RESEARCH RESULTS FOR THE YEAR 2007 'DEVELOPMENT OF PLUM POX VIRUS RESISTANT 'FRENCH PRUNE' ('IMPROVED FRENCH') PLUM.

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OBJECTIVE

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

PROCEDURE

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

'Improved French' 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. A number of lines will be tested in the containment greenhouse for resistance. Molecular tests will be carried out to confirm that the PPV resistance mechanism is active and stable.

Since 1990, the USDA-AFRS Genetic Improvement Research Unit has been developing genetically engineered (GE) plum lines with different novel traits including resistance to PPV. 'HoneySweet' a GE plum highly resistant to PPV, developed in our laboratory, is undergoing the deregulation process in the U.S. We can consistently produce PPV resistant GE plums and have them in the greenhouse to test within 6 months. We propose to adapt this highly efficient transformation system to clonal material of 'French Prune'. Explants used to test regeneration efficiency will include leaves, petioles, internodes and roots. We have successfully developed GE pears and apples from clonal material and recently, a system for transformation of clonal European plum was reported for the Russian plum variety 'Startovaya'. Based on our experience with previous transgenic plum lines we will utilize gene constructs with minimal intellectual property "issues" and constructs with the highest potential for consumer acceptability. Gene constructs will utilize only plant gene sequences (promoters and terminators). We will also investigate the potential of eliminating bacterial genes typically used as transformation "markers" which are a cause of concern for some consumers.

RESULTS

Project funds were received by ARS and officially made available to R. Scorza on August 15, 2007. A postdoctoral research associate who was hired to work on the project began work on

November 1, 2007. The results reported in this narrative represent preliminary results from August 15, 2007 and results of the first detailed experiments starting from November 1, 2007.

'French Prune' budwood was kindly provided by Dave Wilson Nursery and by the National Clonal Repository in Davis, CA. *P. domestica* rootstock was bud-grafted and 'French Prune' buds were allowed to produce shoots. As these shoots developed they were harvested for in vitro tissue culture (Figure 1).

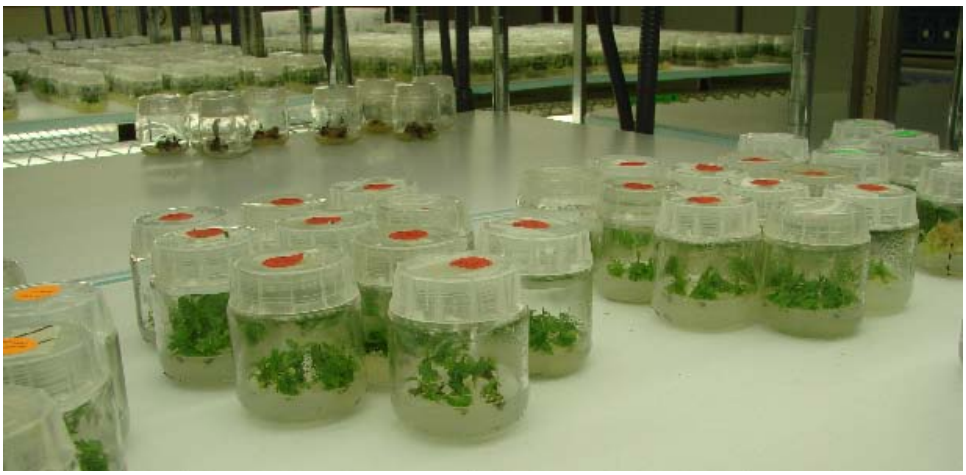

Figure 1. Shoot cultures of 'French Prune' growing in vitro as sources of leaves for regeneration and transformation experiments.

From the established 'French Prune' in vitro cultures leaves were harvested to begin to evaluate media and growth regulator combinations to induce the regeneration of shoots which is critical for the transformation process (Figure 2).

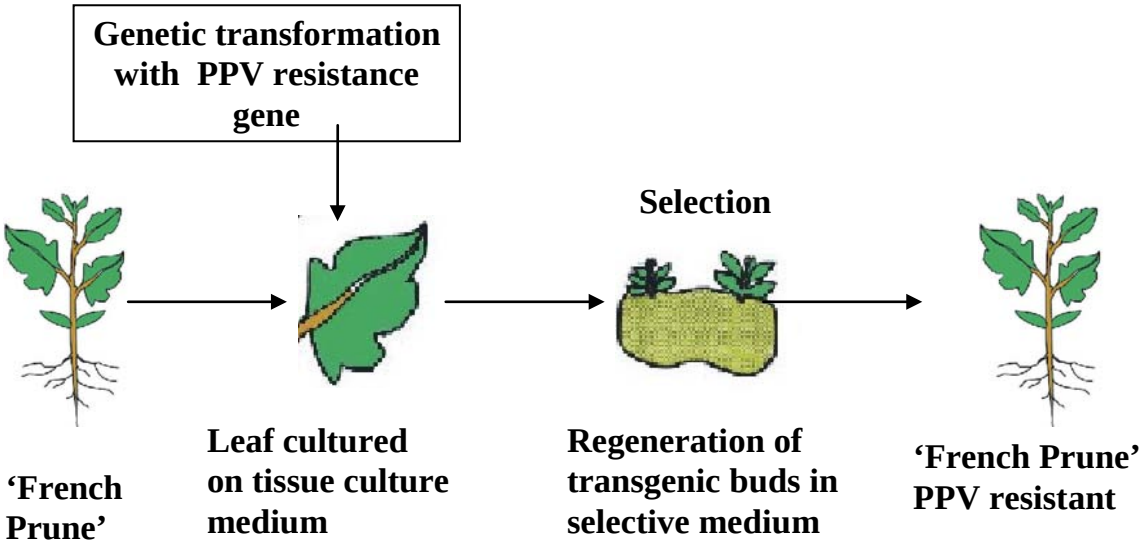

Figure 2. Scheme for the regeneration and transformation of 'French Prune'

