Analysis of Flower Scent of *Freesia* Species and Cultivars

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Abstract

The composition of volatile compounds emitted from flowers of nine *Freesia* species and 16 cultivars was investigated by headspace adsorption/gas chromatography (HA/GC). Yellow-flowered cultivars such as 'Gold Flame', 'Rijnveld's Golden Yellow' and 'Aladdin' emitted volatile compounds abundantly, while 'Rose Marie', 'Blue Heaven' and 'Volcano' showed hardly any emission. A total of 16 volatile compounds were identified. Linalool, which accounted for at least one third of the total peak area, was the dominant volatile compound common to the species and cultivars other than *F. caryophyllacea, F. refracta* and 'Evita'. *Freesia* species and cultivars were separated into three groups based on the composition of these volatile compounds. In Group I linalool was the dominant volatile compound. This group was further separated into three subgroups; In Subgroup I-i, which included three species (*F. alba*, *F. corymbosa* and *F. elimensis*), and six cultivars, fragrance was dominated by only linalool. In Subgroup I-ii, which included two species (*F. fergusoniae* and *F. leichtlinii*) and five cultivars, flowers had sweet fragrance comprised mainly of linalool and some other related monoterpenoids. In Subgroup I-iii, which included two cultivars, fragrance was characterized by limonene, ocimene and α-terpinolene, as well as linalool. In Group II, fragrance was dominated by linalool, 2-phenylethyl acetate and benzyl alcohol, and this group included *F. occidentalis* and 'Rose Marie'. In Group III, which included *F. caryophyllacea* and 'Evita', fragrance was dominated by terpinolene. 'Gold Flame', *F. sparrmannii* and *F. refracta* were outgroups having a characteristic scent.

INTRODUCTION

Flowers of freesia emit a pleasant sweet scent, which gives an important characteristic to this ornamental crop. Harada (1984) extracted the essential oil from the open florets of 'Rijnveld's Golden Yellow' and analyzed the volatile compounds that characterize the sweet scent. Linalool and α-terpineol, which are responsible for the refreshing floral note, were the major components of this freesia oil. Benzothiazole and pyrazine in the basic fraction, β-geranic acid in the acidic fraction and some phenolic compounds in the weak acidic fraction were also detected. Komoda (1995) analyzed the volatile compounds of four freesia cultivars by using the thermal desorption cold trap (TCT) headspace method and found that the major components of the volatiles varied among cultivars. Such chemical studies have already been conducted, but these are restricted to only a few cultivars that are valued for their perfumery properties.

New cultivars with a wide range of perianth colors have been introduced to Japan from the Netherlands in the last two decades, but most of these cultivars have weak or no pleasant scent. Goldblatt (1982) described that the origin of the cultivated freesia lies with *F. alba* which has a typical freesia scent. *F. leichtlinii*, from which yellow color may have originated, also has strong sweet fragrance. The pink coloration may derive from *F. corymbosa* (syn. *F. armstrongii*) which is scarcely scented. In breeding freesia cultivars, floral scent is an important characteristic to be modified. However, the emitting characteristics of scent in *Freesia* species are not clear and their contribution to the scent formation of cultivars has not been discussed.

The purpose of this research was to characterize the composition of flower

volatiles of nine *Freesia* species and 16 cultivars by headspace adsorption gas chromatography.

MATERIALS AND METHODS

Plant Materials

Twenty corms of nine *Freesia* species and sixteen cultivars were planted on September 25, 2002. Plants were grown in a plastic house kept above 5°C during winter. At anthesis from November 2002 to April 2003, inflorescences with 5 cm flower stalks were excised and senescing and unopen florets were removed to adjust the inflorescence weight to 4.0 g (4-10 florets/inflorescence). Cut inflorescences were placed under laboratory conditions at about 20°C.

Trapping of Volatile Compounds

Flowering inflorescences with the stem bases inserted into a 100 ml beaker containing deionized water were placed in a 1.5 liter desiccator with air inlet and outlet lines. Charcoal and Molecular Sieve 5A columns were connected to the air inlet line and a column containing 500 mg of Tenax TA (60-80 mesh, GL Science, Japan), which trapped floral volatiles, was connected to the air outlet. A continuous stream of air released from a cylinder (21% O_2 and 79% N_2) was regulated by a flow controller (GL Science, Japan) at 60 ml min^{-1} and passed through the charcoal filter, Molecular Sieve 5A column, desiccator, Tenax TA column and a flow meter (GL Science, Japan) for 2 hr at room temperature (20-22°C). After volatile compounds were released from Tenax TA by heating and led to a gas chromatography (GC), the columns were aged at 220°C with flushing helium gas for at least 2 hr and used again for the next trapping.

