FINAL RESEARCH REPORT 2012 'DEVELOPMENT OF PLUM POX VIRUS RESISTANT 'FRENCH' PLUM.

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OBJECTIVES

The objectives of this project are to 1) to develop genetically engineered clones of 'French Prune' that are highly resistant to PPV and 2) determine the level of susceptibility of 'French Prune' to PPV.

PROPOSED RESEARCH AND DEVELOPMENT

Since 1990, the USDA-AFRS Genetic Improvement Research Unit has been developing genetically engineered (GE) plum lines with different novel traits including resistance to plum pox virus (PPV). PPV is the most serious disease of plum, causing fruit deformation, premature fruit drop, and ultimately loss of productivity and tree death. PPV has devastated plum production in Europe. It spread to North and South America in the 1990s and continues to spread in Canada, Chile and is currently present in New York after being eradicated in Pennsylvania and Michigan. 'HoneySweet' a GE plum highly resistant to PPV, developed in our laboratory, is undergoing the deregulation process in the U.S. and has been approved by APHIS and FDA. In our laboratory we can consistently produce PPV resistant GE plums from seed and have plants in the greenhouse to test within 6 months. We propose to adapt this highly efficient seed transformation system to clonal material of 'French Prune'. 'French Prune' will be genetically engineered with a piece of a PPV gene to induce a natural plant resistance mechanism called "gene silencing" to specifically destroy PPV. This is the mechanism that is responsible for the high level of resistance of 'HoneySweet' plum. Transgenic 'French Prune' lines will be tested in the containment greenhouse for resistance.

Based on our experience with previous transgenic plum lines we will utilize gene constructs with minimal intellectual property issues and constructs with the greater potential for consumer acceptability. Gene constructs will utilize only plant gene sequences (promoters and terminators) with the exception of the kanamycin antibiotic marker gene which is necessary at this stage of research.

Examination of the PPV susceptibility will be undertaken through inoculation of young propagated trees of 'French Prune' in an approved BL3 bio-containment greenhouse facility at USDA-ARS, Ft. Detrick, Maryland. Under these conditions vegetative tissue symptoms can be evaluated. Budwood will be sent to a European collaborator for field tests and observations of fruit symptoms. These fruit symptom observations will be reported to the CDFB in the future but will be beyond the scope of the proposed project timetable.

RESULTS

In the final stage of the project we concentrated on improving the regeneration of shoots from leaves of 'French Prune'. High regeneration rates are necessary for producing transgenic plants because relatively few cells produced by the leaves cultured in vitro can be transformed (PPV gene inserted) and once the gene for PPV resistance is incorporated into a cell, that cell must then go on to form a shoot. If relatively few cells are transformed with the gene and then few of

the cells form shoots then the odds of developing a shoot with the PPV resistance gene are quite low. We were able to achieve consistent rates of regeneration of shoots from leaves of 'French Prune' at a level of 75%. That is 75% of leaf pieces produced 'French Prune' shoots. Yet the number of shoots produced per leaf remained low in all treatments, averaging 2.5 shoots per leaf (Figure 1). Transformation relies not only on the number of leaf pieces that produce shoots but also on the number of shoots produced per leaf piece. While we have a very good rate of shoot production per number of leaves cultured we feel that it is necessary to significantly increase the number of shoots produced per leaf.

We have made some breakthroughs that we feel have potential for increasing the number of shoots per leaf. A significant breakthrough in the production of shoots per leaf was achieved through the use of a regeneration enhancing gene. We feel that the use of this gene will significantly improve the production of transgenic 'French Prune' shoots. If our results with this method and other methods continue to appear promising we will submit a proposal for supporting a continuation of this project.

We have evaluated vegetative symptoms of PPV on 'French Prune' and while virus titer can reach high levels (Figure 2) symptoms of PPV on leaves of 'French Prune' are mild.

In order to evaluate fruit symptoms of PPV infected 'French Prune' we transferred 'French Prune' budwood to a collaborator in Romania and 'French Prune' trees, and most importantly fruit, can now be evaluated in the field for PPV symptomology. Trees are in the field in Romania and are expected to fruit in the 2014 or 2015 growing season.

