

**AGRICULTURAL RESEARCH FOUNDATION  
INTERIM REPORT  
FUNDING CYCLE 2017 – 2019**

**TITLE:** Use of Somatic Cybridization to Overcome Cytoplasmic Male Sterility in Potato

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**Justification:**

Over the last 50 years, potato breeders have succeeded in introgressing numerous valuable disease resistance genes into elite potato germplasm from wild potato species. However, many of these resistance genes are only present in potato clones with cytoplasmic male sterility (in the absence of male sterility, each potato plant can produce both male (pollen) and female (ovule) gametes, both of which are required for a cross to be successful). Because cytoplasm is inherited from the female parent, and these clones can only be used as female parents, all progeny resulting from these clones are also expected to be male sterile. This prevents plant breeders from making crosses between clones carrying these introgressed genes (neither clone can be the male parent), in turn preventing the development of a potato clone that combines many of these valuable traits.

Somatic cybridization is a technique that can be used to replace the cytoplasm of a potato clone (containing small segments of DNA in chloroplasts and mitochondria), without altering the clone's nuclear genome (which contains the bulk of the genetic information). In somatic cybridization, protoplasts (individual plant cells stripped of their cell walls) of the nuclear donor are fused with protoplasts of the cytoplasm donor, which have been irradiated to disrupt nuclear DNA (Figure 1). If this technique was used to replace male-sterile cytoplasm with cytoplasm that is not known to induce male sterility, fertility would likely be restored to the target clones.

Somatic hybridization, a similar technique in which both nuclei are intact and the fusion product contains the sum of the genes present in the two parents, has been extensively used in potatoes to introgress genes from wild germplasm and to move between ploidy levels in potatoes. Interestingly, somatic cybridization has been used to induce male sterility in citrus for the purpose of developing a seedless mandarin (Guo et al. 2003), and in tobacco for the purpose of hybrid seed production (Atanassov et al. 1998).

The Oregon State University (OSU) Potato Breeding and Variety Development program plays a key role in the Tri-State potato variety development program. OSU breeding efforts focus on the four major market classes: 1) Russets for processing, 2) Fresh market russets, 3) Chipping; and 4) Specialty. Traits of importance include yield potential, biotic stress resistance [Potato Virus Y (PVY), Verticillium wilt, Columbia root knot nematode (CRKN), soft rot, late blight, silver scurf, psyllids, etc.], abiotic stress resistance (drought and heat stress, cold sweetening), cooking quality, low acrylamide level, bruise and shrinkage resistance, storability, internal quality and appearance, and phytonutrient content.

Currently, OSU breeding efforts are more focused on developing new potato varieties with improved resistance to PVY and CRKN. Recently released tri-state variety Payette Russet and advanced selection POR06V12-3 both carries resistance to PVY. For CRKN resistance efforts are being made to develop potato varieties with resistance introgressed from wild potato *Solanum bulbocastanum*. PA99N82-4 is a selection with resistance to CRKN. All the potato selections mentioned above are male sterile and provides a barrier to introgress both resistances (PVY and CRKN) into a single clone. The current proposal aims at developing somatic cybrids to overcome this barrier.

**OBJECTIVES:**

1. Develop Somatic Cybrids to restore male sterility

**PROCEDURES:**

**Procedures**

*Genotypes selected*

A list of male-sterile cytoplasm recipients to be used in this study, and the diseases they hold resistance to are listed in Table 1. Cytoplasm donors will be two clones derived from *Solanum tuberosum* Group Phureja, a group of potatoes indigenous to Columbia which have cytoplasm that rarely induce male sterility.

**Table 1.** Cytoplasm recipients, and which diseases they hold resistance to.

Clone	Resistant
PORO6V12-3	Potato Virus Y Tobacco Rattle Virus Potato Leaf Roll Virus
PA99N82-4	Columbia Root-knot Nematode
Payette Russet	Potato Virus Y

*Somatic cybridization*

Somatic cybridization will be conducted using the methods described by Lightbourn (2004), except protoplasts of the cytoplasm donor will be irradiated with gamma radiation.

*Evaluation of fusion products*

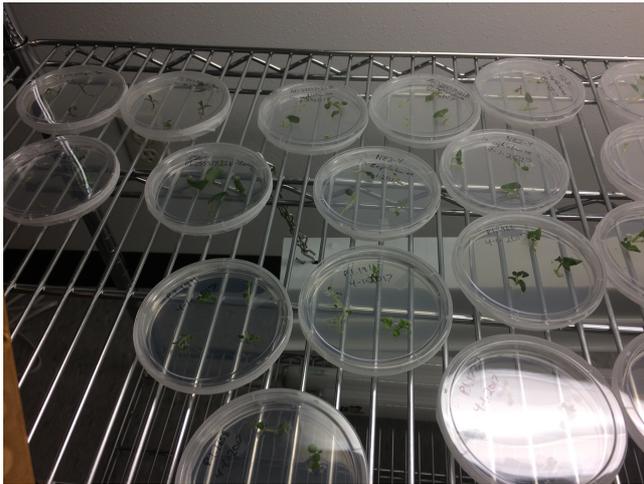
Cytoplasm of plants regenerated from protoplasts will be genotyped using PCR with the primers designed to differentiate the relevant chloroplasts and mitochondria. Pollen from plants that have either mitochondria or chloroplasts from the cytoplasm donor will be used to pollinate the the potato varieties Tacna and Lamoka to assess pollen fertility.

**Update on the Research:**

In our efforts to produce male-fertile somatic cybrid potatoes with key disease resistance genes, we have had mixed results. Using the protocol described in Lightbourn (2004), we were able to produce enough intact protoplasts for to conduct approximately six fusions. By making simple adjustments to this protocol, we expect to increase the yield of protoplasts in future attempts to produce cybrid potatoes, which will greatly increase our

likelihood of success. However, when we attempted to regenerate plants, only a few protoplasts began to divide, and these died approximately one week after fusions. In addition, many of the petri dishes containing regenerating protoplasts became contaminated, and needed to be treated with cephalotaxime or discarded. After talking with scientists familiar with protoplast fusion, we learned that both contamination and protoplast death after several cell divisions are common problems.

We plan on working on this project again in the spring with the facilities in Corvallis rather than initiating the tissue culture work at Hermiston. We are hoping to produce some protoplast fusions this time. Further, we are also exploring the options to get a new equipment to perform Electrofusion.



**Figure:** Plantlets being treated with cephalotaxime prior to electrofusion to reduce risk of contamination

**BENEFITS & IMPACT:**

If this method is successful we will generate plants with disease resistance coupled with male fertility that we can use in the breeding programs.

**ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM:** No additional funding was received

**FUTURE FUNDING POSSIBILITIES:** If we are successful in generating cybrids we will plan on applying for some external grants.

Reference Cited:

Lightbourn GJ (2004) Development of intermonoploid somatic hybrids of potato and their molecular analysis based on polymorphism for retroelement *TST1* (PhD Thesis).