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利用 RAPD 研究桂林桂花品种间的亲缘关系

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摘 要：采用随机扩增多态性 DNA(RAPD)技术，从 100 个随机引物中筛选出扩增效果较好的 20 个引物，分析桂林市 23 个桂花品种的基因组多态性。20 个随机引物共检测到 193 个位点，其中多态位点 114 个，占 59.1%。并进行了聚类分析，构建出树状聚类图，将这些品种划分为 4 个品种群，与传统分类学结果一致。结果表明，以基因型而不是以表现型为基础，分析桂花品种间的区别是可能的。该技术为解决桂林市的桂花品种分类问题提供了重要依据。

关键词：RAPD；桂林；桂花；亲缘关系

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Identifying *Osmanthus fragrans* cultivars in Guilin City and evaluating their genetic relationships by RAPD markers

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Abstract: Random amplified polymorphic DNA(RAPD) markers were applied to analyze 23 *Osmanthus fragrans* cultivars in Guilin City. Among the total 193 fragments generated by 20 selected primers(among 100 primers), 114 appeared to be polymorphic(59.1%). Cluster analysis based on the RAPD results was performed and dendrogram was constructed. These cultivars tested by RAPD were divided into 4 cultivar groups. The result was consistent with that from traditional taxonomy analysis. The RAPD study illustrated that it is possible to analyze intra-cultivar variation of *O. fragrans* cultivars on the basis of genotype rather than phenotype and therefore, offered significant evidences in solving taxonomic problem of *O. fragrans* cultivars in Guilin City.

Key words: RAPD; Guilin; *Osmanthus fragrans*; genetic relationship

Osmanthus fragrans originated from China and belonged to *Osmanthus*. As one of ten traditional famous flowers in China, *O. fragrans* is well known because of its sweet smell (Liu *et al.*, 2000). It is widely cultured in middle and northern semitropical areas. Guilin City is rich in *O. fragrans* (Yang *et al.*, 2000). However, there is insufficient study on abundant resources of *O. fragrans* cultivars in Guilin City. In China, *O. fragrans* had been studied since 1940s (Huang *et al.*, 1949) and played an important role in flower culture. In the past, classical approaches for the identification to

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O. fragrans cultivars were based on morphological and physiological traits. Most morphological traits are easily affected by environmental factors. So it is difficult to assess these traits and their evaluation can be subjective considering that most of these cultivars are related(Liu,2000;Obara-Okeyo *et al.*,1998).

In 1995,the use of isozyme analysis to identify *O. fragrans* cultivars in Henan Province was reported(Chen *et al.*, 1995). But the ability of isozymes to identify cultivars was limited due to lack of sufficient polymorphism. Recently,the random amplified polymorphic DNA technique(Welsh *et al.*,1990; Williams *et al.*,1990) based on the polymerase chain reaction(PCR) has been widely used for cultivar identification. The basis of genetic variability is sequence variation. RAPD are molecular markers that samples and reveals sequence variation by differential amplification of DNA fragments(Zhu *et al.*,1999). There was only one report on RAPD analysis in *O. fragrans* collected from Wuhan City(Zhao *et al.*,1999),which illustrated that genetic diversity between *O. fragrans* cultivars could be measured as RAPD markers diversity. Our objective was to analyze genetic relationships among *O. fragrans* cultivars by RAPD technique and classify these cultivars based on RAPD markers. The study of genetic diversity among cultivars will be of significance in germplasm protection and conservation.

