DEVELOPMENT OF PLUM POX VIRUS RESISTANT 'IMPROVED FRENCH' PLUM

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OBJECTIVES

The objectives of this project are:
1) To develop genetically engineered clones of 'French Prune' which are highly resistant to PPV.
2) To evaluate the PPV susceptibility and symptomology of 'French Prune' under field conditions.

PROCEDURES

Objective 1
Improving the transformation protocol and increasing the rates of regeneration were the primary means of reaching objective 1, to develop genetically engineered clones of 'French Prune' that are highly resistant to PPV.

Improving the transformation protocol and increasing the rates of regeneration focused on three main areas of investigation:
1) Investigation of the timing of 'French Prune' leaf piece exposure to *Agrobacterium tumefaciens* and testing varying concentrations of *Agrobacterium tumefaciens* to which 'French Prune' leaf pieces were exposed.
2) Investigation of the kanamycin antibiotic concentration and timing of exposure for selection of transformed shoots from 'French Prune' leaf pieces.
3) Increasing cell division in 'French Prune' leaf pieces in order to produce a higher number of regeneration shoots that would then be exposed to gene transfer by *Agrobacterium tumefaciens*.

Objective 2
In order to achieve goal 2 'French Prune' plants will be inoculated with the U.S. PPV-D strain at the USDA-ARS Ft. Detrick containment greenhouse and evaluated for virus titer and for leaf symptoms. To evaluate fruit symptoms it will be necessary to fruit 'French Prune' trees in a European country where PPV is endemic.

RESULTS AND CONCLUSIONS

Objective 1
1) We have found that the time of exposure of 'French Prune' leaf pieces to *Agrobacterium tumefaciens* (10 min exposure and 4 days co-cultivation) is satisfactory and longer or shorter times do not significantly improve transformation.

2) A large number of treatments have been investigated for the proper timing of exposure 'French Prune' leaf pieces to kanamycin for the selection of transformed cells. Early selection

with high and low levels of kanamycin and delayed selection with various levels have not proved to be useful in the selection of transgenic shoots. Early selection appears to be too extreme and late selection produces many escaped untransformed shoots. Although we successfully use kanamycin selection for the transformation of plum from seeds, the use of kanamycin for selection in *Prunus* has been reported to be problematic by other researchers. We are currently evaluating the use of an herbicide resistance gene (*bar*) in place of the kanamycin resistance gene for selection of transformed shoots. This gene for selection appears to be promising.

3) Although we have achieved a 64% regeneration rate from leaves of 'French Prune' each leaf piece produces on average 1-2 shoots. This level of regeneration is not ideal for transformation. A higher number of shoots regenerated from each leaf piece would greatly increase the probability of producing transformed shoots. We have tested a large set of conditions to improve the quantity of shoots produced per leaf without significant increase in shoot production. We are testing a novel strategy utilizing KNOX genes. These genes have been isolated from *Arabidopsis* and have been shown to be involved in the formation of shoot meristems. We found that these genes, when inserted into plum seeds, regenerated transgenic plum plants expressing the KNOX gene. Leaves from these otherwise normal looking plum plants produced a very large number of shoots when cultured in vitro. We are attempting to transfer the KNOX gene into 'French Prune' in order to obtain 'French Prune' plants that will produce large numbers of shoots from leaves that can then be readily transformed with genes for PPV resistance and other useful genes.

While significant progress has been made over the course of the funded study, transgenic 'French Prune' plants expressing the PPV resistance transgene have not yet been produced. We are currently pursuing novel and promising methodologies that have already shown signs of success. Funding for the current cycle will allow for the research to continue to June 2011 (receipt of funds to our station and a new hire were in June 2010) and we expect that greater progress, if not complete success, will be achieved for objective 1 by that time.

Objective 2

Serological (ELISA) assays for PPV detection using the U.S. PPV-D strain for inoculation of 'French Prune' under containment greenhouse conditions at the USDA-ARS Ft. Detrick facility have clearly shown that this variety is susceptible to PPV, producing high titer of the virus in inoculated plants. Yet symptoms of PPV on the leaves of these infected plants are mild. PPV symptoms on leaves and fruit do not necessarily correlate and mild leaf symptoms do not insure mild fruit symptoms. We cannot grow fruiting plants in the Ft. Detrick greenhouse, therefore field testing in an area where PPV infection occurs is necessary. Budwood of 'French Prune' was shipped (under APHIS and European permits) to a long-time collaborator in Romania, Dr. Ioan Zagrai. Rootstocks were budded in Romania in 2009 and as of December 2010 trees are in a greenhouse awaiting field planting in the growing season of 2011. Foliage and fruit symptoms and virus concentration will be evaluated by Dr. Zagrai, Head of Research Department, Breeding and Virology Lab, Fruit Research & Development Station, Bistrita, Romania. The transfer of 'French Prune' budwood to Romania will allow us, in the longer term, to ascertain fruit symptoms produced by the PPV-D strain and also other strains that are endemic to Romania. This data will be made available to the CDPB when results are received from Romania.