GC Analysis

The oven of a flush sampler (FLS-1, Shimadzu, Japan) was connected to the injection port of GC (GC-17A, Shimadzu, Japan) and previously heated to 200°C before analysis. The Tenax TA column was placed in the oven with the syringe needle inserted into the GC injector. The volatile components were released and flushed out from the Tenax TA column with helium gas as carrier. GC was equipped with a capillary column coated inside with 0.25 μm film of polyethylene glycol (0.25 mm i.d. x 60 m; DB-WAX, J&W Scientific, USA) and flame ionization detector (FID). The column temperature was maintained at 70°C for 5 min, raised to 220°C at the rate of 3°C min^{-1}, and then maintained at 220°C for 5 min. The injection and detector block temperatures were set at 200°C and 300°C, respectively. The split ratio was set automatically at 1:110 and nitrogen was used as make-up gas. The peak area was measured with a Chromatopak Integrator (C-R8A, Shimadzu, Japan).

Identification of Volatile Compounds

Volatile components were identified by comparing their retention time to that of the authentic standard and by co-chromatography. A standard mixture of major component volatiles was prepared. One microliter of each volatile compound previously reported to exist in the floral scent of freesia, was mixed together in 70 μl hexane. One drop of the mixture was dripped on a watch glass placed in a desiccator, trapped with Tenax TA, and analyzed by GC as mentioned above.

Cluster Analysis

Cluster analysis was conducted using the percentage data of peak area of each volatile compound. Numerical distance was calculated using statistic software STATISTICA™ (Three's Company, Inc.). A dendrogram was constructed by weighted pair-group method using arithmetic average (WPGMA) clustering.

RESULTS AND DISCUSSION

A total of 16 major volatile compounds were detected (Table 1). When the total peak area of each chromatogram was compared to that of 'Rijnveld's Golden Yellow' (100%), emission of volatile compounds was very low in 'Blue Heaven', 'Rapid Yellow', 'Rose Marie' and 'Volcano' while almost the same or higher in 'Tonga', 'Aladdin', 'Cherry', 'Evita', 'Gold Flame' and ' Tanga' (Fig. 1).

Except for *F. caryophyllacea*, *F. refracta* and 'Evita', the dominant volatile compound was linalool that peaked at the retention time (RT) of 36.5 min (Fig. 2) and accounted for at least nearly one third of the total peak area (Table 1). Other monoterpenoids such as limonene (RT 20.7 min), ocimene (RT 21.7 min) and terpinolene (RT 22.5 min) were also detected in most species and cultivars (Fig. 2). Benzyl alcohol (RT 50.0 min), *cis*-3-hexenyl acetate (RT 25.9 min) and/or 2-phenylethyl acetate (RT 55.0 min) were the major volatile constituents specific to some species and cultivars.

Based on the volatile compositions, three groups with some outgroups were generated by cluster analysis (Fig. 3). Group I is the largest cluster that is grouped within a chemical distance of 0.5 and further separated into three subgroups. In Subgroup I-i the dominant volatile compounds was only linalool (percentage of the peak area was higher than 85%) with only a trace level of other components (Table 1). Three species (*F. alba*, *F. corymbosa* and *F. elimensis*) and six cultivars ('Cherry', 'Elegance', 'Rapid Red', 'Sandra', 'Volcano' and 'Blue Heaven') belong to this subgroup (Fig. 3). In Subgroup I-ii fragrance was comprised mainly of linalool and other related compounds, i.e., sabinene (RT 17.8 min), myrcene (RT 18.9 min), limonene, ocimene, terpinolene and α-terpineol (RT 44.0 min), which were detected at higher abundance than the fragrance in Subgroup I-i (Table 1). Two species (*F. fergusoniae* and *F. leichtlinii*) and five cultivars ('Agenta', 'Aladdin', 'Rijnveld's Golden Yellow', 'Souvenir' and 'Tonga') belonged to this subgroup (Fig. 3). The inflorescences in this subgroup emit the typical strong sweet fragrance of freesia. Two cultivars, 'Oberon' and 'Rapid Yellow', which belonged to Subgroup I-iii, had weak fragrance characterized by limonene, ocimene and α-terpinolene, as well as linalool (Table 1).