1 Materials and methods

1.1 Plant materials

23 *O. fragrans* cultivars were all collected from Heishan Botanical Garden in Guilin City of Guangxi Province. Leaves of these cultivars were dried by Silica gel and put in refrigerator. These cultivars were:(1)*O. fragrans*'Yuanban Jingui';(2)*O. fragrans*'Taoye Jingui';(3)*O. fragrans*'Xiangjingui';(4)*O. fragrans*'Jinlian';(5)*O. fragrans*'Zi'e';(6)*O. fragrans*'Mantianxing';(7)*O. fragrans*'Nongchaoer';(8)*O. fragrans*'Qingyun';(9)*O. fragrans*'Yaotiaoshunü';(10)*O. fragrans*'DayeYingui';(11)*O. fragrans*'Xiaoye Yingui';(12)*O. fragrans*'Ruichi Yingui';(13)*O. fragrans*'Zi Yingui';(14)*O. fragrans*'Ruichi Ziyingui';(15)*O. fragrans*'Taoye Ziyingui';(16)*O. fragrans*'Xiaoye Ziyingui';(17)*O. fragrans*'Meixin';(18)*O. fragrans*'Nanxi Dangui';(19)*O. fragrans*'Guifeihong';(20)*O. fragrans*'Zidangui';(21)*O. fragrans*'Danxin';(22)*O. fragrans*'Sijigui';(23)*O. fragrans*'Yueyuegui'.

1.2 DNA extraction

DNA was extracted from 0.5 g of cold and dry leaf. Leaf tissue was ground to a fine powder in liquid nitrogen,and then placed in a 10 mL eppendorf tube. 3 mL 2×CTAB extraction buffer preheated was added to the tube,mixed gently by inversion and incubate at 65 ℃ in a hot water-bath for 30 min. Then 1 mL(1/3 volume)KAc was added and the eppendorf tube was cooled immediately in ice water for 20 min. An equal volume of 24 chloroform:1 isoamyl alcohol(v/v) was used for extraction for 10 min with gentlly but thoroughly mixing and the phases were seprated by centrifugation at 8 000 r/min for 15 min at a room temperature. Collected the upper water and repeated the CI extraction at 4 ℃. Then transfered the upper aqueous layer to a new 10 mL tube with a wide-bore pipette tip. An 2/3 volume of cold isopropanol was added and mixed properly to precipitate the DNA. Centrifuge at 10 000 r/min for 10 min. Discarded the supernatant,washed the pellet with 1ml 75% ethanol twice. Dried the pellet and dissolved in 200 μL TE. The DNA was stored at -20 ℃.

1.3 RAPD analysis

Random primers,Taq DNA polymerase,dNTP were all bought from Sheng Gong Company of Shanghai. the reaction mixture consisted of 1× buffer,2.25 mM $MgCl_2$,0.15 mM dNTPs,0.2 μM primer,1.0 U Taq DNA polymerase and 50 ng genomic DNA per 20 μL reaction volume. The amplification reaction was performed in GeneAmp PCR system 2400(Perkin Elmer Corp. USA) and pro-

grammed for initial heat denaturation at 94 ℃ for 2 min, 40 cycles of 94 ℃ (50 s), 37 ℃ (1 min), 72 ℃ (2 min) followed by an extension period of 8 min at 72 ℃ and then held at 4 ℃. A negative control PCR tube containing all compoents except genomic DNA was included in all the runs. The amplified fragments were separated on 1.5% agarose gel with ethidium bromide (0.5 μg/mL) in 1 × TAE buffer at 50 V. The gel was visualized by illumination with ultraviolet light and photographed.

1.4 Analysis of cultivar relationships

Each amplification fragment generated by PCR was treated as a unit character and scored as present(1) or absent(0). Genetic distances were calculated between all pairs of entries using Nei's coefficient of genetic distance (Nei *et al.*, 1979): F = 2Xab/(Xa+Xb), P = 1 − F; Where F is the pairwise similarity coefficient, Xa and Xb are the total number of bands in cultivar A and B respectively, Xab is the number of bands shared by A and B, and P is the genetic distance between A and B. A dendrogram was prepared for the relationships among the 23 cultivars based on the genetic distance matrix by SAS computer program.

