



## Floral volatile profile of *Dendrobium nobile* (Orchidaceae) in circadian cycle by dynamic headspace *in vivo*.

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The olfactory stimulus used by flowers are composed by a complex mixture of volatile compounds, usually emitted in a well defined ratio. In addition to the ecological interactions, the complexity of floral volatiles has been an inexhaustible source of inspiration and raw material development for the perfume industry. However, due to the fragility of the plant tissue, the study of floral volatiles require suitable techniques, without use of solvent or heating, in such a way that not cause distortion of the authentic composition of the floral scent (1). The aim of this study was to analyze qualitative and quantitatively the variation of the floral volatile profile of *Dendrobium nobile* (Orchidaceae) in circadian cycle by dynamic headspace *in vivo*. Floral scent was collected with adsorbent tubes filled with 3 mg of Porapak Q, based on the methodology developed by Kaiser (2). *D. nobile* flowers were sampled in the laboratory. Twenty flowers were enclosed *in vivo* within a polyester bag (15 cm X 25 cm) and the emitted volatiles trapped in an adsorbent tube through the use of a vacuum pump, rate adjusted to 60 mL min<sup>-1</sup> using a power supply and a flow meter. A second pump with positive flow was used to counterbalance the vacuum. Samples were collected for 4 h in two periods of the day (8 to 12 am and 1 to 5 pm) and at 7 consecutive days with a 50 µL dichloromethane elution of the adsorbent trap, after each sampling. *n*-Octadecane was added to the extract as internal standard (12.4 µg). A 2 µL aliquot of each extract was injected in splitless mode onto Agilent 6890N gas chromatograph fitted with a HP5MS capillary column (30 m X 0.25mm X 0.25 µm), using hydrogen as carrier gas at 1.0 mL min<sup>-1</sup>. Injector was kept at 250 °C and FID at 280 °C. Oven temperature ranged from 40 °C (for 5 min) up to 240 °C at a rate of 3 °C min<sup>-1</sup>. The amount of each compound was calculated by relating its peak area, corrected by the response factor (RF), to that of the internal standard. For GC/MS, samples were injected into Agilent 6890N gas chromatograph coupled to a 5973N mass detector. Helium was used as carrier gas (1.0 mL min<sup>-1</sup>). Compounds were identified by comparison of both mass spectra and linear retention indices with spectral library and literature. In the flowers scent, 32 substances were identified, among which four showed great relevance to the major profile:  $\alpha$ -pinene (65.5 to 211.3 ng), *p*-cymene (24.0 to 116.6 ng), limonene (1.1 to 88.7 ng) and (*E,E*)- $\alpha$ -farnesene (4.1 to 70.4 ng). On average, volatiles release was much more pronounced from 8 to 12 am (388.5 ng) than in 1 to 5 pm (107.7 ng). Furthermore, the ratio of the major compounds undergoes dramatic changes. Between 8 to 12 am, the sum of  $\alpha$ -pinene and *p*-cymene is 2.2 times the sum of limonene and (*E,E*)- $\alpha$ -farnesene, while in the period between 1 to 5 pm, the sum of limonene and (*E,E*)- $\alpha$ -farnesene is 15.6 times less than the sum of  $\alpha$ -pinene and *p*-cymene in the volatile composition of *D. nobile* flowers ( $p < 0.05$ ).

1. Tholl, D. et al. The Plant J., 2006, **45**, 540-560.
2. Kaiser, R. Chimia, 2000, **54**, 346-363.

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