Project title: Regulation of Farnesene Synthesis to Control Scald

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Objectives: (1) Obtain a genomic clone of the apple $\alpha$-FS gene including the promoter and ethylene response element(s); (2) Isolate and characterize the promoter region from the genomic clone of the apple peel HMG2 gene and determine whether expression of HMG2 is regulated by ethylene; (3) Evaluate the correlation of ethylene-induced transcription of genes encoding enzymes in the $\alpha$-farnesene synthetic pathway with accumulation of $\alpha$-farnesene during storage, and with the incidence and severity of scald development; (4) Transform apple shoots with antisense or RNAi constructs of the $\alpha$-FS gene (and the HMG2 gene if time allows) driven by either the CaMV 35S or apple $\alpha$-FS promoter and test transgenic apple plants for suppression of $\alpha$-farnesene production.

As stated last year, research priorities for the proposed objectives were amended. Work on the apple peel HMG2 from ‘Law Rome’ was abandoned after finding that this gene is not up-regulated by ethylene and is unlikely to play a major role in production of $\alpha$-farnesene during storage. In February 2004, postdoctoral Research Associate and Co-PI Steve Pechous left the PQSL after accepting a permanent position. Prior to his departure, in collaboration with Dr. Chris Watkins at Cornell, Steve completed the study proposed under objective 3. Using primers based on the ‘Law Rome’ AFS1 cDNA, the AFS1 cDNA from ‘Idared’ apple was obtained by PCR amplification after reverse transcription of peel tissue mRNA. This established a good experimental system for comparison of ethylene-induced AFS1 gene expression and $\alpha$-farnesene production and oxidation in fruit of the scald-susceptible Law Rome and scald-resistant Idared cvs. A report of this study was recently published (Postharvest Biol. Technol. 35:125-132, 2005). Efforts to clone the AFS1 promoter as proposed under objective 1 were renewed with the hiring in October 2004 of plant molecular biologist Nigel Gapper, who filled an ARS Headquarters-funded postdoctoral position awarded in 2003. Genome walk experiments have yielded a 1.5-kb genomic DNA fragment upstream of the ‘Law Rome’ AFS1 open reading frame. The first difficult and laborious step required for the transformation studies with RNAi constructs of AFS1 proposed under objective 4 was taken this spring; shoots of ‘Law Rome’ and ‘Delicious’ were harvested from a commercial orchard and established in tissue culture. Seizing an opportunity, last fall we entered a collaboration with Dr. Jinhe Bai (Oregon State – MCAREC) to investigate scald control by 1-MCP in ‘d’Anjou’ pear in relation to AFS gene expression and $\alpha$-farnesene synthesis and oxidation. Dr. Gapper has cloned the complete cDNA for Pyrus communis AFS and the study should be completed in several months.
Progress, significant findings, and future plans: Project accomplishments and findings during the past 12 months (06/04 – 06/05) are listed, and future plans and applications are described below:

- 5' and 3' primers were designed based on the cDNA sequences of ‘Law Rome’ and ‘Idared’ apple AFS1 and used with reverse-transcribed peel tissue RNA from scald-susceptible ‘d’Anjou’ pear fruit to obtain a complete Pyrus communis AFS cDNA (PcAFS) by 3'- and 5'-RACE. The open reading frame of PcAFS is 97% identical to the corresponding apple sequences, and the deduced amino acid sequence of the encoded PcAFS protein is 95-96% identical to the sequences of AFS from ‘Law Rome’, ‘Idared’, and ‘White Pearmain’ apple (Figure 1).

- A collaborative study with Dr. Jinhe Bai at OSU-MCAREC compared expression of the pear α-farnesene synthase gene PcAFS, accumulation of α-farnesene and its conjugated trienol oxidation products (CTols), and scald incidence in control and 1-MCP-treated (300 ppb) ‘d’Anjou’ pears stored up to 6 months at –1 °C. PCAFS expression, determined by quantitative RT-PCR using the 18S competimer system (Ambion, Inc.), showed a marked increase about one month earlier in control vs. 1-MCP-treated fruit, continued to increase up to 5 months, and remained about 1.5- to 2-fold higher in the controls from 2 to 5 months of storage (Figure 2). Both α-farnesene and CTols reached very high levels in control fruit (200 and 90 µg/g FW at 3 and 4 months, respectively) then declined after 4 months (Figure 3). Maximum levels of α-farnesene and CTols were 5 and 12-fold lower, respectively, in 1-MCP-treated vs. control fruit. Scald incidence was 100% in controls after 3 to 5 months of storage, whereas 1-MCP-treated fruit were scald-free until 6 months, when scald incidence was 13%.

- In April 2005, branchlets were cut from trees of ‘Law Rome’ and ‘Red Delicious’ growing in a commercial orchard, and young shoots were excised, sterilized, and placed on tissue culture medium. After initial severe problems with contaminant microorganisms from the orchard, it appears that modified procedures have enabled the establishment of viable shoots in sterile culture. These will be maintained and propagated, and ultimately used in transformation experiments with an RNAi construct of AFS1 in Agrobacterium. On the basis of sequencing data, initial efforts to generate an RNAi construct using the ‘Law Rome’ AFS1 cDNA appear to have been successful.

- Genome walk from the 5'-end of the ‘Law Rome’ AFS1 open reading frame using genomic DNA prepared from young leaf tissue yielded about a 1.5-kb fragment of the AFS1 promoter region. Experiments currently in progress will attempt to clone another 1.0 to 1.5 kb upstream of the existing promoter fragment prior to complete promoter sequence analysis and efforts to identify ethylene (and possibly low temperature) response elements. The same strategy will be used to clone the AFS1 promoter from scald-resistant ‘Idared’ apple, with the aim of determining whether differences in regulatory elements of the AFS1 promoters from the two cultivars can account for the very different levels of AFS1 expression during air storage at 0.5 °C. It is also planned to use the ‘Law Rome’ AFS1 promoter to drive the AFS1 RNAi construct in transgenic apple plants, with the rationale that it should serve to silence the gene after fruit are placed in cold storage.

