Appealing to your senses: Regulation of floral volatile synthesis for enhanced fragrance

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Abstract

At The University of Florida, we now have a critical mass of interdisciplinary researchers focused on delivering new varieties of plants that appeal to the senses of consumers. This unique combination of expertise allows us to 1) understand consumer preferences for different horticultural crops and plant characters and 2) determine the biochemical, physiological and molecular basis of these characters. We utilize information gained in these experiments to guide the development of new varieties of flowering plants, fruits and vegetables – a process we refer to as 'Consumer-Assisted Selection'. At the present time, our main focus is directed toward making flowers that smell better for the next generation of consumers of floriculture crops.

Introduction

The University of Florida Floriculture Biotechnology and Genetics Laboratory was established in 1995 with the central focus of bridging the gap between basic and applied science in the field of floriculture. Using molecular biology tools and genetic engineering, we have determined the function of several genes with potential applications in the development of new floriculture cultivars. We utilize *Petunia* x *hybrida* 'Mitchell Diploid' (MD) as a physiological and genetic model plant for much of our basic science work. This plant has proven to be very useful because it is a doubled haploid with a short lifecycle, and it is easily transformed using

Agrobacterium tumefaciens, which facilitates phenotyping the effects of transgenes. We also have a large expressed sequence tag (EST) collection made from several different cDNA libraries of different petunia tissues, which facilitates the discovery of new genes with unknown functions that might be useful for making novel plants. MD flowers are also easy to work with because of their large size, and because they emit high levels of volatile organic compounds. These emitted volatiles give 'MD' a characteristic aroma, which is particularly fragrant at night.

Regulation of floral fragrance synthesis

Floral fragrance in petunia is mainly composed of volatile benzenoids/ phenylpropanoids (FVBPs) derived from the shikimate pathway in the plastid. Utilizing our EST database, we have isolated and characterized several FVBP genes that encode proteins which catalyze reactions directly responsible for production of these emitted volatiles. By reducing their expression in transgenic plants using RNA interference (RNAi), we have determined the function of several FVBP genes through observation of loss of specific volatile organic compounds. This has allowed us to construct a putative biochemical model for FVBP synthesis in petunia (Fig. 1).

FVBP genes are coordinately regulated at the level of transcription in various ways. Analysis of spatial expression patterns of all known FVBP biosynthetic pathway genes has shown us that all genes are highly expressed in the petal limb tissues of the corolla, the known site of volatile emission (Underwood et al., 2005;

