PROGRESS REPORT TO THE MAINE POTATO BOARD
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Project Title

Building Varietal Resistance to Disease using Marker-assisted Selection

Investigators

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Executive Summary

Improved variety resistance will be a key long-term means of managing the new strains of both late blight and PVY that are plaguing the industry. Marker-assisted selection is an effective method of speeding the development of new potato varieties with key disease resistance traits; however, only a few useful molecular markers are currently available to help breeders select potato clones that have desirable traits. In this project, we are developing late blight and PVY resistant potato clones through the use of marker-assisted selection. We will utilize phenotyped and genotyped late blight resistant parental clones to begin identifying likely resistance genes. We will begin developing genetic marker protocols that can be used to speed future breeding, selection and variety development for late blight resistance. When the resulting materials also have a PVY resistant parent in their background, we will use two PVY resistance markers (RYSC3 for Ry_{adg}; YES3 for Ry_{sto}) to speed identification of new PVY-resistant potato clones. Where appropriate we will also use the H1 marker for golden nematode resistance. Maine Potato Board support during 2012 and 2013 allowed us to successfully incorporate the RYSC3 and H1 markers into our breeding efforts. The marker-assisted approach helps us identify resistant clones earlier in the program, thus increasing the chances of retaining resistant clones over several years of field selection and improving the program's chances of releasing useful varieties with resistance to late blight, PVY, and golden nematode. This research will also allow us to more quickly identify resistant clones for use in future crossing programs designed to generate resistant varieties.

Duration of Project

Funding was requested for April 1, 2016 to March 31, 2017 to support the expansion of marker-assisted disease resistance breeding as an integral component of the potato breeding pipeline at Aroostook Research Farm. The goal is to implement simple and effective DNA-based procedures for genotyping previously established breeding lines, new clone selections, and their derivatives for late blight resistance.

Project Objectives:

To reduce the impact of late blight through the development of new varieties and breeding lines with effective resistance to diverse populations of the late blight pathogen

To reduce the impact of PVY and golden nematode by identifying sources of resistance within the program

To use DNA marker-assisted selection strategies for combining known resistances genes for one disease (e.g., late blight resistance in *R1, R3a,* etc.) with others (e.g., PVY resistance in Ry_{adg}, or Ry_{sto}) in common genetic backgrounds that are economically important to the potato industry in Maine and the eastern United States

Grant Received:

$7,000 ($7,000 was requested)

Progress on late blight marker-assisted selection

Late blight resistance is one of the priorities of The University of Maine Potato Breeding Program. Heretofore, resistant clones and varieties have been identified by phenotypic selection against field populations of late blight. With this research, we have continued moving toward a more strategic, genetic-based selection process that maximizes opportunities for 'pyramiding' resistance genes. Resistant clones and varieties from within the ME program and from the USDA-ARS Beltsville potato breeding program have been identified as important candidates. Example late blight resistance sources that are being examined include: B0718-3, Barbara, Dakota Trailblazer, Defender, Dorita, Elba, J117, Jacqueline Lee, NY121, Missaukee, Pirola, Stirling, Tollocan, Torridon, and Yukon Gem. In all, more than 60 varieties and clones are being studied.

Protocols for eleven resistance genes derived from *Solanum bulbocastanum, S. demissum, S. microdontum,* and *S. phureja* have been gathered from the literature. All available markers for *S. demissum R*-genes are being utilized: *R1, R2, R3a, R3b, R8,* and *R10*. The *R1* and *R2* genes have been completely sequenced, so the identification of these genes should be very reliable. The protocols for the other four genes are associated with nearby chromosomal markers. The protocols for *R3a, R3b,* and *R8,* were used in a study to show that the Mastenbroek differential clones MaR8 and MaR9 are actually stacked with multiple late blight genes derived from *S. demissum* (Kim et al., 2012). The marker associated with the *R10* gene is 0.05 cM away, indicating extreme likelihood that the gene is present when the marker is. The *S. bulbocastanum* hybrid showing aphid resistance as well as late blight resistance, Sbu8.5, is known to have the

RB/*Rpi-blb1* gene, and is being tested for the *Rpi-blb2* and *Rpi-blb3* genes. All three genes have been completely sequenced, so these markers are highly reliable. The results of this study are the start of a late blight genetic profile for each variety and clone.

Plant DNA extractions for late blight resistances have been refreshed, and PCR-based marker testing is continued from fall 2015. Marker tests are ongoing daily, and late blight marker testing for gene identification should be complete by the end of this grant term. Additionally, almost 40 of these varieties and clones will continue to be phenotyped in leaf assays against US8, US11, and US24. They will be measured for disease amount and rate. Most of the clones and varieties have records of resistance against US8 published in the literature. If my results for the US8 echo these publications, then the results of the tests against US11 and US24 will also be valid. I am waiting for these races to be delivered from Cornell University; testing will continue through the spring, with plans of completion by July.

The results of the genetic and phenotypic data of each variety and clone will be combined to create a genetic late blight resistance profile. The profile will be established by examining the relationships between resistance and certain gene combinations. While many combinations of resistance genes have been shown to be additive, a study from 2014 indicates the possibility that some resistance genes interact to suppress resistance (Stirnweis et al., 2014). The authors suggested that this was a reason that a resistance gradient exists in resistance crosses. If there are any patterns of suppressive and additive resistance, we will be able to detect them as we look at the genotyping and phenotyping data together. The outcomes of this analysis will highlight the effectiveness of certain gene combinations, and may help potato breeders be more efficient at the crossing block. I am anticipating this analysis (and my dissertation) to be complete by the end of this calendar year. The disease genetic profile that we establish now will have long-term benefits for the industry as a whole; as new races of late blight and other pathogens attack the crop, we will be able to refer to these profiles for novel combinations of resistance.

Progress on PVY marker-assisted selection

Resistance to PVY has become a high priority in The University of Maine Potato Breeding Program as concerns about the disease have increased in the industry. With the reduced funding that was available for the proposed project, we focused our efforts on new marker development for late blight resistance. Where resistance sources are also likely sources of PVY resistance, we are using DNA-based marker technology to evaluation the likelihood of stacked late blight and PVY resistance genes; however, we have moved the routine use of DNA-marker based technology for selection of PVY resistant clones to other funding sources and will report on that effort in our overall potato breeding program report.

New lab equipment

Some of the funding requested for this year was for the purchase of a blue-light transilluminator. This instrument reads electrophoresis gels without the use of dangerous

carcinogens. We purchased the instrument and was able to switch to a safer, and unexpectedly faster, protocol for the 2016 season. There is virtually no difference in data collection, and we are very thankful to the Maine Potato Board for supporting the safety of our lab workers.

References

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