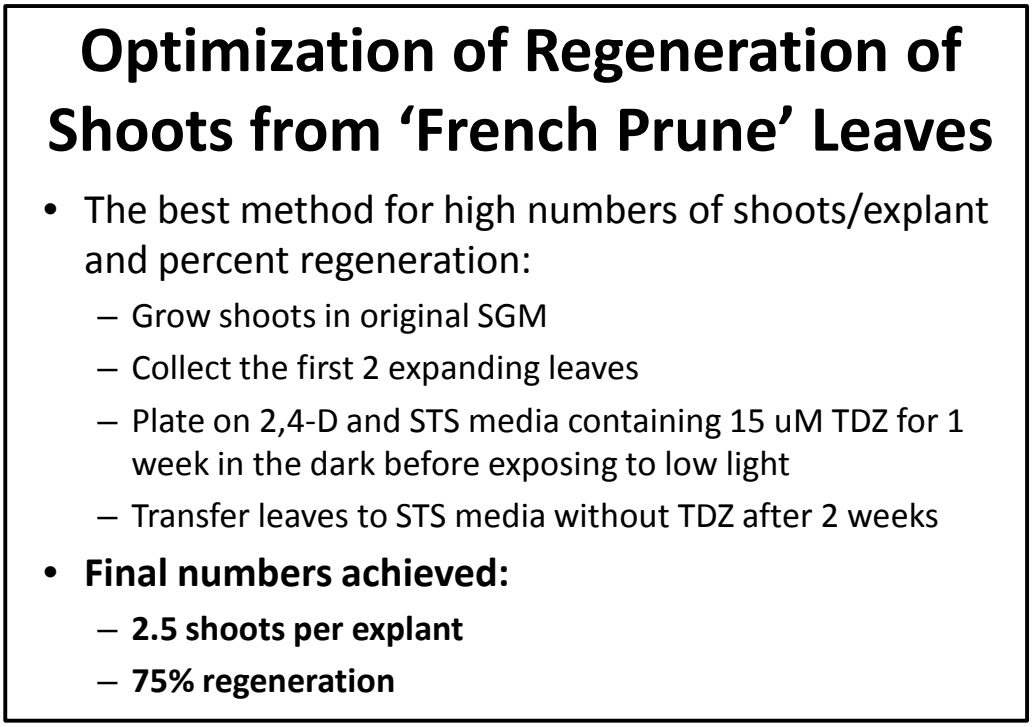

Figure 1. Protocol for optimization of regeneration of shoots from leaves of 'French Prune'.

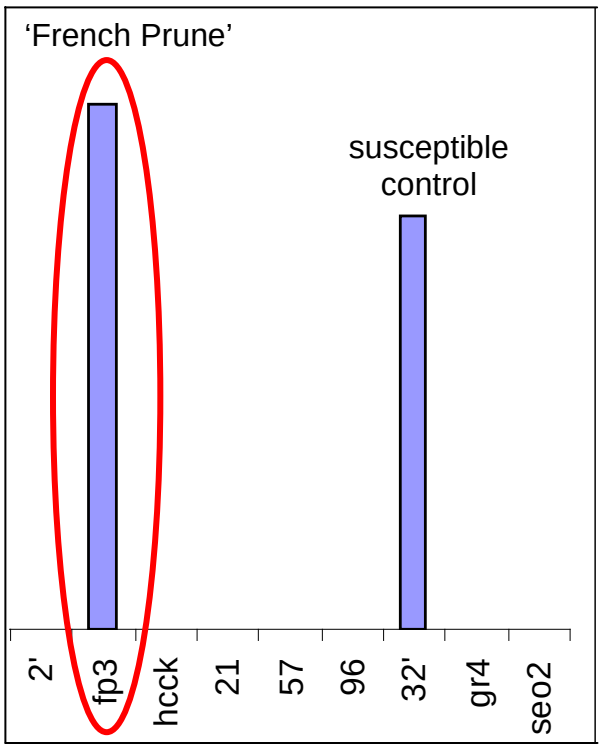

Figure 2. Quantative PCR analysis of 'French Prune' PPV infection. Bars indicate a high level of infection in 'French Prune' and the susceptible control. Absence of bars indicates little of no infection of the resistant transgenic test plants.

Project publications:

Petri, C. and R. Scorza. 2010. Factors affecting adventitious regeneration from in vitro leaf explants of 'Improved French' plum, the most important plum cultivar in the USA. Ann. Appl. Biol. 156: 79-89. 2010.

Srinivasan, C., Z. Liu, R. Scorza. 2011 Ectopic expression of class 1 *KNOX* genes induce adventitious shoot regeneration and alter growth and development of tobacco (*Nicotiana ttabacum* L.) and European plum (*Prunus domestica* L.). Plant Cell Rept. 30: 655-664.