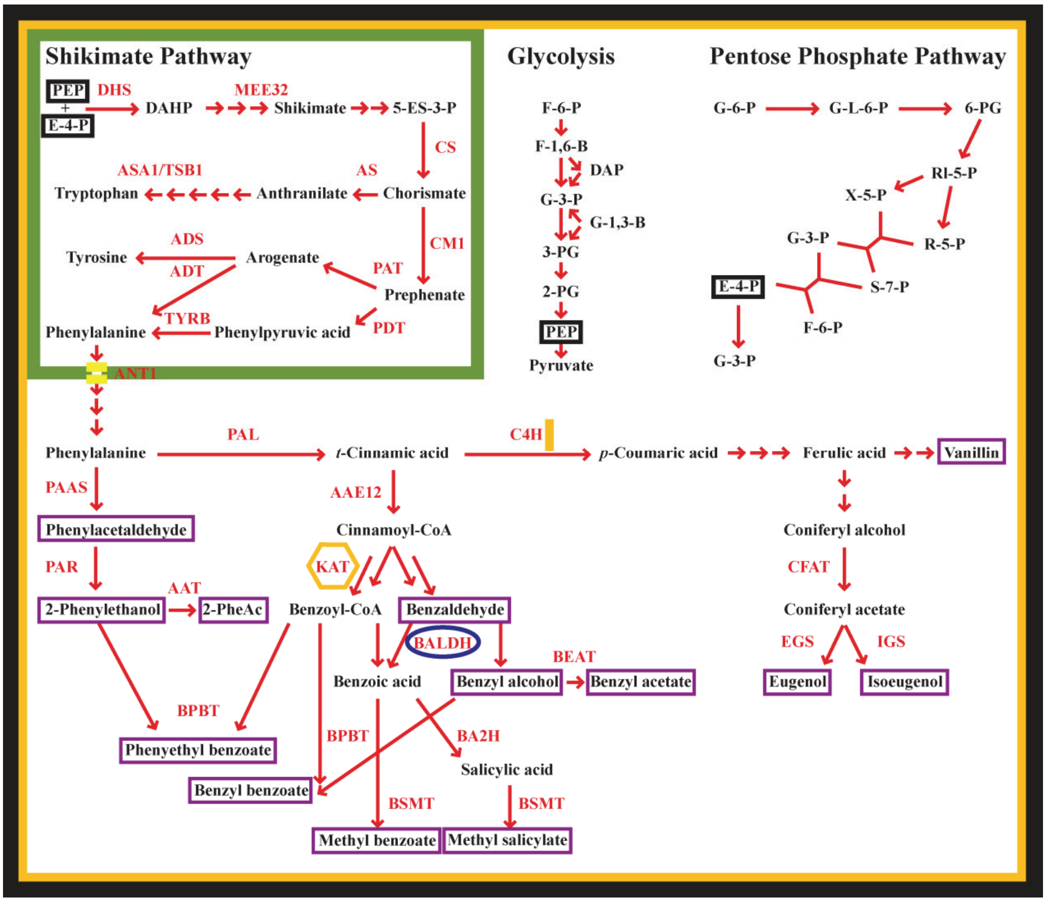

Fig. 1. A biochemical model for synthesis of floral volatile benzenoids/ phenylpropanoids in *Petunia* x *hybrida* ‘Mitchell Diploid’.

Colquhoun et al., 2010a). Temporal transcript analysis has revealed that FVBP transcript begin to accumulate in the corolla at the start of anthesis when the flower begins to open. At this developmental stage, the flower becomes receptive to pollination and also capable of emitting fragrance. It is also at anthesis when the corolla becomes sensitive to ethylene which ultimately leads to corolla senescence. Finally, FVBP transcripts are also regulated by an endogenous circadian/diurnal rhythm. Maximum mRNA accumulation of FVBP genes occurs in the mid-late afternoon, which preceeds maximum emission of volatiles around midnight (Colquhoun et al., 2010a).

After spending several years cloning and characterizing genes encoding enzymes responsible for direct synthesis of volatile compounds, we decided to go 'upstream' in the shikimate pathway to determine if similar levels of regulation occur. These efforts led to the isolation and characterization of two genes encoding chorismate mutase (*PhCM1* and *PhCM2* respectively) (Colquhoun et al., 2010b). One of the genes, *PhCM2*, is not regulated in a manner similar to previously isolated FVBP genes. It is expressed in all plant tissues and it is not temporally, developmentally or hormonally regulated. Conversely, *PhCM1* is transcriptionally regulated in a similar manner to previously described FVBP genes. Knock-down (RNAi) *PhCM1* transgenic plants show great reductions in some volatiles such as methyl benzoate and phenylacetaldehyde, but not others such as eugenol and isoeugenol. Thus, *PhCM1* appears to be specific to the process of floral volatile biosynthesis, while *PhCM2* likely has a 'housekeeping' function to maintain phenylalanine synthesis for other cellular processes.

Since we observed that several important FVBP genes share similar patterns of transcriptional regulation, it was logical for us to next search for genes encoding transcription factors that might be responsible for controlling volatile synthesis. Bioinformatic analysis of all known petunia transcription factors identified several candidates of interest. After screening these genes to find which ones are predominantly expressed in flower petal tissues, we were able to isolate several interesting MYB transcription factor genes. Two of these, *PhMYB4* and *PhMYB24* appear to play significant roles in regulating important processes in petunia flowers. Both genes are expressed predominantly in flower petal tissues, and both are developmentally regulated in these tissues in a similar manner to the previously isolated FVBP biosynthetic pathway genes. Using RNAi to knock down expression of *PhMYB4* led to the observed phenotype of a shift in the composition of emitted floral volatiles. Compared to MD plants, *PhMYB4*-RNAi plants produced less methyl benzoate, but more isoeugenol. Closer investigation showed us that *PhMYB4* acts normally by suppressing the expression of *PhC4H*, which catalyzes the conversion of cinnamic acid to p-coumaric acid. We took a similar approach and made transgenic plants with reduced expression of PhMYB24. Analysis of these plants revealed the striking phenotype of producing flowers that did not open and go through normal anthesis. Apparently PhMYB24 controls expression of several important genes involved in phenylpropanoid production, and also cell wall modification. Thus, this gene is defined as a 'gatekeeper' to the last stages of floral development.

Why is this important?

Our overall goal of this research is to ultimately gain enough information to assist us in breeding new varieties of plants with enhanced fragrance. It is not likely that transgenic plants will make it to US markets any time soon, but we should be able to make gains with new gene markers and analytical techniques. The other part of the equation we are working on is centered on understanding which fragrances humans prefer most. This will be important in making the next generation of fragrant flowers with maximum consumer appeal. We have been utilizing human sensory panels to determine if there are discernable differences between transgenic plants with altered floral volatile synthesis. Not surprising, humans can easily differentiate between normal and engineered plants. In recent work, we have been using facial recognition technologies to quantify human hedonic responses to determine which visual and olfactory characteristics have the greatest influence on human behavior. By analyzing our students at the University of Florida, we feel this approach will be useful in determining which color and fragrance combinations will have the greatest appeal to this next generation of affluent consumers of floral crops. Using a 'Consumer Assisted Selection' approach, we are confident that we can create iconic plants with widespread consumer appeal.

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