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# Scent profiling of *Cymbidium ensifolium* by electronic nose

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ABSTRACT

The performance of electronic nose (E-nose) for Chinese *Cymbidium* scent profiling has been evaluated. Changes in scent profiles of two *Cymbidium ensifolium* cultivars have been monitored at different flowering stages (initial flowering, full flowering, and terminal flowering) and different times combined with two gas collecting devices. Samples were collected by static headspace (SHS) method. How E-nose can be used for pattern recognition and for studying the releasing of flower scent were proposed. Data obtained were subjected to principal component analysis (PCA) and discriminant function analysis (DFA). PCA was performed on the initially instrumental data to explore the structure of each data set and such result showed that the sensory data contained information related to the cultivar and to time spots. DFA was performed to improve the results, leading to clear separations between the sample groups. Gas collecting device did not seriously affect the result of PCA and DFA. Relative aroma intensity (RAI) was proposed as an alternative concept to compare scent intensity between samples on different time points. These results demonstrate the potential application of the E-nose to evaluate the scent profile of flower.


## 1. Introduction

Chinese orchid (*Cymbidium* spp.), the king of fragrant plants, has an extremely high ornamental value and economic value (Chen and Tang, 1982). *Cymbidium* has been considered as one of the most important potted flowers in East Asia for centuries. It has been cultivated since ancient times (widely practiced during the Song Dynasty in China, 960–1279 AD) and has been used in many situations due to its potential benefits for temperament and health (Chen and Tsi, 1998).

Scent, odor or aroma is an important flower quality attribute. Flower odors are produced by the specific combination of many different volatile molecules, each one in different concentrations (Dudareva and Pichersky, 2006). The scent is detected when its volatiles enter the nasal passages and are perceived by receptors of the olfactory system (pronasal) (Meilgaard et al., 1991; Nuener-Jehle and Etzweiler, 1991; Pierce1 and Halpern, 1996). Sometimes, it is impossible to analyze trace amount of scent by conventional techniques due to the complexity of the volatile compounds. Lack of scent characteristic of Chinese orchid is a recurrent complaint of breeders and consumers. Modern analysis and tools like the electronic nose (E-nose) can perceive odor in a similar way to that of the human olfactory system. Among commercially available E-nose instruments, oxide semi-conductor (MOS) sensors are most widely used. They consist of an array of sensors that react differently in the presence of volatiles. Unlike most chemical sensors, which are designed to detect specific gas molecules, the sensors in the E-nose are not specific to a particular volatile but are compatible with a large range of organic vapors (Marsili, 2002). Each sensor with varying selectivity and sensitivity responds to a wide range of chemical compounds. And fragrances and scents were qualitatively and quantitatively evaluated finally. However until now, little attention has been paid to the application of E-nose on flowers. Discrimination of three lily groups and their interspecific hybrids with an E-nose were reported by Fukai and Abe (2002). To explore the fine particular flavor of *Cymbidium*, it is necessary to know the scent profile. E-nose might be a suitable tool to fulfill this expectation.

Adequate isolation and pre-concentration of the odor-active analytes are mandatory and critical steps in the methodologies for the application of E-nose (Sides et al., 2000). The simplest way to assess the chemical composition of a scent is direct analysis of a portion of the air in contact with the odor source, without any other sample treatment. However, considering the typical trace or ultratrace levels of the components, it is impossible to apply static headspace (SHS) to chemical-fragrance analysis. Nonetheless, when techniques and devices with adequate detection limits (thresholds) are available, SHS can be particularly suitable because of its inherent simplicity (Augusto et al., 2003). Some authors

*Abbreviations:* DFA, discriminant function analysis; DHS, dynamic headspace; E-nose, electronic nose; SHS, static headspace; GSJ, cylindrical glass specimen jar; MOS, oxide semi-conductor; PCA, principal component analysis; PSC, polycarbonate specimen container; RAI, relative aroma intensity.

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even pointed out that, for these samples, both analysis time and detection limits of SHS were better than those obtained using a commercial dynamic headspace (DHS) analyzer (Clarkson and Cooke, 1996).

This work represents the first attempt to verify the feasibility of studying the floral scent law of diurnal variation and flowering stage variation by E-nose, and to evaluate the effectiveness with different gas collecting devices and statistical analysis methods. In addition, parameters evaluating the scent profile of different cultivars on different time spots are investigated.

## 2. Materials and methods

The experiment was divided in two steps: (I) according to SHS method, the sample is sealed in a modified specimen container/jar for 30 min. Headspace gas was collected by a gas tight syringe thereafter; (II) volatiles were injected into the E-nose manually.