Table 1　Sequences of 20 random primers and numbers of RAPD markers

Primer No.	Sequence(5'-3')	Numbers of RAPD markers
S22	TGCCGAGCTG	7
S43	GTCGCCGTCA	10
S88	TCACGTCCAC	9
S92	CAGCTCACGA	10
S166	AAGGCGGCAG	12
S193	GTCGTTCCTG	9
S252	TCACCAGCCA	10
S408	TCTGTTCCCC	13
S505	GACCTAGTGG	9
S514	CAGGATTCCC	11
S1142	AATCCGCTGG	10
S1216	CCTTGCGCCT	6
S1340	ACACTCGGCA	8
S1452	AAGAGGGCGT	7
S1495	CACGAACCTC	12
S1515	CCCACACGCA	13
S2025	GGGCCGAACA	11
S2110	GTGACCAGAG	7
S2120	ACCCTGAGGA	9
S2124	GTTCCCGACA	10

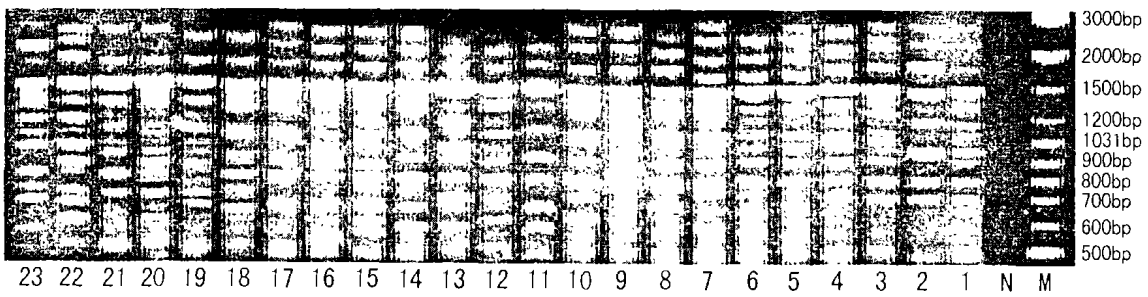

Fig. 1　RAPD patterns amplified by primer S2025

M: DNA marker, 100bp DNA ladder; N: Negative control numbers refer to the corresponding names of *O. fragrans* cultivars.

2　Results and analysis

2.1 Results of DNA amplification

20 random primers were selected from 100 primers because they could amplificate clear, stable and repeatable bands. The total of 193 distanct major RAPD bands, 114 (59.1%) out of which were polymorphic, were consistently generated from 20 primers. Between 5 to 14 bands were scored perprimer with an average of 9.6 bands (Table 1). Sizes of amplified fragments ranged from 400 to 3 000 bp. Different primers had different amplification results. Each cultivar gave unique amplification products to distinguish it from the other tested genotypes when several primers were considered. It fully indicated abundant polymorphism existed between genomes. As shown in Fig. 1, the polymorphism of 23 filaments was very high and the result was stable after being repeated for many times.

2.2 Analysis of phylogenetic relationships among cultivars

Genetic distances derived from pairwise similarity coefficients among the 23 *O. fragrans* culti-

vars are summarized in Table. 2. The values of genetic distance ranged from 0. 054 8 to 0. 576 4, which illustrated that the genetic difference among *O. fragrans* cultivars was very distinct. The smallest genetic distance was found between *O. fragrans*'Zi'e' and *O. fragrans*'Nongchaoer', which had a genetic distance coefficient of 0. 054 8. Obviously, they had close phylogenetic relationship. The remotest relationship took place between *O. fragrans*'Nanxi Dangui' and *O. fragrans*'Yueyuegui' with the genetic distance of 0. 576 4. The relationships between *O. fragrans*'Nanxi Dangui' and *O. fragrans*'Sijigui', *O. fragrans*'Guifeihong' and *O. fragrans*'Yueyuegui', *O. fragrans*'Danxin' and *O. fragrans*'Sijigui' were also remote, whose values of distances were larger than 0. 5.

