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Lilium floral fragrance: A biochemical and genetic resource for aroma and flavor

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ABSTRACT

Hybrid *Lilium* (common name lily) cultivars are among the top produced domestic fresh cut flowers and potted plants in the US today. Many hybrid *Lilium* cultivars produce large and showy flowers that emit copious amounts of volatile molecules, which can negatively affect a consumer's appreciation or limit use of the plant product. There are few publications focused on the biochemistry, genetics, and/or molecular regulation of floral volatile biosynthesis for *Lilium* cultivars. In an initial pursuit to provide breeders with molecular markers for floral volatile biosynthesis, a total of five commercially available oriental and oriental-trumpet hybrid *Lilium* cultivars were selected for analytical characterization of floral volatile emission. In total, 66 volatile molecules were qualified and quantitated among all cultivars. Chemical classes of identified volatiles include monoterpene hydrocarbons, monoterpene alcohols and aldehydes, phenylpropanoids, benzenoids, fatty-acid-derived, nitrogen-containing, and amino-acid-derived compounds. In general, the floral volatile profiles of the three oriental-trumpet hybrids were dominated by monoterpene hydrocarbons, monoterpene alcohols and aldehydes, while the two oriental hybrids were dominated by monoterpene alcohols and aldehydes and phenylpropanoids, respectively. Tepal tissues (two petal whirls) emitted the vast majority of total volatile molecules compared to the reproductive organs of the flowers. Tepal volatile profiles were cultivar specific with a high degree of distinction, which indicates the five cultivars chosen will provide an excellent differential genetic environment for gene discovery through comparative transcriptomics in the future. Cloning and assaying transcript accumulation from four floral volatile biosynthetic candidates provided few immediate or obvious trends with floral volatile emission.

1. Introduction

Many angiosperm species synthesise and emit volatile organic molecules from floral tissues. These floral bouquets of volatile compounds can be as simple as a few chemically related volatile molecules to a mixture of a hundred volatile molecules produced from divergent metabolic pathways (Dudareva et al., 2013). One of the most well studied floral volatile functions is the role in sexual reproduction through pollinator attraction (Kessler et al., 2008; Klahre et al., 2011). For some angiosperm species, when a flower reaches sexual maturity, the recruitment of an active insect pollinator becomes paramount. This can be accomplished by broadcasting a chemical signal that will attract the pollinator to the flower, and increase the probability of pollen contact. However, the requirement for the correct pollinator based on flower structure, size, and/or location is fundamentally important (Andrews et al., 2007; Strauss and Whittall 2006).

This paradigm also holds true for human attraction to flowers. In the 1993 book *The Culture of Flowers*, Sir John Rankine Goody clearly illustrated the human fascination with flowers via anthropologic examples of human flower usage: in written language, social rituals, art works, and food stuffs (Goody, 1993). Breeding of ornamental angiosperm species dates back to the eighteenth century, and has produced countless angiosperm cultivars with varied traits (i.e. color, size, fragrance, longevity, and disease resistance). The continued generation of flower cultivars (e.g. roses, lilies, petunias, tulips, etc) speaks directly to the variation in human preference for flowers and plants. Three consumer

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segments have been identified with unique desired floral characteristics that motivate buying preferences through consumer-assisted selection methods (Levin et al., 2012). One of these segments is most interested in the floral trait of fragrance. In order to understand and characterize the molecular and biochemical pathways leading to floral volatile biosynthesis a model organism amenable to molecular biology studies is needed.

Functional research into floral fragrance arguably began with the characterization of gene products responsible for the synthesis of floral volatile compounds in the model organism *Petunia* x *hybrida* cv. 'Mitchell Diploid' (MD) (Boatright et al., 2004; Negre et al., 2003; Underwood et al., 2005; Verdonk et al., 2005). MD is a laboratory generated, homozygous diploid species (Mitchell et al., 1980) that emits approximately 12 floral volatile benzenoids/phenylpropanoids (FVBPs). Many of the protein encoding genes in the FVBP metabolic network have been identified and characterized at some level. Several aspects of this research have been translational in nature. For example: identification of the core β-oxidative pathway for benzoic acid biosynthesis (Colquhoun et al., 2012; Klempien et al., 2012; Qualley et al., 2012; Van Moerkercke et al., 2009), identification of novel enzymatic activities like a decarboxylation-amine oxidation reaction (Kaminaga et al., 2006; Tieman et al., 2006), and understanding of a complex and coordinated gene network (Colquhoun and Clark, 2011; Colquhoun et al., 2010a; Van Moerkercke et al., 2012a, 2011, 2012b; Verdonk et al., 2005). Much of this research was aided by the relatively simple cellular environment of the MD corolla limb tissue. The corolla limb tissue is mostly devoid of pigments, only a few cell layers thick, and predominantly expresses the FVBP gene network. However, the limited number of volatile compounds produced by MD flowers has confined the ability to translate much of this research into other plant products like fruits and vegetables, which can emit hundreds of different volatile compounds. Previous analysis of *Lilium* cv. 'Casa Blanca' established the presence of a diversity of volatile compounds synthesized from several independent pathways such as terpenes, phenylpropanoids, and nitrogen-containing compounds (Oyama-Okubo et al., 2011). The presence of compounds from diverse metabolic volatile pathways positions lilies as a potential model system for the elucidation of gene products responsible for the synthesis of volatiles which can build upon the information derived from *Petunia* x *hybrida* cv. 'Mitchell Diploid.' Therefore, information derived from the lily system could be translational to understanding the synthesis of volatiles from a wider range of important agricultural crops such as blueberry (*Vaccinium Cyanococcus*) and strawberry (*Fragaria*).