### 2.1. Samples

Two different cultivars with varying scent characteristics of *C. ensifolium*, namely 'Xiaotaohong' and 'Hehuasu' cultivated at the Research Center of Chinese orchid, Zhejiang University (China), were set in two different gas collecting devices before determination: (I) rectangular polycarbonate specimen container (PSC, 130 L, 45 cm × 45 cm × 65 cm) with a rubber plug and (II) cylindrical glass specimen jar (GSJ, 1.5 L, Ø10 cm × 28 cm) with a rubber plug. In the first case, the container can accommodates four pots of orchids, which more reflect the real flowering situation and has more practical application value. In the second situation, the jar only contains scape and 200 mL water which keep the scape activity, so the background value of scent is the smallest and it reflects the most accurate profile of scent. The scents were kept in sealed syringes until E-nose measurements were carried out. And the headspace gas collected in sealed blank collecting device was used as control.

The analyses were performed at time 8:30, 10:30, 12:30, 14:30, and 16:30 on three flowering stages. Considering different comprehensive factors, the division of various stages of flowering is as follows:

(1) Initial flowering: less than 1/3 flowers of anthotaxy in bloom.
(2) Full flowering: between the period of initial flowering and terminal flowering.
(3) Terminal flowering: more than 1/3 flowers of flowers of anthotaxy deflorated.

### 2.2. E-nose system

The use of E-nose system in flower scent studying can be of interest in order to complement human sensory analysis and offers a technique easier to implement than other instrumental analysis techniques. All samples were analyzed on αFOX 4000 E-nose equipped with 18 metal oxide semi-conductors (MOS) (2006). A combination of several sensors provides a unique olfactive picture or finger print of the samples (Pearce and Sonchez-Montaries, 2003). Inside the E-nose system, a constant dry air carrier gas flow (set to 150 mL/min) is used to sweep the gas sample injected through the three chambers in contact with sensors. Typically, each sample analyzed is tested in three replicates prepared from the same sample. The data extracted from sensor responses can be used to detect, characterize, identify and eventually quantify the volatiles.

**Table 1**
Analytical conditions.

| | |
|---|---|
| Headspace generation | |
| Headspace generation time | 30 min |
| Headspace generation delay | >30 min |
| Headspace injection | |
| Injected volume | 2000 μL |
| Injected speed | 2000 μL/s |
| Acquisition parameters | |
| Acquisition time | 120 s |
| Acquisition period | 1.0 s |
| Acquisition delay | 300 s |

### 2.3. Statistical analysis

To simplify data processing, the responses from 18 sensors (18 dimensions) can be processed using only the maximum resistance changes $[\max(R_0 - R)/R_0]$, where $R_0$ is the baseline resistance and $R$ is the maximum resistance reading during the vapor exposure of each sensor (Gutierrez-Osuna et al., 2002). The data were processed using principal component analysis (PCA) and discriminant function analysis (DFA), applying the statistical program Alpha Soft Version 11.0 in order to identify and quantify the samples (2006).

PCA is a statistical technique with unsupervised learning that allows reduction of multidimensional data to a lower-dimensional approximation, while simplifying the interpretation of the data. This allows the user to interpret the data easily. In addition, the samples can be classified without prior information on the samples. Conversely, DFA require prior knowledge about the samples. The models obtained from DFA analysis were validated using a modified cross-validation (leave-one-out) method. In this validation method, the datasets obtained during measurements were randomly divided into a training set, used for building up the model (learning process), and a validation set, used for validation purposes.

In addition, as the florescence of cultivars varied and the problem of background value, the statistical software SAS (version 8.0) (1999) was used to calculate the relative aroma intensity, i.e. the Euclidean distance between response values of the samples and the controls, which make the comparison of scent intensity between different cultivars and different time points.

## 3. Results

### 3.1. E-nose analytical conditions

As with any analytical instrument, it is essential that E-nose is in exactly the same state for each sample, or, at the very least, differences among arrays in space and time should not prevent an analysis of sufficient sensitivity being carried out to discriminate among the samples (Collier et al., 2003). A number of preliminary tests were made in order to find optimum analysis conditions that were acceptable for all of the samples. By using PCA analysis, the optimal analytical conditions were found for this study. The analytical conditions are summarized in Table 1. With these conditions, the sensors were successful in study of floral scent profiling. And the measurements of E-nose were performed under the same conditions through the entire experiment.

From Fig. 1, we can see that when flower numbers ≥4, a clear distinction between the control and 'Xiaotaohong' could be found. And, the E-nose shows a certain degree to differentiate flower scent at different concentrations. Clusters formed for each sample from replicates are small which points out to a very good reproducibility of the tests. Moreover, in PCA plot, the distribution of number 4–8–12 moved from the II quadrant to the III quadrant and the gap to the control in I quadrant was larger. The difference between samples with various flower numbers was not obvious ($P > 0.05$), this