed as generally expected, stunting the plant growth thus extending time between cutting, the overall look of the plants was determined to be unacceptable for the program. The incidence of Callus and unusual growth on some of our most widely used varieties, as well as some poor recovery results did not inspire confidence in this method for preserving our mother stock.

Alternative methods have been assessed for time maintenance and feasibility. The new chosen method uses salicylic acid as a osmotic inhibitor, as well as cooler temperatures (5-7°C). The cold incubator is equipped with lights to maintain slow growth. The plants are then maintained on a 4-6 month cutting cycle. The appropriate incubator has been ordered and the new chosen method will be initiated this fall.

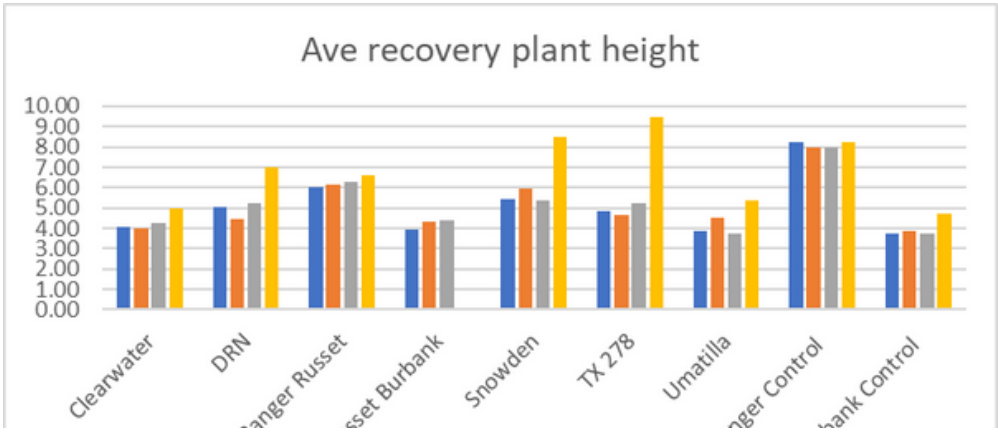


Sub-objective B: Establish a long term mother stock reserve

The goal of this objective is to create a backup mother stock inventory that can be recalled or refreshed without maintenance cutting for up to 2 years

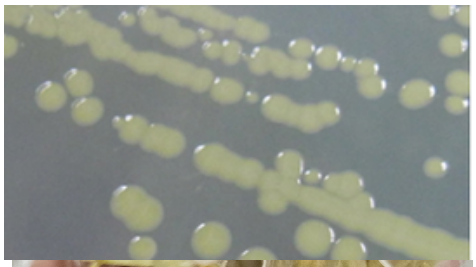
MIM (minituber induction media) is used for storage of mother stock. This is beneficial because it reduces the possibility of contamination. The process stores plants that develop in darkness to induce micro-tuber formation. The plants are then moved to a cold incubator (7°C), and kept for up to 2 years. Currently 4 recovery events have been evaluated, 3, 6, 9 and 12 months. The plants are removed from the cold room, grown at Room temp with 16/8 hr day night light cycles for 6 weeks, and then cut to normal media. Recovery plants are growing as expected.

This process is vital for asset redundancy. In the case of a catastrophic event (mass contamination, or overheating) we have a system in place to recover all our current varieties without needing to import and quarantine until proven clean.

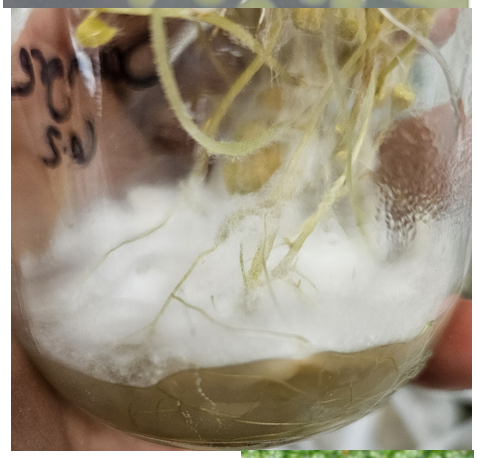


Sub-objective C: Establish a protocol for preventing and treating microbial and insect contamination.