Group II was represented by 'Rose Marie' and *F. occidentalis* with fragrance comprised mainly of linalool, 2-phenylethyl acetate and benzyl alcohol (Table 1). This group was connected to Group I at a chemical distance of 2.4. In Group III fragrance was dominated by terpinolene (71-85%) instead of linalool; 'Evita' and *F. caryophyllacea* belonged to this group (Table 1) which is connected to Group I and II at a distance of 4.7 (Fig. 3). Volatiles emitted from inflorescences of 'Gold Flame', *F. sparrmannii* and *F. refracta* were characterized by terpinolene (43%), *cis*-3-hexenyl acetate (44%) and 2-phenylethyl acetate (46%), respectively. These three cultivars and species were outgroups.

The chemical data obtained by HA/GC in this study confirms the results of previous studies on some freesia cultivars (Harada, 1984; Aida et al., 1993; Komoda, 1995). The results also suggest that *Freesia* species is more chemically diverse than cultivars, because most of the cultivars used in this experiment were categorized into Group I.

It has been pointed out that the composition of flower volatiles greatly depends on the method of collection. Headspace adsorption with Tenax TA has been widely applied to the trapping method of volatiles in horticultural crops, ex. peach (Jia and Okamoto, 2001), *Cyclamen* (Ishizaka et al., 2002) and *Gypsophila* (Doi et al., 2002). Volatile compounds that characterize the top note of fresh samples can be directly trapped by this method. Furthermore, it is easy to check the amount of compounds emitted in different periods.

In this study linalool and other monoterpenoids were detected as the components of headspace volatiles of freesia inflorescences. Terpenoids, especially monoterpenoids and some sesquiterpenoids are very common constituents of floral scent (Knudsen et al., 1993). Mettal et al. (1988) reported that several monoterpenoids including limonene, myrcene and ocimene are regular constituents of daffodil scent, but linalool, which is dominated in freesia scent, is not. Monoterpenoids in plants are derived from geranyl

pyrophosphate (GPP) originating from mevalonic acid via isopentenyl pyrophosphate (IPP), while, sesquiterpenoids are derived from farnesyl pyrophosphate (FPP) formed by condensation of GPP and IPP (Chappell, 1995). Several enzymes catalyzing these biosynthetic pathways have been defined. Linalool is produced as an intermediate on the metabolic pathway of monoterpenoids. Linalool synthase, a monoterpenoids synthase that catalyzes the formation of an acyclic monoterpene alcohol, linalool, was first isolated and characterized in *Clarkia breweri* flowers, which emit linalool in higher abundance (Pichersky et al., 1995; Dudareva et al., 1996). The purpose of the production of linalool and its oxides in the transmitting tissue is not clear, but they may attract insects, or may be related to a defense mechanism since linalool is relatively toxic to animals and microorganisms (Bruneton, 1995).

Goldblatt (1982) reported that *F. leichtlinii* was used for introducing the yellow coloration into modern freesia cultivars. Results obtained here showed that most cultivars having yellow or orange perianths are grouped into the same cluster of *F. leichtlinii* and *F. fergusoniae*, both of which have a strong sweet scent. This result strongly supports the description of Goldblatt (1982) and suggests that the strong sweet scent of yellow-flowered cultivars may originate from *F. leichtlinii* and/or *F. fergusoniae*. The chemical constitutions of freesia volatiles, among which linalool and terpinolene seem to be the key compounds, is useful for classifying freesia cultivars and for detecting their lineage. Basic knowledge on the relationship among *Freesia* species and cultivars will contribute to developing molecular markers specific to the characteristic sweet scent of freesias in future breeding work.

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Tables

Table 1. Volatile composition of nine *Freesia* species and 16 cultivars analyzed by a HA/GC method.

