Special Research Report #304: Plant Breeding & Genetic Engineering

Floriculture Genomics: Basic Tools for Crop Improvement though Biotechnology

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BACKGROUND

American Floral Endowment
FUNDING INDUSTRY SOLUTIONS THROUGH RESEARCH AND EDUCATION

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Four years ago, we started an effort in functional genomics and DNA sequencing has led to the development of one of the largest collections of genes expressed in ornamental plants (petunia) in the world. After conducting focused microarray experiments, we have isolated several genes that we have identified to have potential utility for use in genetically engineering plants with altered hormone responses, altered floral fragrance, and a variety of other potentially valuable characteristics. We have also isolated several genes that appear to be expressed in specific floral tissues (ie. petals), and many of these contain DNA sequence that shares little or no similarity to other genes of known function in any other plant. We are currently engineering many of these genes to either over-express and/or knock out their expression in transgenic plants in an effort to uncover their biological function. Results have been promising to date, leading to the isolation of genes involved in the synthesis of several interesting biochemicals: methylbenzoate, a chemical giving flowers a sweet fragrance (Negre et al., 2003; Underwood et al, 2005); isoeugenol - clove oil – a chemical giving flowers a spicy fragrance; benzylbenzoate, used commercially as an insecticide ; 2-phenylethanol (rose oil) and beta-ionone, the two major constituents of rose fragrance. As a result of these efforts, we have produced transgenic plants with altered fragrances that are perceived differently by humans, as measured by sensory panelist analysis of engineered flowers.

MATERIALS AND METHODS

1: DNA sequence data was collected from approximately 11,500 genes expressed in petunia flowers. A database of clones and sequences has been produced that is now available to other floriculture researchers.

2: DNA microarrays were constructed to allow for identification of groups of genes transcriptionally regulated during ethylene regulated flower senescence.

3: Expression of these senescence-related genes was characterized in order to identify components of the senescence signaling pathways and determine the most appropriate genetic targets for the manipulation of senescence.

RESULTS

In order to satisfy all objectives set forth in this proposal, the following significant milestones of this project were achieved:

1 – Construct Petunia cDNA libraries from various floral tissues – This milestone was fully achieved in years 1 and 2 of the project with the production of three flower cDNA libraries. These libraries contained genes from flowers at different developmental stages, flowers treated with ethylene, and pollinated flowers as a means to supply all genes expressed during development and floral senescence. However, we added to this objective by constructing additional cDNA libraries from petunia leaf tissue, early development fruit (1-5 days after pollination) and ripening fruit (16-24 days after pollination).

2 – Obtain DNA sequence of 10,000 random cDNAs – This milestone was fully achieved in years 1-3 of the project with

the continual sequencing of random cDNAs isolated from our flower cDNA libraries. Additional sequence was obtained from these libraries over the last two years, resulting in the production of DNA sequence data from an additional 1500 cDNAs. This now constitutes the world's largest collection of DNA sequence information for the species Petunia.

3 – Construct cDNA microarrays for use in isolating flower specific genes – This milestone was fully achieved in years 2-3 of the project (see significant accomplishments below), but we continue to proceed with construction of microarrays for use in gene expression analysis for a broader range of research collaborators. In the past year, the data produced as a result of this project in cooperation with The Institute for Genomics Research (TIGR) has been used to produce a set of 70-base pair oligonucleotides representing the entire set of petunia genes with collaborators at the Ohio State/USDA-Wooster. These oligonucleotides will be used to produce a standard petunia microarray that can be distributed to a wider range of scientists for use in the next year.

4 – Make DNA sequence available for public use – This was not an original objective of the project. However, we were fortunate enough to work with the computer engineering department at UF in year 2 of the project to produce our own DNA sequence database. In year 3, we worked with the Solanaceous Genome Network at Cornell/USDA to make these data available to a broader scientific community. Last year, we were successful in making all of the DNA sequence data, along with potential functional data for each gene available for public use through TIGR. This group was responsible for constructing a petunia gene index, and for annotating the sequence for submission to NCBI GenBank.

5 – Isolate DNA promoter elements from flower specific genes – This was not an original goal of the project, but plans to obtain promoter elements were added later. Last year, we achieved our first progress for this objective with the isolation of 3 flower petal specific promoter elements from genes that were determined to be expressed exclusively in petunia flower petals. We are currently fusing these promoter elements to genes involved in ethylene perception to determine if we can increase flower longevity by reducing ethylene sensitivity exclusively in flower petal tissues of transgenic plants. If that proves to be a successful approach, we will continue to fuse these promoters to other genes of interest.

6 – Prove the in-vivo function of petunia genes with potential commercial utility by knocking out their expression and analyzing phenotypes in transgenic plants - Last year, the Clark lab was able to successfully prove the function of two genes involved in floral fragrance, and we currently have three additional genes that we believe will prove to be involved in floral scent biosynthesis. Once these transgenic plants are made, we hope to use them in experiments to determine their biological function. Work is still ongoing in the Jones lab to isolated genes involved in floral senescence, but several senescence related genes encoding cysteine proteases have been isolated as a result of microarray experiments that are logical candidates for having a role in protein degradation during petal senescence.

IMPACT TO THE INDUSTRY

This project will produce an unprecedented number of approaches for experiments focused on determining the function and regulation of virtually any gene expressed in a petunia plant. By determining the function of interesting genes expressed in petunia flowers throughout all stages of development and senescence, we can now get a better understanding of their role in important physiological processes in floriculture crops. This knowledge will also help us to determine the potential for manipulation of particular genes for commercial utility

through the production of the next generation of transgenic plants. As a result of this project, we will continually develop new technologies that will be useful in developing important research collaborations that are vital to supplying the floriculture industry with new innovations. It is our ultimate goal to take promising new technologies discovered during this research into other floriculture crops in the not too distant future, and make flowers smell better, last longer on retail shelves, and have fewer insect and disease pests.

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