Most contemporary *Lilium* flowers are stimulating to the human senses of vision and olfaction, and are very popular with the home gardener and people that enjoy plants in general. *Lilium* species and cultivars are among the top produced domestic fresh cut flowers and potted plants (Vilsack and Clark, 2009). Presumably, due to societal popularity and the genus biology itself, *Lilium* species have been a source of interest to humans for at least 3000 years according to Minoan civilization paintings like The Prince of Lilies. More recently, the Madonna lily is said to be the basis for the fleur-de-lys, which is first historically associated with the French monarchy. Today, *Lilium* species and hybrids can be found most anywhere, and are represented in many colors, shapes, and sizes.

Despite the overall popularity of *Lilium* plants, consumers have communicated specific aspects of some lilies that are undesirable. Many *Lilium* flowers produce excessive amounts of floral volatile organic compounds that may be overpowering for some consumers or uses. Few scientific studies have addressed the different volatile chemicals that specific lily flowers produce and emit, the variation of volatile compounds emitted throughout the *Lilium* genus, or even individual volatile biosynthetic pathway elucidations. However, it seems that lily flower fragrance may be a breeding trait of interest if it were quantitative and much more tenable from a biochemical, molecular, and genetic level.

In the recent past, research using a pharmacological inhibitor, aminooxy acetic acid (AOA), of a specific plant enzyme, phenylalanine ammonia-lyase (PAL), resulted in a significant reduction of total floral fragrance from an oriental hybrid lily cultivar (Oyama-Okubo et al., 2011). The results of this study were confusing since the pharmacological inhibitors used to reduce floral fragrance emission inhibit the enzymatic ability of a key protein in the phenylpropanoid pathway, but the lily cultivar used for these experiments produced and emitted phenylpropanoids, terpenoids, and nitrogen-containing volatile compounds. Therefore, the mechanism of action would be surprising and has not been elucidated currently. With that said, this work did lay a foundation for what volatile organic compounds can be produced in an oriental hybrid lily flower.

The work presented here focused on the analytical characterization of volatile compounds emitted from *Lilium* flowers. The overall goals of this work were to understand the diversity of floral volatile compounds, quantitate the amounts of floral volatiles, and to begin to address any opportunities for gene function identification.

2. Results and discussion

Numerous oriental hybrid *Lilium* cultivars emit a strong floral fragrance, which can be an overall negative feature for consumers. *Lilium* breeders and industry leaders have communicated the desire to reduce the amount of floral fragrance via molecular breeding efforts. However, relatively little is known about *Lilium* floral fragrance from the perspectives of genetics, biochemistry, and molecular regulation. To begin to explore this issue, four hybrid *Lilium* cultivars (Tarrango, Belladonna, Robina, and Conca d'Or) were chosen with strong floral fragrance and one cultivar (Santander) with a weak floral fragrance (Fig. 1A–E). The qualitative determination of strong and weak fragrance was an anecdotal observation. All plants and flowers were grown, harvested, and shipped using common practices standard to the industry.

2.1. Floral volatile emission is differential between tepals and reproductive organs

The initial testable hypothesis was that the individual floral tissues from a single cultivar (Fig. 1E and F) produce and emit varying amounts of total volatile molecules. Similar to the anatomical separation and volatile collection conducted with petunia flowers (Underwood et al., 2005), the relatively large *Lilium* flowers were excised from the stalk and separated spatially into four tissue types: distal tepals (sepals), proximal tepals (petals), stamens, and carpels. Subsequent to biological staging and anatomical separation, volatile molecules were collected and analyzed from these tissues using a dynamic push/pull head space collection system (Underwood et al., 2005) and gas chromatography mass spectrometry (GC–MS) methods (Schmelz et al., 2001).

Significantly higher amounts of total volatile molecules were detected from tepal tissues compared to reproductive organ (stamen and carpel) tissues (Fig. S1). The reproductive organs of all cultivars emitted relatively low levels of total volatiles ranging from approximately 350 ng * gFW^{-1} * hr^{-1} emitted by the carpel of 'Conca d'Or' to approximately 11,000 ng * gFW^{-1} * hr^{-1} emitted by the carpel of 'Tarrango'. Total volatiles from 'Conca d'Or' tepal tissues were detected at approximately 145 times greater than total volatiles from all reproductive organs. Conversely, total volatiles from tepal tissues of 'Santander' were detected at approximately seven times greater levels than total volatiles from the reproductive organ tissues. Total volatile amounts from tepal