Group	I-i									I-ii						
Species and cultivars / Volatiles (RT min)																
ƒ¿-Pinene (15.7 j	tr	0.8	tr	0.3	0.2	0.1	1.1	0.5	1.7	0.4	0.3	0.8	0.1	0.2	tr	0.2
Sabinene (17.8 j	tr	-	tr	tr	tr	-	tr	-	0.4	2.6	1.0	2.6	2.7	1.9	1.7	1.7
Myrcene (18.9 j	1.1	0.2	1.6	0.7	0.7	0.3	0.6	2.6	0.6	3.1	1.4	2.5	2.2	1.7	1.4	1.8
Limonene (20.7 j	0.3	-	0.3	0.5	0.3	0.1	0.2	0.5	0.8	5.0	3.4	4.1	3.8	3.1	2.4	3.3
Ocimene (21.7 j	0.1	-	0.1	tr	0.2	-	tr	0.3	tr	4.9	0.5	3.9	2.9	2.7	2.3	1.8
Terpinolene (22.5 j	0.7	-	0.5	0.5	0.4	0.2	1.7	0.7	0.4	0.4	0.5	0.4	2.9	0.2	0.3	0.8
Hexyl acetate (24.0 j	0.1	tr	0.1	tr	0.2	1.1	0.1	0.6	-	0.3	1.0	0.2	0.2	0.1	0.1	0.2
cis -3-Hexenyl acetate (25.9 j	tr	-	-	tr	0.1	0.8	-	-	-	tr	-	0.5	0.1	0.1	tr	0.1
Linalool (36.5 j	94.5	96.4	93.8	92.5	93.4	94.9	94.2	87.8	91.3	71.0	72.6	73.3	75.8	78.9	84.8	82.9
ƒ¿-Terpineol (44.0 j	0.1	-	tr	tr	tr	tr	tr	-	tr	2.4	3.5	5.1	2.4	4.2	3.0	1.2
ƒÀ-Selinene (45.5 j	-	-	0.1	4.7	-	-	-	-	-	tr	0.2	-	0.4	tr	tr	-
Dehydro-ƒÀ-Ionone (46.0 j	-	tr	-	-	-	-	-	-	-	-	-	-	-	-	-	tr
2-Phenylethyl acetate (48.0 j	0.1	-	tr	-	tr	-	-	-	-	tr	0.3	-	-	-	-	-
Benzyl alcohol (50.0 j	0.1	-	0.1	-	tr	-	-	0.1	0.4	0.2	0.2	-	0.1	-	tr	-
2-Phenylehtyl alcohol (55.0 j	tr	-	tr	-	-	-	-	-	0.8	0.4	0.2	-	0.1	tr	tr	tr
ƒÀ-Ionone (55.5 j	-	-	-	-	-	-	-	-	0.9	0.1	tr	-	0.1	-	-	tr
Total	97.0	97.4	96.7	99.2	95.5	97.6	98.0	93.2	97.2	90.8	85.1	93.4	93.7	93.3	95.9	94.0

Group	I-iii		II		III				
Species and cultivars / Volatiles (RT min)									
ƒ¿-Pinene (15.7 j	0.1	0.3	0.7	1.5	-	0.2	0.2	2.4	-
Sabinene (17.8 j	4.6	2.2	-	tr	0.2	0.1	tr	-	-
Myrcene (18.9 j	3.4	6.5	0.3	tr	-	0.7	0.4	-	9.8
Limonene (20.7 j	6.5	7.2	tr	tr	-	0.4	0.2	-	7.3
Ocimene (21.7 j	5.6	4.2	-	-	3.2	3.0	1.4	-	2.2
Terpinolene (22.5 j	2.6	0.9	0.8	3.5	84.7	71.2	43.1	-	2.8
Hexyl acetate (24.0 j	0.3	0.8	-	-	-	0.4	0.1	-	1.5
cis -3-Hexenyl acetate (25.9 j	-	-	-	-	-	tr	tr	43.8	-
Linalool (36.5 j	59.7	64.8	48.5	33.1	-	16.6	53.2	53.8	8.3
ƒ¿-Terpineol (44.0 j	8.1	6.0	0.3	tr	-	0.7	0.2	tr	1.5
ƒÀ-Selinene (45.5 j	-	-	-	3.6	-	tr	tr	-	3.0
Dehydro-ƒÀ-Ionone (46.0 j	-	-	0.4	-	-	0.2	-	-	11.6
2-Phenylethyl acetate (48.0 j	-	tr	34.5	19.9	-	0.1	-	-	46.0
Benzyl alcohol (50.0 j	tr	tr	9.3	8.1	-	tr	-	-	2.8
2-Phenylehtyl alcohol (55.0 j	0.2	-	-	-	1.4	tr	tr	-	-
ƒÀ-Ionone (55.5 j	0.1	-	-	-	tr	-	-	-	-
Total	91.2	93.0	94.8	69.7	89.5	93.6	98.7	100	96.8

tr: percentage of peak area is less than 0.1 % .
[1]'Rijnveld's Golden Yellow'