Table 2 Genetic distances among 23 *O. fragrans* cultivars

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	0.000 0													
2	0.078 2	0.000 0												
3	0.109 7	0.114 5	0.000 0											
4	0.112 9	0.063 1	0.118 1	0.000 0										
5	0.228 5	0.216 2	0.218 3	0.204 7	0.000 0									
6	0.216 1	0.227 5	0.220 3	0.198 7	0.067 9	0.000 0								
7	0.203 7	0.219 6	0.218 3	0.207 7	0.054 8	0.090 4	0.000 0							
8	0.238 2	0.227 8	0.230 6	0.228 1	0.078 4	0.093 7	0.083 2	0.000 0						
9	0.237 1	0.229 7	0.220 8	0.219 2	0.092 1	0.096 2	0.106 5	0.091 7	0.000 0					
10	0.226 5	0.232 7	0.229 1	0.221 7	0.089 3	0.092 1	0.097 3	0.088 6	0.057 2	0.000 0				
11	0.230 6	0.228 2	0.216 1	0.218 4	0.091 6	0.069 3	0.090 2	0.089 1	0.102 7	0.091 3	0.000 0			
12	0.226 8	0.223 6	0.218 5	0.219 7	0.089 0	0.091 4	0.092 5	0.061 7	0.098 2	0.092 1	0.089 4	0.000 0		
13	0.204 8	0.216 7	0.209 9	0.201 6	0.138 2	0.126 1	0.132 7	0.137 8	0.127 3	0.138 4	0.142 7	0.126 8	0.000 0	
14	0.203 7	0.210 8	0.211 7	0.187 3	0.132 1	0.130 9	0.131 4	0.142 3	0.129 1	0.140 2	0.132 5	0.125 7	0.092 9	0.000 0
15	0.210 6	0.217 3	0.209 8	0.190 7	0.128 7	0.139 2	0.125 1	0.128 3	0.130 2	0.146 3	0.131 4	0.158 1	0.098 0	0.097 1
16	0.209 9	0.211 3	0.210 7	0.176 5	0.126 3	0.130 8	0.131 5	0.132 9	0.140 5	0.145 1	0.132 8	0.149 3	0.081 6	0.091 3
17	0.207 5	0.208 1	0.210 4	0.182 5	0.151 7	0.143 4	0.129 3	0.140 7	0.127 1	0.137 8	0.131 2	0.147 8	0.102 7	0.074 8
18	0.187 7	0.180 3	0.196 4	0.179 3	0.312 8	0.332 7	0.323 1	0.318 3	0.330 7	0.328 1	0.319 8	0.320 7	0.327 6	0.319 7
19	0.195 1	0.182 4	0.183 1	0.180 2	0.306 9	0.321 5	0.327 3	0.317 5	0.332 1	0.317 3	0.318 1	0.326 3	0.321 5	0.322 5
20	0.189 3	0.187 1	0.192 4	0.190 7	0.310 3	0.330 8	0.316 7	0.317 8	0.326 9	0.317 4	0.316 5	0.322 5	0.323 7	0.316 8
21	0.181 5	0.180 9	0.179 1	0.152 4	0.300 8	0.318 7	0.317 5	0.309 9	0.324 4	0.309 3	0.318 5	0.319 2	0.313 9	0.318 3
22	0.415 3	0.418 1	0.420 3	0.432 8	0.272 1	0.324 7	0.373 3	0.316 9	0.270 6	0.272 6	0.311 9	0.271 7	0.267 5	0.263 3
23	0.457 1	0.447 3	0.450 2	0.441 9	0.298 2	0.296 1	0.306 4	0.298 5	0.268 3	0.270 4	0.315 3	0.267 4	0.258 1	0.260 2

	15	16	17	18	19	20	21	22	23
15	0.000 0								
16	0.067 8	0.000 0							
17	0.098 5	0.092 8	0.000 0						
18	0.328 4	0.321 7	0.321 3	0.000 0					
19	0.317 9	0.320 8	0.318 8	0.060 3	0.000 0				
20	0.320 7	0.318 3	0.316 4	0.125 8	0.101 7	0.000 0			
21	0.316 7	0.320 1	0.313 9	0.118 2	0.130 1	0.078 2	0.000 0		
22	0.259 9	0.260 2	0.271 4	0.510 3	0.476 2	0.482 9	0.464 9	0.0000	
23	0.256 3	0.254 8	0.269 1	0.576 4	0.567 1	0.570 3	0.5501	0.107 5	0.000 0

The Single Linkage method cluster analysis was carried out by using SAS software based on the genetic distances of 23 samples. A dendrogram was developed(Fig. 2). The 23 *O. fragrans* cultivars were divided into 4 clusters at 0. 15 similarity level. Of all the 4 groups, 3 (*Latifolius* Group, *Thunbergii* Group and *Aurantiacus* Group) were Autumn Division, and the other(*Fragrans* Group) was *Fragrans* Division. Their relationship groups based on the RAPD results were basically in accord on the traditional taxonomy. So RAPD analysis worked efficiently in this study.

