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Improving Scent Production in Flowers

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BACKGROUND

Traditional breeding of many ornamentals has unintentionally selected against scent due to the negative correlation between longevity and fragrance. The lack of distinctive scent in many modern floricultural varieties has been recognized as one of the major problems in the floriculture industry. Engineering transgenic plants with improved scent quality would ameliorate this problem. However, it requires an understanding of the molecular and biochemical basis of floral scent production, availability of cloned genes encoding enzymes involved in the biosynthesis of floral volatiles, and promoters directing the expression of these genes to the proper tissue (petals) and at the proper stage of development. We have investigated the molecular changes that affect the level of scent emission in snapdragon, isolated new genes involved in the formation of volatile compounds, and isolated and analyzed the BAMT (***S***-adenosyl-L-methionine: benzoic acid carboxyl methyltransferase) and LIS (linalool synthase) promoters, which could be potentially used to produce transgenic cut flowers with improved novel scents.

MATERIALS AND METHODS

Floral scent emitted from flowers of 50 snapdragon varieties was determined by headspace analysis in combination with gas chromatography and mass spectrometry. New genes involved in floral scent production were isolated using a functional genomic approach. The function of the genes was confirmed by overexpression in *E. coli* followed by enzyme assays. The BAMT promoter region was isolated from a genomic DNA library. The putative full-length BAMT and LIS promoter regions (2kb) were translationally fused to the GUS-NOS reporter gene and transformed into *Petunia Mitchell* using *Agrobacterium tumefaciens.* Ten independent transgenic lines were identified containing the GUS gene under control of the BAMT or LIS promoters.

RESULTS

Regulation of low to moderate emission of methylbenzoate

We found that out of 50 analyzed cultivars, only three – **'**Sonnet White', 'Potomac White' and 'Potomac Pink' - did not emit methylbenzoate or emited it at very low levels. Analysis of BAMT gene expression revealed that the lack or very low emission of methylbenzoate emission in 'Potomac White' and 'Sonnet White' was due to the absence of BAMT gene expression. In contrast, expression of BAMT mRNA in 'Potomac Pink' was relatively high and comparable to its expression in 'Maryland True Pink', the cultivar with the highest methylbenzoate emission. We found that 'Potomac Pink' contains two forms of the BAMT gene, an active and inactive (BAMT*) with 10 changes in the amino acid sequence. Analysis of BAMT and BAMT* gene expression, activity, and methylbenzoate emission in 14 individual plants from the F2 population revealed that some interactions existed between BAMT and BAMT* genes. An increasing relative amount of BAMT* caused a decrease in overall BAMT activity. This could result in the formation of inactive heterodimers, which are rapidly degraded.

Isolation and characterization of new genes

Using a functional genomic approach we isolated and characterized a cDNA that encodes a salicylic acid carboxyl methyltransferase (SAMT) from *Antirrhinum majus*. This gene catalyzes the formation of the volatile ester methylsalicylate from salicylic acid and S-adenosyl-L-methionine (SAM). It can also produce another volatile ester, methylbenzoate, from benzoic acid and SAM.

Using the same approach, we isolated and characterized three closely related cDNAs, which encode two myrcene synthases and an (*E*)-β-ocimene synthase. The two myrcene synthases were catalytically active yielding a single monoterpene product, myrcene; however, only one was expressed at a high level in flowers and contributed to floral myrcene emission. (*E*)-β-Ocimene synthase was highly similar to myrcene synthases and produced predominantly (*E*)-β-ocimene (97% of total monoterpene product) with small amounts of (*Z*)-β-ocimene and myrcene. Analyses of tissue-specific, developmental and rhythmic expression of these monoterpene synthase genes in snapdragon flowers suggested that regulation upstream of individual metabolic pathways coordinates terpenoid and phenylpropanoid emission.

BAMT and LIS promoters

Our results showed that when the 5'-flanking regions of the *LIS* or *BAMT* gene were translationally fused to the β-glucuronidase (GUS) reporter gene and introduced into petunia plants by *Agrobacterium*-mediated transformation, strong expression of GUS was found in floral organs with the maximum level in petal tissue. This indicates that these promoters retain their tissue-specific expression pattern in a heterologous system. We previously had shown that the expression of BAMT is highly petal-specific and occurs in the epidermal cell layer.

Expression patterns of the LIS promoter- GUS fusion gene

CONCLUSIONS

1. Low emission of methylbenzoate in 'Potomac White' and 'Sonnet White' is the result of down-regulation of BAMT gene.
2. In 'Potomac Pink' there are two forms of the BAMT gene, an active and inactive, and interactions between them at the protein level lead to low methylbenzoate emission.
3. Regulation upstream of individual metabolic pathways coordinates terpenoid and phenylpropanoid emission.
4. Isolated BAMT and LIS promoters retain their tissue specificity.

IMPACT TO THE INDUSTRY

New isolated genes SAMT, myrcene synthase and *(E)*-β-ocimene synthase which are responsible for the formation of methylsalicylate, myrcene and *(E)*-β-ocimene could be used for generation of transgenic plants with new scent compounds. The 35S promoter gives rise to gene expression in most tissues at all stages of development. Isolated petal-specific BAMT and LIS promoters could replace the previously used 35S promoter for manipulation of the output of volatile compounds using recombinant DNA technologies.

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