Publications in past 12 months:


Figure 1. Alignment of deduced amino acid (AA) sequences encoded by α-farnesene synthase genes cloned from fruit peel tissue of three apple cultivars and ‘d’Anjou’ pear. AA substitutions are in bold type (chemically similar) or white bold type with gray highlighting (chemically dissimilar).

White Pearmain MEFRVHLQADNEQKIFQNQMKPEPEASYLINQRRSANYKPNIWKNDFLDQSLISKYDGDE 60
Idared MEFRVHLQADNEQKIFQNQMKPEPEASYLINQRRSANYKPNIWKNDFLDQSLISKYDGDE 60
Law Rome MEFRVHLQADNEQKIFQNQMKPEPEASYLINQRRSANYKPNIWKNDFLDQSLISKYDGDE 60
d’Anjou MEFRVHLHADNEQKIFQNQMKPEPEASYLINQRRSANYKPNIWKNDFLDQSLISKYDGDE 60

White Pearmain YRKLSEKLIEVKYLISATMDLVLAKLELIDSVRKLGLANLFKEIKEALDSDIAAIESDN 120
Idared YRKLSEKLIEVKYLISATMDLVLAKLELIDSVRKLGLANLFKEIKEALDSDIAAIESDN 120
Law Rome YRKLSEKLIEVKYLISATMDLVLAKLELIDSVRKLGLANLFKEIKEALDSDIAAIESDN 120
d’Anjou YRKLSEKLIEVKYLISATMDLVLAKLELIDSVRKLGLANLFKEIKEALDSDIAAIESDN 120

White Pearmain LGTRDDLYGTALHFKILRQHGYKVSQDIFGRFMDEKGTLENHHFAHLKGMLELFEASNLG 180
Idared LGTRDDLYGTALHFKILRQHGYKVSQDIFGRFMDEKGTLENHHFAHLKGMLELFEASNLG 180
Law Rome LGTRDDLYGTALHFKILRQHGYKVSQDIFGRFMDEKGTLENHHFAHLKGMLELFEASNLG 180
d’Anjou LGTRDDLYGTALHFKILRQHGYKVSQDIFGRFMDEKGTLENHHFAHLKGMLELFEASNLG 180

White Pearmain FEGEDILDEAKASLTLALRDSGHICYPDSNLSRDVHSLLELPFHRVRQWFVDVKWQINAYE 240
Idared FEGEDILDEAKASLTLALRDSGHICYPDSNLSRDVHSLLELPFHRVRQWFVDVKWQINAYE 240
Law Rome FEGEDILDEAKASLTLALRDSGHICYPDSNLSRDVHSLLELPFHRVRQWFVDVKWQINAYE 240
d’Anjou FEGEDILDEAKASLTLALRDSGHICYPDSNLSRDVHSLLELPFHRVRQWFVDVKWQINAYE 240

White Pearmain KDICRVNATLLELAKLNFNVVQAQLQKNLREASRWWANLGADNLKFARDRLVECFSCAV 300
Idared KDICRVNATLLELAKLNFNVVQAQLQKNLREASRWWANLGADNLKFARDRLVECFSCAV 300
Law Rome KDICRVNATLLELAKLNFNVVQAQLQKNLREASRWWANLGADNLKFARDRLVECFSCAV 300
d’Anjou KDICRVNATLLELAKLNFNVVQAQLQKNLREASRWWANLGADNLKFARDRLVECFSCAV 300

White Pearmain LRNGCISSVSVLLVSFSSTHETGKMAFLHNEDDLYLISLIVRLNNDLGTSAEQ 480
Idared LRNGCISSVSVLLVSFSSTHETGKMAFLHNEDDLYLISLIVRLNNDLGTSAEQ 480
Law Rome LRNGCISSVSVLLVSFSSTHETGKMAFLHNEDDLYLISLIVRLNNDLGTSAEQ 480
d’Anjou LRNGCISSVSVLLVSFSSTHETGKMAFLHNEDDLYLISLIVRLNNDLGTSAEQ 480

White Pearmain ERGSFSSIVYMREVNAEETARKNIKGMIDNAWKKVNGKCTTNQVFPLLSSFMNATN 540
Idared ERGSFSSIVYMREVNAEETARKNIKGMIDNAWKKVNGKCTTNQVFPLLSSFMNATN 540
Law Rome ERGSFSSIVYMREVNAEETARKNIKGMIDNAWKKVNGKCTTNQVFPLLSSFMNATN 540
d’Anjou ERGSFSSIVYMREVNAEETARKNIKGMIDNAWKKVNGKCTTNQVFPLLSSFMNATN 540

White Pearmain MARVASHLYKDGDGFQDEGKPRTHILSLFPQPLVN 576
Idared MARVASHLYKDGDGFQDEGKPRTHILSLFPQPLVN 576
Law Rome MARVASHLYKDGDGFQDEGKPRTHILSLFPQPLVN 576
d’Anjou MARVASHLYKDGDGFQDEGKPRTHILSLFPQPLVN 576
Figure 2. Relative quantitative RT-PCR of PcAFS1 in control and 1-MCP-treated fruit using universal 18S competimer oligos and 18S primer pairs at a 9:1 ratio.
Figure. 3 Accumulation of α-farnesene and its conjugated trienol oxidation products in peel tissue of control and 1-MCP-treated (300 ppb) fruit of scald-susceptible ‘d’Anjou’ pear over 5 months of storage in air at -1 °C.