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Linalool and linalool oxide production in transgenic carnation flowers expressing the *Clarkia breweri* linalool synthase gene

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Abstract

Most modern cut-flower cultivars, including those of carnation (*Dianthus caryophyllus*), lack distinct fragrance. Carnation cv. Eilat flowers produce and emit various fragrance compounds, including benzoic acid derivatives and sesquiterpenes, but not monoterpenes. Based on GC-MS analysis, benzoic acid, benzyl benzoate, phenylethyl benzoate, methyl benzoate, *cis*-3-hexenyl benzoate and β-caryophyllene are the major fragrance compounds, representing ca. 60% of the total volatiles generated by these flowers. The level of these compounds increases dramatically during petal development. To evaluate the possibility of producing monoterpenes in carnation cv. Eilat, we generated transgenic plants expressing the linalool synthase gene from *Clarkia breweri* under the regulation of the CaMV 35S constitutive promoter. The product of this gene catalyzes the production of the monoterpene linalool from geranyl diphosphate. Headspace GC-MS analysis revealed that leaves and flowers of transgenic, but not control plants, emit linalool and its derivatives, *cis*- and *trans*-linalool oxide. GC-MS analysis of petal extract revealed the accumulation of *trans*-linalool oxide but not linalool. The emission of linalool by the transgenic flowers did not lead to detectable changes in flower scent for human olfaction.

Abbreviations: DMAPP – dimethylallyl diphosphate, GPP – geranyl diphosphate, IPP – isopentenyl diophosphate, LIS – linalool synthase, *nptII* – neomycin phosphotransferase II gene

Introduction

The evolutionary success of many plants depends on their ability to produce and emit specific volatile compounds. These compounds attract pollinators and seed dispersers. They also determine flower and fruit scent and flavor and hence dictate consumer appeal. Moreover, an intriguing role for volatile substances in plant defense against insects has been described (Dudareva and Pichersky 2000; Phillips and Croteau 1999; McGarvey and Croteau 1995; Thaler 1999). Despite the importance of scent, many modern cut flowers lack distinct fragrance. Since for many years selection for longevity was a major target, and the loss of fragrance was observed, some negative correlation between these traits has been suggested (Barletta 1995). Nevertheless, the real reason for the loss of fragrance during the course of the breeding programs is still unknown. The research into fragrance focused for many years on its chemical elucidation, coupled with chemical synthesis to produce the large quantities demanded by the industry. Indeed, thousands of structures are known (De Luca and St Pierre 2000; Knudsen et al. 1993); however, the biochemical pathways leading to their production are less detailed (Dudareva et al. 2000). Most fragrance compounds belong to three major groups: phenylpropanoids, fatty acid derivatives and terpenoids (Croteau and Karp 1991). To

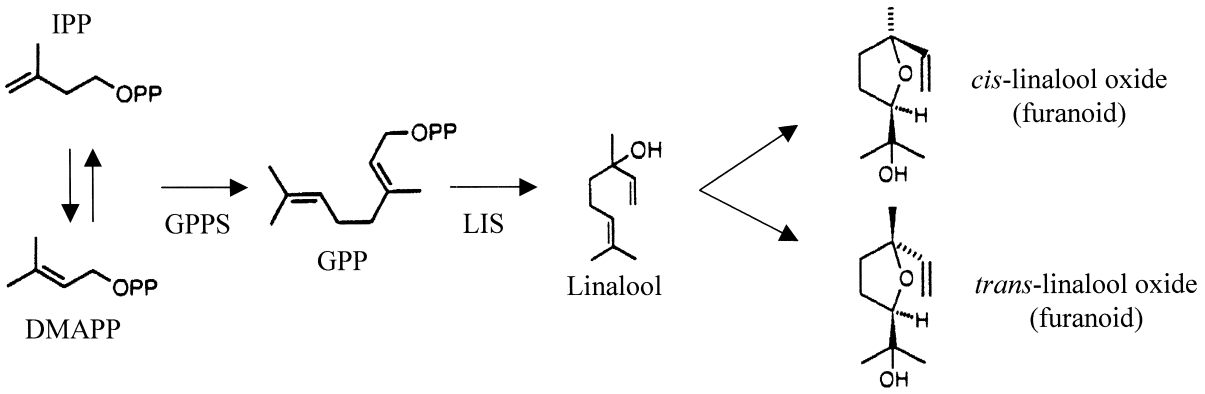

Figure 1. Schematic presentation of the pathway leading to the synthesis of linalool and its derivatives, *cis*- and *trans*-linalool oxide. Abbreviations: dimethylallyl diphosphate (DMAPP), isopentenyl diophosphate (IPP), geranyl diphosphate synthase (GPPS), geranyl diphosphate (GPP), linalool synthase (LIS).

date, only a few genes directly involved in fragrance production have been characterized, one of which is the *Clarkia breweri* linalool synthase gene (*lis*) (reviewed in Dudareva and Pichersky (2000)). The gene product, LIS, is targeted to plastids and catalyzes the conversion of geranyl diphosphate (GPP) to the monoterpene, *S*-linalool, in a single step (Figure 1) (Dudareva et al. 1996). GPP, the precursor of monoterpenes, is in turn produced by GPP synthase from isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP) in both the plastids and cytosol of photosynthetic and non-photosynthetic cells (Phillips and Croteau 1999).

Carnation (*Dianthus caryophyllus*) is one of the major contributors to the cut-flower market and a commercial leader in terms of number of stems sold worldwide. As such it is an important target for the breeding of new cultivars with novel characteristics (Jensen and Malter 1995). All commercial carnation cultivars, except one, have been generated via classical breeding approaches (Tanaka et al. 1998). As in other ornamentals, the main traits in carnation targeted by these programs were vase life, disease resistance, and flower color and architecture. Since scent was never a target for carnation breeders, modern carnation varieties, with few exceptions, lack distinct fragrance (Zuker et al. 1998). Traditionally, carnation flowers possess a spicy/clove odor, which is determined by eugenol. In some old varieties, eugenol contributes up to 85% of total headspace volatiles (Clery et al. 1999). Some modern varieties, on the other hand, produce low levels of eugenol but higher levels of benzoic acid derivatives (methyl benzoate and benzyl benzoate) and the sesquiterpene β-caryophyllene. While the aforementioned volatiles dominate *Dianthus* scent, monoterpenes in general, and linalool in particular, have been detected in only a few varieties and even then, at very low levels (Buil et al. 1983; Clery et al. 1999).

In the present paper, a carnation variety lacking detectable levels of monoterpenes was transformed with the *C. breweri lis* gene. Molecular and detailed fragrance analyses revealed that ectopic expression of *lis* leads to the production of linalool and its derivatives' *cis*- and *trans*-linalool oxide in the transgenic plants.

Materials and methods

Plant material

Carnation (*Dianthus caryophyllus* L.) cv. Eilat plants were propagated vegetatively by cuttings and grown under standard greenhouse conditions. For fragrance analysis, plants (cv. Eilat and transgenic) were grown in a phytotron at day/night temperatures of 22/16 °C under long-day conditions. The photoperiod was lengthened to 16 h (from 6:00 to 22:00) by light from incandescent lamps at a photosynthetic photon flux density of 5.3 μmol m^{-2} s^{-1}. Carnation flower development was divided into three stages: young–petals just starting to emerge from the bud (6 days before anthesis), intermediate – half-open flower (3 days before anthesis), mature-open flower at anthesis.