Title: Cryotherapy of potato stocks in the SLV – 2014 Proposal

Investigators: Robert D. Davidson and Caroline Keller
Department of Horticulture and Landscape Architecture
Shepardson Bldg, CSU and San Luis Valley Research Center

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Nature and Objectives:
The approaches to producing clean, disease-free seed potatoes are constantly changing and improving. It is imperative that these improvements be routinely adopted by the programs at the SLV Research Center to efficiently and effectively service the Colorado potato industry. One of the challenges faced by the CSU Cultivar Development program and the Potato Certification Service Tissue Culture facility has been the clean-up and propagation of potato stocks containing virus diseases. Among the most challenging is Potato Virus S.

The current protocol for establishing tissue culture stocks (mother accession plants) requires the initiation of sprouts from clean tubers into tissue culture, full disease testing of the resulting plantlets, and, if plants are found with virus problems, a series of clean-up or therapy steps to rid the accessions of virus. The base level initiation takes four to six weeks to obtain clean tubers, two to three weeks for sprout growth and another week plus for testing. After that time, if virus is present in the accessions, there is an additional three to four months where the plantlets are increased, put into heat therapy and grown on a media containing a viracide agent, excised and grown back into plants which can be tested, and subjected to a six to eight week greenhouse grow-out with additional testing to verify freedom from the infection. All of these steps take a lot of time and labor commitment, not to mention that they are not always successful. When dealing with 30 + new accessions each year from the CSU Breeding Program and other growers, this becomes quite challenging to say the least.

Recent literature suggests that use of cryotherapy for virus clean-up, especially in potato, has been quite successful and is much less time consuming. A normal tissue culture laboratory is quite capable of handling these techniques and producing the media necessary for these procedures to work. Secondly, after establishing a clone bank stock, finding and utilizing novel techniques for medium term storage and distribution to the producers of the clean clones/cultivars is very beneficial.

Objectives:

1. Receive training in current cryopreservation techniques and then utilize these techniques and those discussed in the literature to develop a cryotherapy program at the SLVRC, PCS tissue culture laboratory. Utilize these techniques to do a comparative study between the current protocols and the benefits of using cryotherapy. (Recently, Davidson and Keller received training in cryopreservation techniques from Dr. Gayle Volk and her technician, Remi Bonnart at the National Center for Genetic Resources Preservation (NCGRP), 2/24-26/2014). A cooperative agreement between the CSU/PCS lab and the Plant Germplasm Preservation Research Unit will be developed to share findings and continue work on cryotherapy methodologies.

2. To work on developing the 'microbille' technology for utilizing tuber sprouts as a medium term storage technique (1-1.5 years) and compare to the current use of medium term storage with minimal medium to produce small microtubers in culture (2-3 years). Additionally, to utilize this technology to provide a quick and reliable mechanism to distribute clean potato cultivars in a much easier manner than use of tissue culture plantlets. The 'microbille' technique was observed by Davidson on a trip to Switzerland, but there was little time for discussion, yet it appears to have great potential for the Colorado program.

Methods, Procedures and Facilities:

Objective 1: *Cryotherapy:* Following the recent reports in the literature, cryopreservation techniques for potato will be utilized with varying vitrification levels of meristematic tissue to clean stocks of virus infection. This involves removing larger meristem segments from infected tissue culture plants, dessicating them with increasing levels of sucrose in the media, attaching the meristems to foil strips, freezing the meristem segments in liquid nitrogen, and then placing the meristems on recovery media for re-growth. After a period of five to eight weeks, plants should be at an appropriate size for testing. Timing, labor, costs, success of clean-up, etc. will be evaluated against the tissue culture lab's current techniques and a position statement will be formulated for the Cultivar Development program and the CCPGA.

Objective 2: *Microbille technology:* Evaluate the use of a 'microbille' type of storage versus current tissue culture options of regular increases of tissue culture mother plants and use of minimal media to produce microtubers. Microbille technology consists of removing small sprouts from clean tubers, disinfecting the sprouts, encapsulating the individual sprouts in an alginate bead, and storing these beads in a cold storage environment. This process may allow for very rapid initiation of new germplasm (2 weeks versus 4-6 weeks), storage of material for up to 1 ½ yr and very rapid distribution of cultivars to the producers. It would be expected that over a two week period all of the start-up material for the labs could be generated and stored in the cold. When the growers needed material, five to ten of the microbille beads could be given to the producer for implant in their own media. This would preclude the need for constant cutting of the clone bank material and produce viable, clean, tissue culture plants within a one to two week time frame rather than the current ten week and more time frame necessary with the microtubers from the minimal media storage.

Resource needs at the SLVRC:

All resources necessary to conduct these projects are currently available or will be purchased as needed.

Competitiveness enhancement for Colorado potato producers and Extension/outreach plan:

The proposed research will make the tissue culture lab more efficient and will allow this facility to complement the potato breeding program in a better manner while helping with grower orders in a more timely fashion. Additionally, more efficient disease clean-up will reduce costs and increase productivity as numbers of clones for clean-up continue to increase. Outreach will take place by use of a series of targeted newsletters to tissue culture labs and a more efficient distribution of the tissue culture material through one-on-one visits with producers.

Potential for Leveraging Outside Funding:

There is no potential for generating outside funds with these procedures, but there has been a good deal of interest by other state's tissue culture labs (Texas, Idaho, and Oregon). It would be expected that if these techniques are successful, a workshop of some sort would be provided for interested parties to learn the procedures.

Time line for Proposed Research:

The time line for this research is expected to take place in the short-term (one yr).

Budget for 2014: $12,000

Labor: Part time (450 hours or about 2.5 months)	$5,000
Various equipment and supplies including a dissecting scope, liquid nitrogen containers, liquid nitrogen, and materials for cryopreservation	$7,000

Selected References:

1. Cong-Linh, L.E. 2012. Le conservatoire des varieties de pommes de terre cultivees en Suisse. Poster located at Station de recherché Agroscope Changins-Wadenswil ACW, Nyon, Suisse.

2. Wang, Q., Y. Liu, Y. Xie, and M. You. 2006. Cryotherapy of potato shoot tips for efficient elimination of Potato Leafroll virus (PLRV) and Potato Virus Y (PVY). Potato Research 49:119-129.

3. Wang, Q.C., B. Panis, F. Engelmann, M. Lambardi, and J.P.T. Valkonen. 2008. Cryotherapy of shoot tips: a technique for pathogen eradication to produce healthy planting materials and prepare healthy plant genetic resources for cryopreservation. Annals of Applied Biology 154:351-363.

4. Yin, Z., C. Feng, B. Wang, Q. Wang, F. Engelmann, M. Lambardi and B. Panis. 2011. Cryotherapy of shoot timps: a newly emerging technique for efficient elimination of plant pathogens. Proc. First IS on Cryopreservation in Hort. Species. Eds: B. Panis and P. Lynch, Acta Hort. 908: 373-383.