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Floriculture Genomics - Identifying Genetic Targets to Delay Flower Senescence

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BACKGROUND

The postproduction quality of flowering horticultural crops is often limited by the longevity of individual blooms or flower senescence. Senescence represents the last stage of flower development and results in the wilting, color fading, and abscission of flower petals. The senescence process in many flowers is regulated by the plant hormone ethylene. Genetically engineered plants that are insensitive to ethylene have significantly delayed flower senescence, but also have reduced seed germination, decreased adventitious rooting and increased susceptibility to disease. The goals of this research were 1. to identify components of senescence pathways that would allow us to specifically delay senescence without affecting other aspects of growth and development, and 2. to determine if these genes (or gene products) are regulated by ethylene.

MATERIALS & METHODS

Genes from *Petunia x hybrida* with homology to genes that have been shown to be involved in senescence or programmed cell death in other plants or animals were identified from the Petunia BlastQuest EST database of Dr. David Clark (Univ. of FL) and approximately 40 cDNA clones were obtained from freezer stocks held in my laboratory.

Changes in the transcript abundance of these genes were investigated using polymerase chain reaction (PCR) and RNA gel blot analysis. Biochemical and morphological investigations were used to confirm the gene expression data from selected genes. Comparative analysis was conducted in wild type *Petunia x hybrida* 'Mitchell Diploid' (i.e. ethylene-sensitive) and transgenic ethylene-insensitive petunias (35S:*etr1-1*; Z00-35-10 or 44568) to identify components of the senescence program that are dependent on ethylene.

RESULTS

The senescence of unpollinated flowers is delayed by approximately 8 days in *etr1-1* transgenic petunias (*Fig. 1*).

Proteases and nucleases

A prominent process during the senescence of petals is the degradation of proteins and nucleic acids by protease and nuclease enzymes respectively. This allows the plant to recycle nutrients like nitrogen and phosphorus from the dying petals and remobilize them to developing tissues. To determine ethylene's role in these processes we studied gene expression and enzyme activity of several proteases and nucleases (Langston et al., 2005; not shown) in petunia corollas.

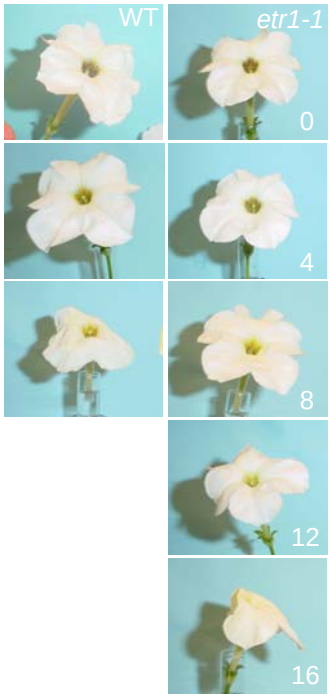

Figure 1. Flower senescence of wild type (WT) and transgenic etr1-1 petunias.

Protein content and protease activity

The protein content of corollas decreased during aging of both wild type and *etr1-1* flowers (*Fig. 2A*). Increased protease activity corresponded with declining protein content of the corolla (*Fig. 2B*). These studies indicate that protein degradation is a component of the senescence

program in both ethylene-sensitive and -insensitive petunias. Inhibitor studies indicated that the majority of the protease activity was from the cysteine protease class of protease enzymes (data not shown).

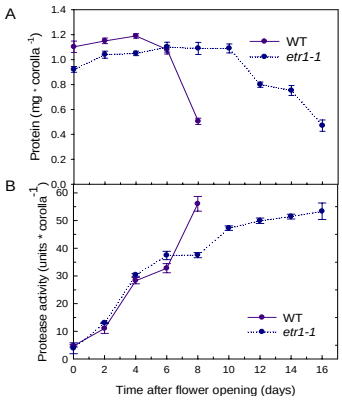

Figure 2. Changes in protein content and protease activity in corollas of wild type (WT) and transgenic etr1-1 petunias.

