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Emitted and endogenous floral scent compounds of *Prunus mume* and hybrids

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ABSTRACT

Prunus mume is the only species of *Prunus* known to produce a strong floral fragrance. Most interspecific hybrids between *P. mume* and other species of *Prunus* lack the fragrance. The analysis of variations in emitted and endogenous compounds among genetically close cultivars is a powerful approach for revealing the mechanisms underlying floral scent emission. Compounds emitted by flowers from five cultivars were collected using the static headspace method, and endogenous compounds in the flowers were extracted with ethyl acetate. Samples were analysed quantitatively and qualitatively using gas chromatography-mass spectrometry. The result showed that benzenoids were the dominant compounds, of which benzyl acetate was the principal component contributing to the floral scent of *P. mume*. A clustering analysis of the floral volatiles from the different cultivars suggested that the scent traits of hybrids are related to the taxonomic relationship between their parents. The correlations between the amount of the endogenous and emitted compounds revealed that benzyl acetate had a stronger tendency to be volatile than the other compounds and the volatilisation rate of volatile compounds varied greatly among different cultivars. The importance of the biosynthetic pathway and the function of benzaldehyde are discussed.

1. Introduction

Volatile compounds in plants can be classified into three major classes: terpenoids, phenylpropanoids/benzenoids and fatty acid derivatives (Pichersky et al., 2006). The metabolism of phenylpropanoids/benzenoids comprises a series of complex branching biochemical pathways. The starting points in the biosynthesis of benzenoid volatiles are benzyl alcohol, benzaldehyde, and benzoic acid (Boatright et al., 2004). Benzyl acetate is synthesised by the reaction of benzyl alcohol and acetyl-CoA (Dudareva et al., 1998). Meanwhile, benzyl alcohol and benzoyl-CoA are combined into benzyl benzoate (Boatright et al., 2004). Eugenol, another volatile belonging to the phenylpropanoid class, is synthesised in two steps from coniferyl alcohol (Dexter et al., 2007). The biosynthesis and emission of one compound is easily influenced by its precursors which are located in the same metabolic pathway (Aranovich et al., 2007). Because some crucial intermediary metabolites are nonvolatile, both

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the volatile compounds and the nonvolatile precursors must be investigated to reveal the relationship among these compounds.

Floral scent is an important characteristic for attracting pollinators (Dudareva and Pichersky, 2000) and also enhances the value of ornamental plants and cut flowers. However, many cultivated flowers have lost their scent (Clery et al., 1999; Vainstein et al., 2001). *Prunus mume* Siebold & Zucc. is a famous ornamental and fruit tree in East Asian countries. *P. mume* blooms in early spring in Southern China and has a unique fragrance in contrast to other species of *Prunus* (Chen, 2010). Hybrids between *P. mume* and other *Prunus* trees are highly cold tolerant and have been introduced into Northern China. However, the unique and pleasant fragrance of *P. mume* does not exist in most hybrids. Given the differences in the volatile compounds between *P. mume* and its interspecific hybrids, it can be used as a model to research the heredity of scent in the *Prunus* genus.

In this study, a comprehensive analysis of volatile compounds emitted by five cultivars of *Prunus* genus was undertaken. The endogenous as well as volatile compounds of the flowers were analysed independently to investigate their contributions to the composition of the floral scent. The emission of the principal compounds is discussed based on the benzenoid biosynthesis pathway.

2. Materials and methods

2.1. Plant materials

Five cultivars grown on the campus of Beijing Forestry University, Beijing, China were selected for the study. These cultivars include (1) *P. mume* 'Fenhong' – strong-scented (Chen and Chen, 2009), (2) *Prunus armeniaca* L. 'Caoxing' – non-scented (3) *P. mume* 'Yan Xing' – light-scented, a hybrid between *P. armeniaca* 'Caoxing' and *P. mume* 'Fenhong' (Zhang, 1987), (4) *Prunus cerasifera* Ehrh. 'Pissardii' – non-scented, and (5) *P. mume* 'Meiren' – non-scented, a hybrid between *P. cerasifera* 'Pissardii' and *P. mume* 'Fenhong' (Chen, 2010). Branches with flower buds before floral opening were clipped and placed in a beaker filled with distilled water during the early spring of 2012. These branches were incubated under a 12L/12D photoperiod at 20 °C in a climate-controlled growth chamber until the flowers opened (Mortensen and Gislerød, 1999).

2.2. Floral scent collection

Volatile compounds emitted from the flowers were collected through static headspace adsorption (Kolb and Ettre, 2006). Sorbent tubes (CAMSCO, Houston, TX, USA) filled with 200 mg of Tenax GR (60/80 mesh) were pretreated with flowing nitrogen for 2 h at 270 °C to remove impurities from the sorbent (Agelopoulos and Pickett, 1998). Fully opened flowers (McTavish et al., 2000) were detached from their branches, weighed, and then sealed into a gas sampling bag (Ted-050, Plastic Film Corp, Romeoville, IL, USA). After the bag was vacuumed using a mini pump (GSP-300FT-2, Gastec Corporation, Japan), the bag was filled with 3 L of clean air purified by activated carbons. The bag with flowers was placed in a culture box for 1 h at 20 °C in light. Subsequently, volatile chemicals from the headspace of the flowers were trapped into the sorbent tubes at a flow of 200 mL min^{-1} using a mini pump (Kong et al., 2012). Gas sampling bags without flowers were used as controls. The procedure was repeated three or four times.

2.3. Preparation of endogenous compounds

The fully opened flowers of the five cultivars were frozen in liquid nitrogen. A 0.5 g flower sample was ground using a mortar and pestle, and the resulting powder was extracted with 1.5 mL of ethyl acetate. This extract was dehydrated with anhydrous sodium sulphate, and benzyl propionate was added as an internal standard (Drøhse Høgedal and Mølgaard, 2000; Kondo et al., 2006).

2.4. Gas chromatograph–mass spectrometry (GC–MS) analysis

The sorbent tubes were desorbed using a commercial automatic thermal desorption system (PerkinElmer Turbo Matrix 650, PerkinElmer, Waltham, MA, USA) at 260 °C for 10 min. A flow of helium transferred the desorbed substances into a cold trap (−25 °C), which was then rapidly heated (40 °C s^{-1}) to 300 °C to inject the sample into the chromatographic column. The total split ratio was 4.6%. The samples were analysed using a gas chromatograph (PerkinElmer Clarus 600) coupled to a mass spectrometer (PerkinElmer, Clarus 600T). The GC instrument was fitted with a DB-5 capillary column (30 m × 0.25 mm × 0.25 μm). The column oven program started at an initial temperature of 40 °C for 2 min, after which the temperature was increased to 180 °C at 4 °C min^{-1}. The temperature was maintained at 180 °C for 3 min, increased to 270 °C at 20 °C min^{-1}, and then held at 270 °C for 3 min. Helium was applied as the carrier gas. MS was used with an electron impact ion source at an interface temperature of 220 °C, and the mass scan ranged from 29 amu to 500 amu (Esteban et al., 1996). For the endogenous extract analysis, the same GC–MS instrument was used and the same analysis parameter was adopted; only the method of sample injection was changed. The injector temperature was 250 °C, and injection was performed in splitless mode. One microlitre of the sample was injected for GC–MS analysis and the solvent delay was 3.0 min.