PROJECT NUMBER: New-ARS

TITLE: Genetic Transformation of Pear for Fire Blight Resistance and Growth Habit Improvement

YEAR INITIATED: 2000-2001 CURRENT YEAR : 2001-2002
TERMINATING YEAR: 2002-2003

PERSONNEL:
Project Co-leaders: Richard L. Bell, and Ralph Scorza, USDA-ARS,
Appalachian Fruit Research Station, Kearneysville, WV

JUSTIFICATION:

Fire Blight

Fire blight is the most serious disease of pears. All of the major pear cultivars grown in North America are highly susceptible, and infection can result in severe short- and long-term economic losses. Resistant clones of the major cultivars would significantly reduce both the incidence and severity of infections. Genetic transformation offers a means of introducing antibacterial genes into currently important cultivars without changing their other important characteristics. This approach has already been used by researchers at Cornell University to improve the level of fire blight resistance of M.7 and M.26 apple rootstocks, and 'Gala' and 'Galaxy' apple cultivars, and by French INRA researchers to transform 'Passe Crassane' pear. The genes used by this and other research groups are responsible for producing natural and synthetic "lytic peptides" which disrupt the bacterial cell membrane.

Four antimicrobial genes have been obtained from Demeter Biotechnologies, Ltd., as part of a Cooperative Research and Development Agreement. These genes are advanced generation synthetic derivatives of those used by the Cornell and INRA teams. We have assayed the peptides produced by these genes for activity against the fire blight organism, and based on these assays, have selected one gene for initial use. At the present time, our first transformation experiments have produced over a dozen transformed clones of 'Bartlett'. These clones are being tested to determine the levels at which the peptide is being produced in the plants, and the degree of improvement in resistance to fire blight.

Numerous additional transformed clones of 'Bartlett' may be necessary in order to select those which express high levels of resistance to fire blight. In addition to the genes, various gene promoters, which control whether, where, and when genes are expressed, need to be used in transformation to determine which will result in the highest level of resistance. Signal peptides, which move the antibacterial peptides produced by the genes out of the plant cell, into contact with the bacterial cells, are also being tested.

Dwarf Growth Habit

Production efficiency of deciduous tree fruits has been dramatically improved through the use of dwarfing rootstocks to reduce tree size. Additional efficiencies have been associated with alterations in growth habit, particularly the introduction of spur-type mutants of apple cultivars. Reduced tree stature in pears has been achieved through the use of quince (*Cydonia oblonga* L.) as a dwarfing rootstock, and more recently, by several semi-dwarfing 'Old Home' x 'Farmingdale' clonal rootstocks. However, quince rootstocks are not well adapted to most of the major pear production regions of North America, and the degree of size reduction of the 'Old Home' x 'Farmingdale' rootstocks is inferior to quince. Genetically dwarfed scion cultivars have not been commercially introduced.

We have previously transformed (i.e. inserted a gene into) 'Beurre Bosc' pear with a gene, rolC, which causes dwarfness. Studies are being conducted on the three transgenic clones to determine if the degree of dwarfing is economically useful. Additional independent transgenic clones containing rolC and other growth altering genes are needed to fully assess the feasbility of transgenic dwarf pear trees.

OBJECTIVES:

Fire Blight Resistance: Determine degree of gene expression of each transformed clone of Bartlett', and determine degree of resistance to artificial inoculation with the fire blight pathogen. Additional transgenic clones will by developed.

Dwarf Growth Habit: Produce additional independent transformed clones, using *rolC* and other genes known to affect plant growth habit (for example, *ipt*, *rolA*, *rolB*, and/or *PhyA*), to properly assess the utility of producing genetically dwarfed clones of major pear cultivars. Characterize growth habit alterations of transgenic clones.

PROCEDURES:

Fire Blight Resistance

Transformation: The basic transformation procedure will be that previously employed (Bell, R. L. et al. 1998. HortScience 33:461; Scorza, R. et al. 1998. HortScience 33:461; Bell, R. L. et al. 1999. J. Amer. Soc. Hort. Sci. 124:570-574.) Immunoassays using peptide-specific antibody will be used to assess the amount of peptide produced in the transgenic plants. Controls will include shoots regenerated from leaf cultures not inoculated with the Agrobacterium, and shoots from proliferation cultures.

Evaluation of fire blight resistance: Ten shoots per transgenic clone will be rooted in vitro then transferred after rooting to a sterile soilless potting mix, and acclimated to the greenhouse. These self-rooted plants will be grown and used as a source of budwood for tests of fire blight susceptibility. Trees (20 of each clone) will be produced by budding onto Bartlett seedling rootstock in the greenhouse. Each tree will be inoculated using the

cut-leaf procedure with a 107 cfu/ml suspension of a mixture of virulent strains of Erwinia amylovora, and disease progress measured at weekly intervals until symptom progression ceases.

Dwarf Growth Habit

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Evaluation of growth habit: Three methods of plant production will be used for the investigation of growth habit alteration: 1) transgenic trees will be own-rooted and compared with own-rooted 'Bosc' and 'Bosc' on 'Bartlett' rootstock; 2) transgenic clones will be used as rootstock for 'Bosc' and 'Bartlett'. Controls will be 'Bartlett' and 'Bosc' on 'Bartlet' seedling rootstock and on quince; 3) Transgenic 'Bosc' will be budded onto 'Bartlett' seedling rootstock with 'Bosc' on 'Bartlett' seedling as control.

Growth, precocity, productivity and fruit quality will be evaluated for all trees produced above through standard nursery and field trials.

ANTICIPATED BENEFITS AND INFORMATION TRANSFER: Within the one-year period to be covered by the current funding proposal, we anticipate that transgenic clones of 'Bartlett' and 'Beurre Bosc' will be produced and their transgenic status verified. During this funding period preliminary tests of reaction to fire blight will be completed on those clones transformed with lytic peptide genes. Preliminary evaluations of growth in the greenhouse can be made on transgenic 'Beurre Bosc' containing growth altering genes.

The projected products of the proposed research are transgenic clones of 'Bartlett', with significantly improved levels of resistance to fire blight. These clones will significantly reduce crop and tree losses to this disease, and may allow the elimination of prophylactic sprays. Dwarf and semi-dwarf clones of 'Bartlett' and 'Beurre Bosc' and/or dwarfing rootstocks developed will allow for higher density production systems, reduced pruning, and more efficient pear production.

Information developed during the project will be reported to the WTFRC, to colleagues at scientific meetings, and published in scientific journals. Fire blight resistant transgenic clones will be made available to nurseries for commercial propagation.

BUDGET:

Salary and Benefits:
One Technician (full-time, temporary) $25,000

(ARS funding will cover research supplies and equipment, greenhouse and field costs, travel and meeting expenses.)