



ANNUAL PROGRESS REPORT JUNE 2003

Project Title: Floriculture Genomics- Identifying Genetic Targets to Delay Flower Senescence

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Project Justification and Impact:

During the first year of this project approximately 4500 unique cDNAs from petunia have been sequenced and a petunia genomics database has been created. This project has resulted in numerous collaborations including the one between Dr. David Clark and myself. The petunia database has provided thousands of interesting genes which are involved in all aspects of growth and development, and it will take many labs to characterize these genes, genetically engineer plants, and evaluate these plants for Horticultural performance and commercial utility. Biochemical studies in my laboratory have identified a number of enzymes involved in flower senescence. We have utilized the petunia genomics database to identify genes that encode these enzymes so we could study the transcriptional regulation (gene expression) of these genes during senescence. Our full-length sequence data and expression profiles will help build the petunia database and add to its usefulness as a Floriculture resource.

Petunia is the leading Floriculture model system because it can be easily transformed using *Agrobacterium tumefaciens*. This allows us to increase or decrease the expression of a gene of interest and observe the resulting phenotypes in transformed plants. These plants can then be used to determine the function of genes of interest. Seeds from these plants will also be a valuable resource to other researchers. Ultimately, the promising new technologies discovered during this research will be transferred to other floriculture crops of commercial importance.

Goal and objectives:

Goal: *To use functional genomics to identify components of the senescence signaling pathways and engineer the most appropriate genetic targets for the manipulation of senescence.*

Objective 1: Use the petunia genomics database to identify genes regulating senescence.

Objective 2: Characterize the temporal and spatial expression profiles of target genes.

Objective 3: Create and analyze transgenic plants to determine the function of target genes.

Experimental plan:

Genes regulating senescence

Flower senescence is accompanied by large increases in the activity of hydrolytic enzymes that function in the disassembly of macromolecules (proteins, nucleic acids, carbohydrates, lipids) and remobilization of their constituents from the petals to sink

tissues like seeds or young leaves. In the first year of this study we focused on genes encoding proteases, enzymes that degrade proteins. In the next two years we will expand this study to include genes encoding endonucleases (enzymes that degrade nucleic acids, both RNA and DNA) and lipases and lipoxygenases involved in the degradation of membrane lipids. We have currently identified 12 such genes from the petunia genomics database. In petunia flowers the degradation of nuclear DNA occurs during the later stages of senescence when the petals are wilted. This corresponds with the appearance of a nuclease with activity against both double-stranded DNA and single-stranded DNA. This activity is not detected in nonsenescent tissues and appears to be senescence specific (Langston and Jones, 2003). Sequence data can be used to determine which of the endonuclease genes identified from the petunia database encodes this protein.

Senescence is a form of programmed cell death (PCD). Cell death has been most extensively studied in animals and many genes have been identified that play a role in the initiation and execution of the cell death process. Few of these genes have been identified in plants, and we have been able to identify only a few of these from the petunia database. It is likely that many animal PCD genes will not have similar genes in plants, but it is also possible that their sequences are different enough that we cannot identify them based on sequence data alone. Most genes have specific regions or domains that help define their function. Evolutionarily these are highly conserved, because changes here would make the protein non functional and could be lethal. In contrast, other parts of the gene's sequence may change and not affect the final protein. Because of these potential sequence differences it can be difficult to identify genes that encode the same proteins in plants and animals. We will therefore identify conserved PCD-related domains from animal genes and use these domains to search the petunia database to identify those genes that might have a function in programmed cell death. I will refer to these genes as the PCD-related genes and the initial set of 12 genes described above as the senescence-related genes.

Expression profiles:

The expression of the senescence-related and PCD-related genes will be investigated to determine where in the flower and the rest of the plant the gene is expressed and to determine patterns of expression during pollination-induced and natural senescence. This will be done using RNA gel blots, a common technique in my laboratory. The DNA clones will also be fully sequenced. The sequence data available for most genes from the database is partial, and full-length sequences will aid in the characterization of the proteins encoded by these genes. Expression studies will also be conducted using *etr1-1* (ethylene insensitive) petunias. These studies will identify 1. genes that require ethylene for their expression, 2. genes that are expressed independent of ethylene, and 3. genes where the timing of their expression is regulated by ethylene (i.e. up regulation is delayed in *etr1-1* flowers). For example, up regulation of the senescence-specific nuclease activity detected in petunia corollas (PhNUC1) was delayed in *etr1-1* flowers, indicating that ethylene modulates the timing of PhNUC1 induction but is not an absolute requirement for its activation (Langston and Jones, 2003).

Functional analysis using transgenic petunias:

Transgenic petunias that have modified expression of senescence- and PCD-related genes will be created to confirm their function in flower senescence. A target gene can be decreased (knocked out) within the plant using antisense or RNAi technologies. Genes that are believed to be involved in the initiation or execution of the senescence

process (mRNA levels increase during senescence) will be knocked out. Those that might function as repressors of senescence (i.e. the protease inhibitors) will be over expressed to increase the amount of the inhibitor in the flower.

The first genes to be modified in transgenic plants will be the protease and protease inhibitor genes identified in year 1 of this study. Those protease genes that are up regulated during senescence will be knocked out and the 2 protease inhibitors that are down regulated (potential senescence repressors) during senescence will be over expressed in the flower. Thirty to fifty independent T₀ lines will be generated using *Agrobacterium tumefaciens* mediated transformation. These plants will be grown to flowering and screened for a delayed flower senescence phenotype. After selfing the T₀ lines, the T₁ plants will also be screened for the phenotype of increased flower longevity to show that the trait is heritable. Total protein and protease activity changes during flower senescence will be determined. The effects of modifying 1 gene on the expression of other genes within the protease gene family and the effects of over expressing a protease inhibitor on the expression of protease genes will be assessed using RNA gel blots. Horticultural performance of the lines with delayed flower senescence will be evaluated to determine their true commercial utility. Time to flowering, flower number and size, leaf senescence, fruit maturation, seed set, and adventitious rooting of cuttings will be evaluated on all selected genetic lines. Additional genes as identified in the second year of study will also be transformed into plants in the third year of the study.