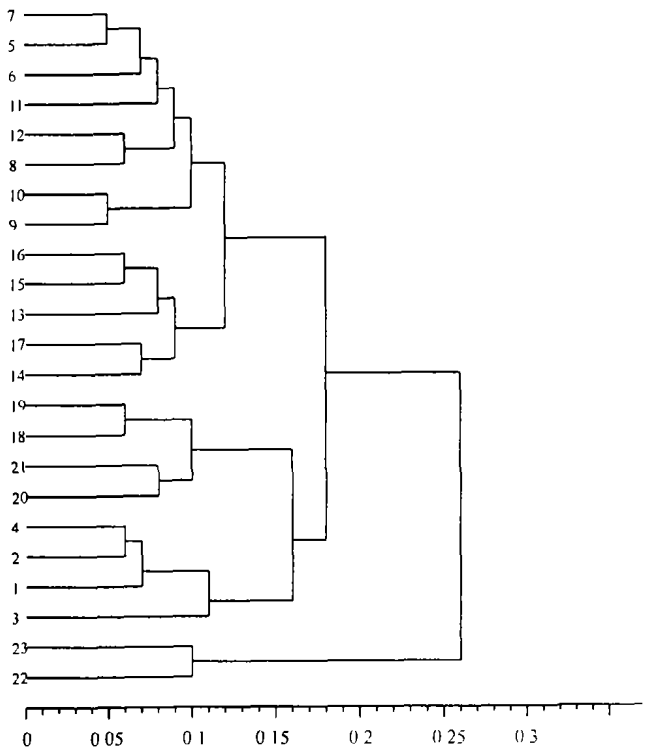

Fig. 2 Dendrogram based on RAPD markers amplified from 23 *O. fragrans* cultivars

3 Discussions

Using the inforescence types and blossoming habits as the first criterion, and the flower colors as the second one, Xiang Qi-bai *et al.* proposed a system in which two divisions and four groups were recognized, namely, Fragrans Division and Autumn Division. Fragrans Division is more primitive than Autumn Division, while the evolutionary sequence from primitive to advanced in Autumn Division is *Latifolius* Group, *Thunbergii* Group and *Aurantiacus* Group(Zang *et al.*, 2002). Although our conclusions in this study held out their viewpoints, there was difference about cultivar divisions in cultivar group. As it was shown in the dengrogram, cultivar of *O. fragrans*'Taoye Jingui' and *O. fragrans*'Jinlian'was clustered at 0.063 1. The result showed the closer relationship exited between the two cultivars. As far as physiological traits are concerned, the ovary of *O. fragrans*'Taoye Jingui' is sterile, but O. *fragrans*'Jinlian'is fertile. This trait is regarded as the main basis in classification of *O. fragrans* cultivars. However, according to our results, the genetic distance between them is very small. Maybe the samples tested in our study is insufficient, and if we have enough samples, we will obtain a large number of genotype and offer more foundation for analysis of genetic relationships among *O. fragrans* cultivars.

RAPD is now widely used in the study of plant systematic evolution, phylogenetic relationship and genetic polymorphism(Lu *et al.*, 2002). Although the stability and reliability of RAPD technique is suspected, We think if reaction conditions are optimized and all reagents are fixed and guaranteed same in all the runs, the stable and repeatable results will be easily obtained. Our results have shown that RAPD based classification of *O. fragrans* cultivars is an alternative and complementary approach to the traditional methods for studying *O. fragrans*.