The goal of this objective is to develop a protocol with microbial controls to be used to prevent and/or treat contamination from multiple sources, but focused on insect vectored contamination.



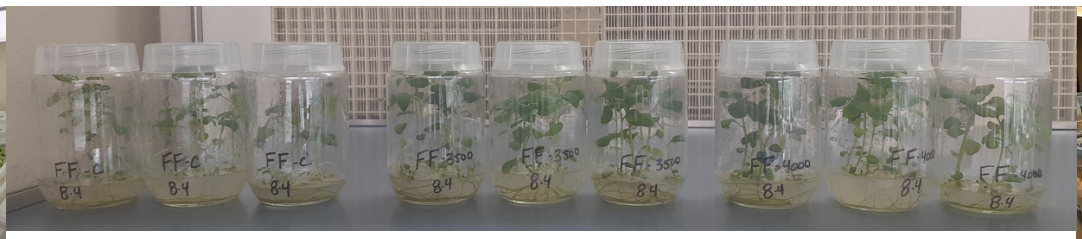
Contamination in tissue culture is one of the largest threats to the wellbeing of the mother stock. Even with precautions(aseptic technique, parafilm, sticky cards, bug bombing), sometimes insects sneak their way into the jars. They spread bacteria and fungus, which can be propagated. The lab has countered this by adding both an insecticide and antibiotics to the media.



The insecticide we use is Orthene, a water soluble insecticide that works systemically (through out the plant). We use 1 mL of orthene solution/L of media. The Orthene used in the lab is 13g (75% active ingredient) mixed into 100mL distilled water. It is used cyclically so a build up resistance does not occur.

When microbial contamination does appear, antibiotics are added to the media. We tested several antibiotics at multiple concentrations to see where the optimum level of microbial control with the fewest side effects to the plants is. The best antibiotic we have found is **carbenicillin at a rate of 100ug/L.** This antibiotic can be used safely at a fairly high rate with minimal negative effects on the tender plantlets. Other antibiotics tested had much lower tolerances, and in some cases resulted in poor growth and vigor that persisted when plants were cut to fresh media.

Objective 2: Convert plant growth lights from fluorescent to LED



The goal of this objective is to increase efficiency, and reduce long term costs of both energy and cooling costs required in the TC room.

The tissue culture room lighting has been in place for over 20 years. The fluorescent lighting currently in place puts out a large amount heat requiring an AC unit to run full time to keep the plants at optimal temperatures. It also requires diligence to replace burned out bulbs, and keep the spectrum balance in place for said plants.
A Shift to LED lighting has been discussed for a while now, but looking into what the best options are has been daunting. There are so many choices and many of the LED grow lamps designed for greenhouses are purple. It was decided that the purple lighting was not ideal for our program as the color made identifying contamination much more difficult.

MSU facilities sourced adjustable LED lighting, adjustments on the lights include 3 color spectrums and 3 dim levels. Experiments were done on 9 varieties (5 of the most common russets, and 4 specialty lines) to determine if the LED lighting creates superior plants, and if so, what color spectrum is best.

LED lighting produced plants that were greener, stouter, and had overall better vigor. Plant height was better under fluorescent light early in the experiment, but as the experiment progressed, the LED lighting plants caught up and about half of the selected lines were taller.

The chosen LED lights are currently being installed in the tissue culture room, set to a median color spectrum and median intensity.

Field Trip: University of Wisconsin - Madison

Tissue culture staff got the opportunity to attend a conference in Madison Wisconsin.
While there, we had the opportunity to observe the tissue culture system they have in place.
UW-Madison has successfully utilized both the MIM mother stock Long term storage, and the Salicylic acid extension media for maintaining WI's potato mother stock. They utilize Percival cool incubators and a positive pressure growth room for recovery that helps keep contamination to a minimum. We look forward to having similar success with the protocols we are adopting to improve efficiency, cost and manpower to keep our mother stock clean and accessible.