Figures

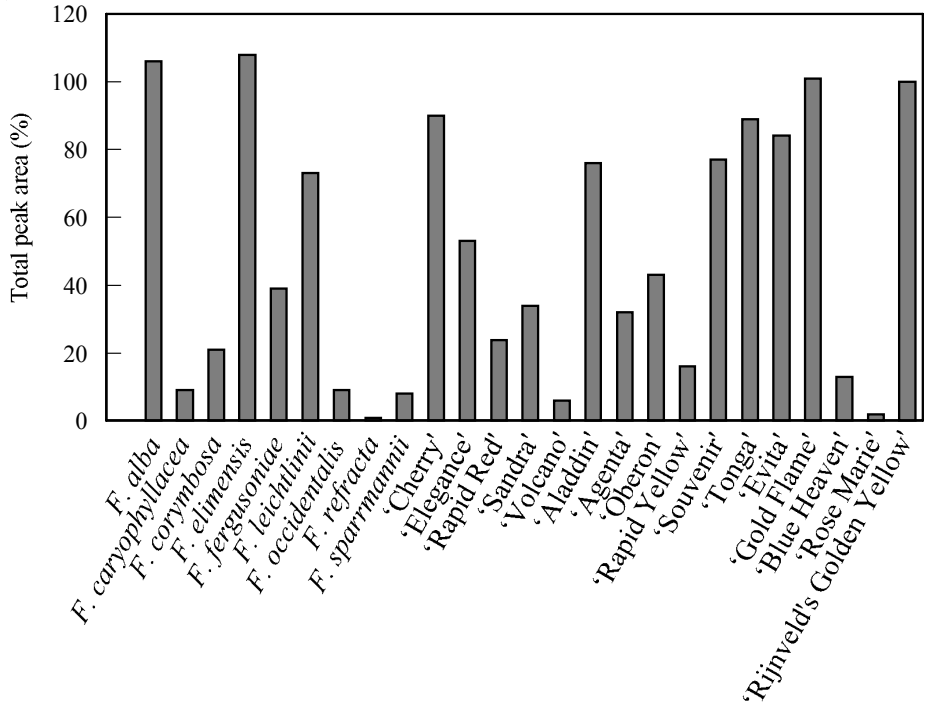

Fig. 1. Total peak areas in gas chromatograms of headspace volatiles of *Freesia* species and cultivars. Values are relative to 'Rijnveld's Golden Yellow' at 100.

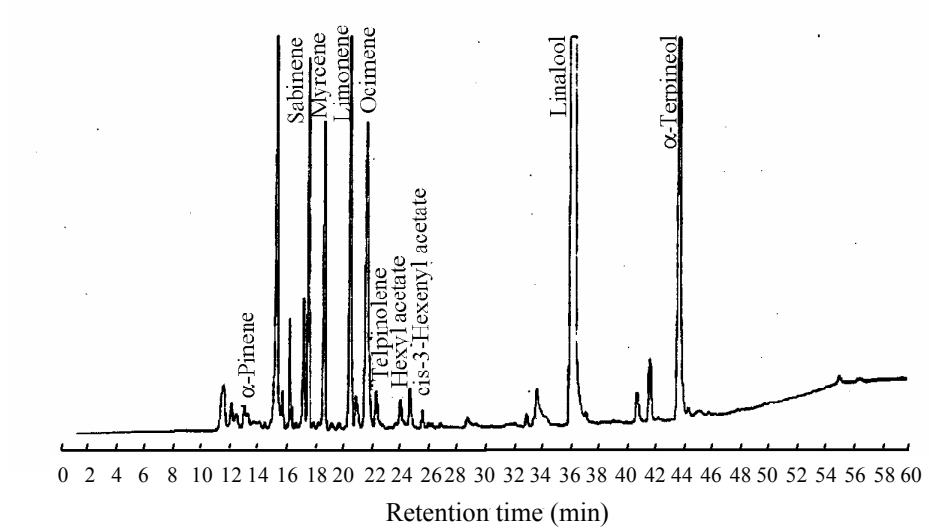

Fig. 2. Gas chromatogram of headspace volatiles of 'Rijnveld's Golden Yellow'.

