

Variation of fragrance constituents in the leaves of *Prunus*

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Received 27 November 2004; accepted 22 July 2005

Abstract

Hexane extracts from the leaves collected from 77 individual trees of the subgenera (*Cerasus*, *Padus*, *Laurocerasus*, and *Prunus*) in the genus *Prunus* were analyzed using gas chromatography, and the variations of linalool, phenethyl alcohol, and coumarin were investigated.

The content of coumarin was 0.00–2.94 mg/dw; the relative content was 0.0–78.4%. The individual trees were divided into two groups, based on whether the relative content of coumarin exceeded 6%. In *Pseudocerasus*, *Prunus verecunda* (Koidz.) Koehne and the Yamazakura and Kanhizakura groups showed relatively high coumarin content. *Prunus × introrsa* cv. *Introrsa* (Tsubakikanza-kura), which is an interspecific hybrid between *Prunus campanulata* Maxim and *Prunus pseudocerasus* Lindley, showed the highest coumarin content among all the samples. *P. campanulata* Maxim and *P. pseudocerasus* Lindley showed low coumarin content, whereas most interspecific hybrids with *P. campanulata* Maxim and all interspecific hybrids with *P. pseudocerasus* Lindley showed high levels of coumarin.

These results suggest that the production of coumarin in the leaves of *Cerasus* trees is genetically controlled.

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Keywords: *Prunus*; *Cerasus*; *Prunus × introrsa* cv. *Introrsa*; *Prunus pseudocerasus*; Fragrance constituents; Coumarin

1. Introduction

Sakura (cherry) that often appears in *Manyooshu* (the oldest existing collection of Japanese poetry) is one of the flowers most associated with the Japanese since ancient times. Sakura is generally classified as Rosaceae, Amygdalaceae, *Prunus*, and *Cerasus* (Ohba, 1989; Kawasaki, 1991). About 10 types of Sakura are classified as species indigenous to Japan (Honda and Hayashi, 1982; Kawasaki, 1993; Katsuki, 2001), and many types of Sakura are also classified as interspecific hybrids and cultivars in Japan (Honda and Hayashi, 1982; Kawasaki, 1993; Katsuki, 2001). The most famous Sakura is ‘Someiyoshino,’ which is considered an interspecific hybrid between *Prunus pendula* and *Prunus lannesiana*. This flower blooms throughout the Japanese archipelago for about a month in spring, beginning in southernmost Japan (Okinawa and Kyushu) and ending in Hokkaido to the north.

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Past research has confirmed that benzaldehyde, benzyl alcohol, phenethyl alcohol, linalool, and coumarin are present in Sakura flowers, *Prunus mume* (Sieb.) Sieb. et Zucc. flowers, and *Prunus persica* Batsch flowers (Horiuchi and Misumi, 1989; Kurihara, 1998). It is also reported that the cultivars of *P. lannesiana* emit a pleasant smell in Japanese cherries, such as those with fragrant flowers found in the Tama Forest Science Garden, with 34 genealogies classified into three types (A, B, and C) based on the differences in fragrance as preserved in this garden (Hatakeyama et al., 1988; Horiuchi, 1991). The young leaves of *P. lannesiana* have also been used as a decorative wrapping for sakura-mochi, a traditional Japanese-style confection (tinted the color of Sakura and which emits the leaf's fragrance). The constituent that emits this fragrance is known to be coumarin (Watanabe, 1998).

Coumarin has been used for various purposes as a flavor ranked with vanillin. Its use as a spice was recently restricted by law, but it is still synthesized from *o*-cresol as an important aromatic mainly used in soap and tobacco products (Watanabe, 1998).

This study investigated the variations in five fragrance constituents of flowers and leaves of *Prunus* (benzaldehyde, benzyl alcohol, phenethyl alcohol, linalool, and coumarin).

2. Experimental method

2.1. Sample material

Samples were collected from 77 individual trees from July to October (when the cherry leaves reach full growth) at three locations (Table 1). Samples were collected from six individual trees in Ooyama Park, Tsuruoka, Yamagata Prefecture, on July 17, 2002, three individual trees in the seedling field on the experimental plantation at the Yamagata University Agriculture Faculty, Tsuruoka, Yamagata Prefecture, on September 20, 2002, and 68 individual trees in the cherry preservation forest of the Tama Forest Science Garden, Forest and Forestry Products Research Institute, Hachioji, Tokyo, on October 2, 2002.

2.2. Extraction

Five grams of each sample were cut into pieces, to which hexane was added for extraction at room temperature. Hexane extracts were obtained by concentrating the extracted solution.

2.3. Gas chromatography analysis

The hexane extract from each of the samples was liquefied by adding the same volume (ml) of hexane as the dry weight (g) of the sample. One microliter of each liquefied extract was analyzed using a Hitachi G-3000 gas chromatograph with a TC-WAX 25-m capillary column for 60 min at temperatures ranging from 150 to 245 °C, incremented in 4 °C-steps per minute.

2.4. Identification of the hexane extract constituents

Preparations of the five constituents (benzaldehyde, benzyl alcohol, phenethyl alcohol, linalool, and coumarin) purchased on the market were subjected to the spike test using the gas chromatograph to confirm that the retention times matched those of the hexane extracts. A JMS-SX102A instrument (manufactured by JEOL Ltd.) was used for GC–MS analysis to identify the hexane extracts.

2.5. Discriminant analysis

Excel Statistics 97 (SSRI Co., Ltd. for Windows 95) was used for discriminant analysis.

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