Protease gene expression

Nine cysteine protease (CP) genes were identified from the petunia BlastQuest EST database. Transcript (mRNA) from all nine CPs was detected in corollas (*Fig. 3*). Six of the nine CPs (*PhCP2, PhCP3, PhCP5, PhCP8, PhCP9, PhCP10)* increased during corolla senescence, suggesting that they have a function in the senescence program. Four of the senescence-associated CPs had delayed increases in *etr1-1* corollas that corresponded to the wilting of these flowers. In wild type flowers ethylene regulates the timing of transcript increases and in *etr1-1* flowers these increases are delayed in the absence of the ethylene signal. These experiments support a role for ethylene in initiating the timely onset of senescence, but suggest that senescence does occur in the absence of this ethylene signal. In contrast, we were able to identify genes that did not fit this model. *PhCP3* and *PhCP5* increased at 8d after flower opening in *etr1-1* flowers. The fact that the pattern of expression in *etr1-1* corollas was similar to that in wild type corollas despite the delay in flower senescence exhibited by these flowers suggests that the up-regulation of these genes is not dependent on ethylene. *PhCP10* was the only CP that was specific to senescing tissues. Transcript was only detected in senescing corollas and leaves (leaf data not shown).

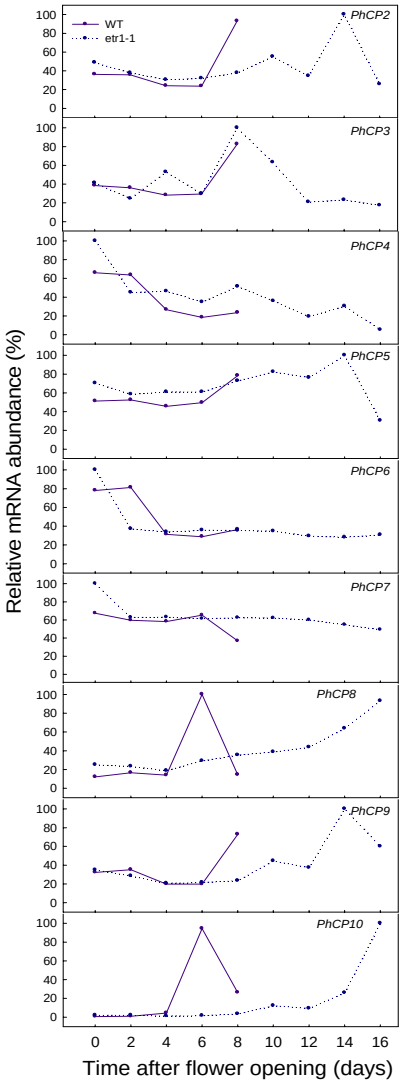

Figure 3. Changes in cysteine protease gene expression in corollas of wild type (WT) and transgenic etr1-1 petunias.

CONCLUSIONS

1. We have identified senescence up-regulated genes and those that are specifically expressed only in senescing tissues (leaves and flowers).
2. We have confirmed a primary role for ethylene in regulating the timing of flower senescence, but have also identified components of the senescence program that are independent of ethylene.

IMPACT TO THE INDUSTRY

Molecular and biochemical studies of senescence lead to a greater understanding of the pathways that regulate plant senescence and allow us to identify genetic targets that can be used to delay senescence and improve postproduction quality by conventional breeding or genetic engineering.

PUBLICATIONS

1. Jones M.L., G.S. Chaffin, J.R. Eason and D. Clark (2005) Ethylene Sensitivity Regulates Proteolytic Activity and Cysteine Protease Gene Expression in Petunia Corollas. Journal of Experimental Botany. In Press.

2. Langston B.L., S. Bai and M.L. Jones (2005) Increases in DNA Fragmentation and Induction of a Senescence-Specific Nuclease are Delayed during the Senescence of Ethylene-insensitive (*etr1-1*) Transgenic Petunias. Journal of Experimental Botany. 56:15-23.

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