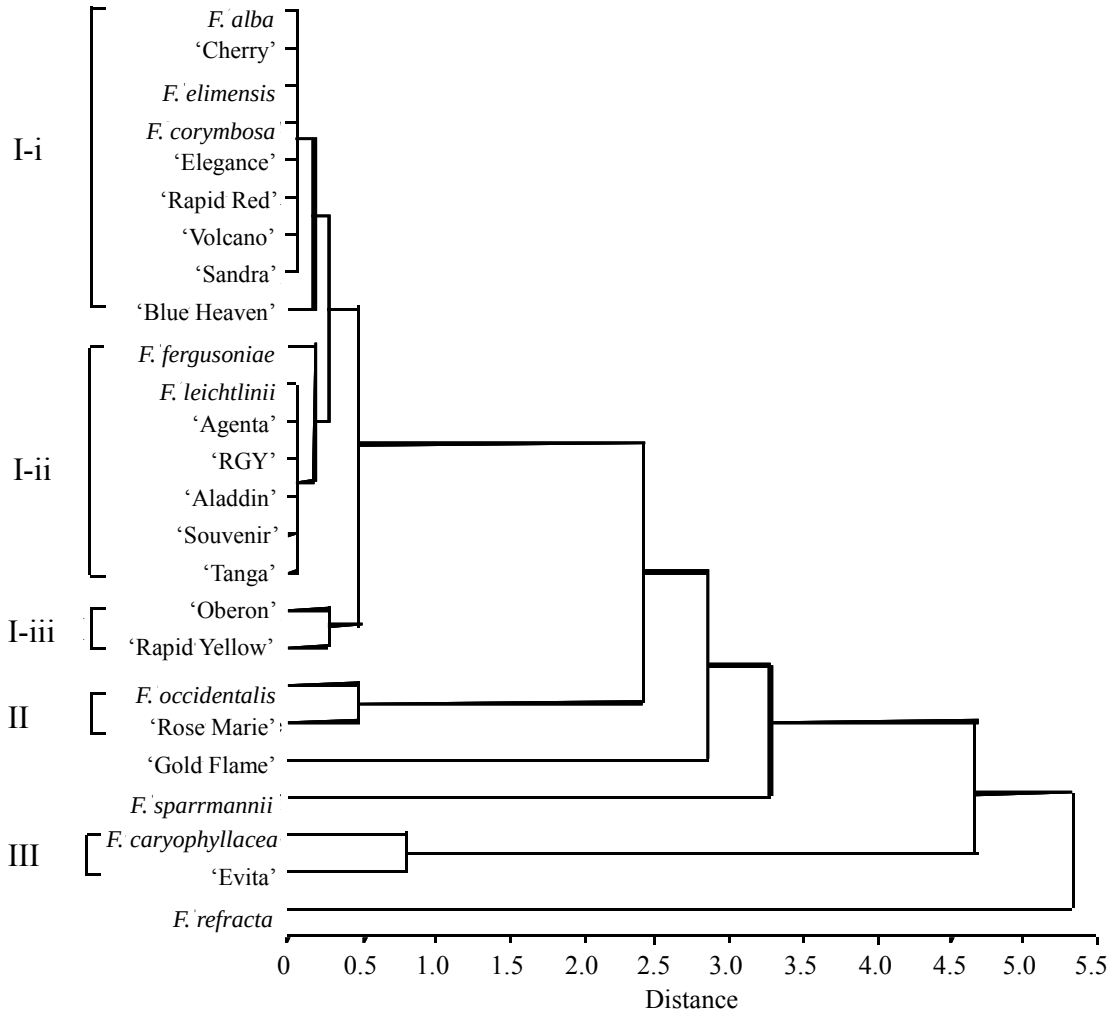

Fig. 3. Cluster analysis based on the volatile composition of 9 species and 16 cultivars of *Freesia*. 'RGY': 'Rijnveld's Golden Yellow'.