A series of factors known to affect regeneration were evaluated in order to optimize the process. These have included growth regulators (plant hormones), growth media nutrients, chemicals used to set up the gelling of the tissue culture medium and the ratio of hours darkness to light. Optimization of these factors has led to an average of 50% of the leaf explants regenerating one to several shoots each. These early results are encouraging because this indicates that 'French Prune' has the capacity to regenerate in vitro. There are many fruit varieties that have been found to be thus far impossible to regenerate and this makes the ability to genetically engineer these varieties nearly impossible. The work currently underway is to increase the rate of regeneration of 'French Prune' shoots from leaves which will improve the potential for the transfer of PPV resistance genes into this variety.

In addition to the work on regeneration we are developing the transformation technologies for 'French Prune'. We are testing methods for selection of the transformed shoots, currently using the kanamycin antibiotic resistance gene which has been classified as exempt from tolerance in raw agricultural commodities by EPA and is the gene in genetically engineered soybeans, corn, cotton, etc.

We are testing 4 genetic "constructs" for providing PPV resistance, 2 of which contain a gene promoter (gene promoters control the expression of the PPV resistance gene) called *CAB* that is from peach instead of from a plant virus (the 35S promoter commonly used in genetically engineered crops is from the cauliflower mosaic virus) (Figure 3, also see Hily et al, J. Amer. Soc. Hort Sci 132:850-858. 2007). Some of these constructs have already produced PPV resistant plums. They are based on gene silencing as the resistance inducing mechanism. This mechanism has been extremely effective in providing high level, long-term, stable resistance in the genetically engineered plum variety 'HoneySweet' (Malinowski et al Plant Dis. 90:1012-1018. 2006).

In the future we would like to test the possibility of eliminating the antibiotic resistance gene and produce transgenic plants without selection. We can now do that using plum seed material which regenerates at much higher levels than do leaves at this point in time.

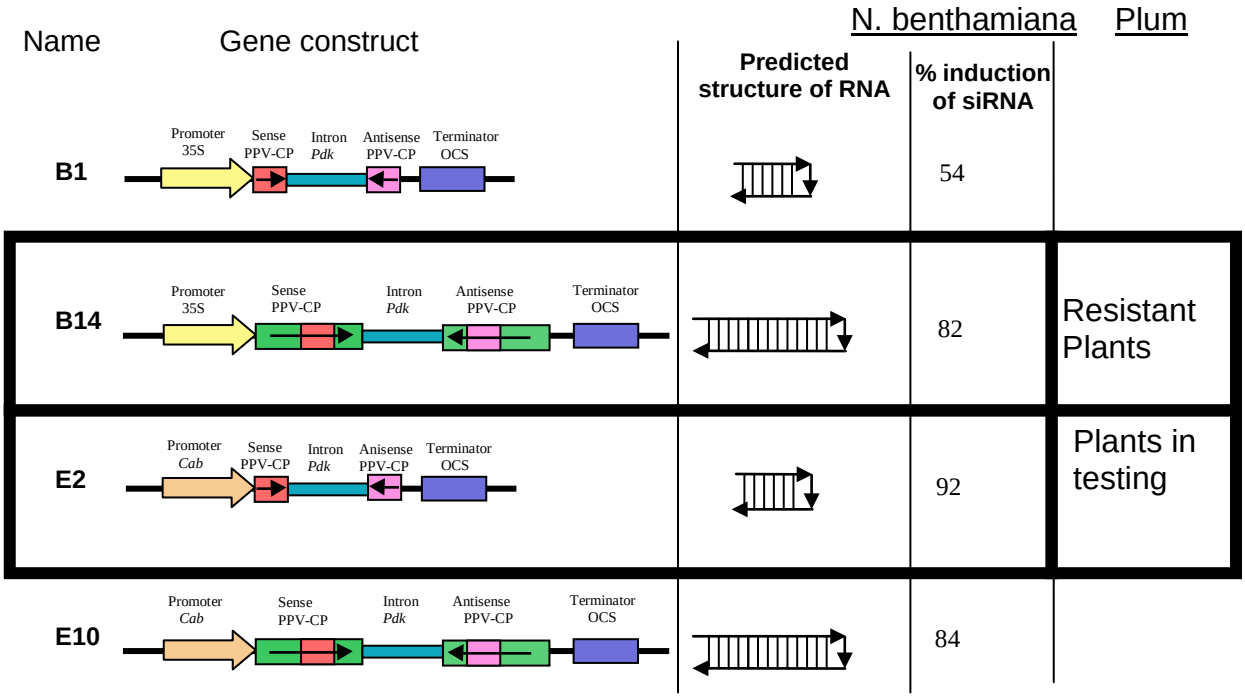

Figure 3. Gene constructs being tested for providing PPV resistance in 'French Prune'.

In order to test the susceptibility of 'French Prune' to PPV and to evaluate symptoms we have inoculated plants with PPV Pennsylvania D strain under containment conditions at the USDA facility at Ft. Detrick Maryland. These plants will be evaluated in late December. We have commitments from colleagues in the Czech Republic, Poland, Romania, and Spain to test 'French Prune' trees in field plantings under natural conditions of aphid transmission of PPV. In some test sites the D strain of PPV predominates and in some, the M and the REC strains are predominant so these plantings will provide an overall view of the symptomolgy in leaves and fruit of 'French Prune' infected with PPV. We are making arrangements for transfer of budwood to the European test sites in the summer of 2008 but there may be a possibility of winter 2007-2008 shipments for greenhouse budding at some locations.