References:

Liu YL, Xiang QB. 2000. studies on the classification of *Osmanthus fragrans* cultivars[A]. 20 years of science and technology development of Chinese flowers[C]. *Science Press*, 1: 631－642. (in chinese)

Yang KM, Zhu WJ. 2000. *Osmanthus fragrans* Lour.[M]. Shanghai: Science and Technology Press, 8－12. (in chinese)

Huang YY, Huang DL. 1949. Theories of flowers[M]. Shanghai: New century press. (in chinese)

Liu YL. 2000. Studies on resources and classification of *Osmanthus fragrans* cultivars[J]. *J Jiangsu For Sci and Tech*, **27**: 27－31. (in chinese)

P. Obara-Okeyo & S. Kako. Genetic diversity and identification of *Cymbidium* cultivars as measured by random amplified polymorphic DNA (RAPD) markers[J]. *Euphyica*, 1998, **99**: 95－101.

Chen JY, Ning YX, Zhao CH, *et al.* 1995. Studies on the isoperoxidase of *Osmanthus fragrans* varieties in Henan[J]. *Acta Hort Sin*, **22**(2): 176－180. (in chinese)

Zhu C, Liu FY. 1999. To apply RAPD in classification and differentiation of cultivar of *Osmanthus fragrans* Lour.[J]. *Guihaia*, **19**(2): 190－192. (in chinese)

Zhao XL, Yao CH. 1999. Preliminary RAPD analysis of *Osmanthus fragrans* cultivars[J]. *J Huazhong Agr Univ*, **18**

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续表 1

种名 Species	习性及用途 Habit and purpose	种名 Species	习性及用途 Habit and purpose
鸡矢藤 *Paederia cavaleriei*	缠绕,垣,常绿	光叶叶子花 *Bougainvillea glabra*	缠绕,廊架,常绿观花
广西鸡矢藤 *P. pertomentosa*	缠绕,垣,常绿	叶子花 *B. spectabilis*	缠绕,廊架,常绿观花
毛鸡矢藤 *P. scandens* cv. *Tomentosa*	缠绕,垣,常绿	羽叶喜林芋 *Philodendron bipinnatifidum*	气生根,岩垣,常绿
毛钩藤 *Uncaria hirsute*	缠绕,廊架,常绿	深裂喜林芋 *P. elgans*	气生根,岩垣,常绿
大叶钩藤 *U. macrophylla*	缠绕,廊架,常绿	红柄喜林芋 *P. erubescens*	气生根,岩垣,常绿
钩藤 *U. rhynchophylla*	缠绕,廊架,常绿	缎叶喜林芋 *P. sloriosum*	气生根,岩垣,常绿
华钩藤 *U. sinensis*	缠绕,廊架,常绿	黑金喜林芋 *P. martianum*	气生根,岩垣,常绿
马兜铃 *Aristolochia debilis*	缠绕,廊架,常绿	攀援喜林芋 *P. scandens*	气生根,岩垣,常绿
海南马兜铃 *A. hainanensis*	缠绕,廊架,常绿	喜林芋 *P. imbe*	气生根,岩垣,常绿
大叶马兜铃 *A. shakangii*	缠绕,廊架,常绿	大花清明花 *Beaumontia grandiflora*	缠绕,廊架,常绿观花
大血藤 *Sargentodoxa cuneata*	缠绕,廊架,常绿	白花鱼藤 *Derris alborubra*	缠绕,廊架,观叶果
毛叶轮环藤 *Cyclea barbarta*	缠绕,垣,常绿	毛鱼藤 *D. elliptica*	缠绕,廊架,观叶果
密花轮环藤 *C. densiflora*	缠绕,垣,常绿	锈毛鱼藤 *D. ferruginea*	缠绕,廊架,观叶果
海南轮环藤 *C. harnanensis*	缠绕,垣,常绿	亮叶揭阳鱼藤 *D. fordii* var. *lucida*	缠绕,廊架,观叶果
四川轮环藤 *C. sutchuenensis*	缠绕,垣,常绿	揭阳鱼藤 *D. fordii*	缠绕,廊架,观叶果
轮环藤 *C. racemosa*	缠绕,垣,常绿	粉叶鱼藤 *D. glauca*	缠绕,廊架,观叶果
广西轮环藤 *C. sutchuenensis* cv. *Sessilis*	缠绕,垣,常绿	边荚鱼藤 *D. marginata*	缠绕,廊架,观叶果
金钱吊乌龟 *Stephania cepharantha*	缠绕,垣,常绿	密锥花鱼藤 *D. thyrsiflora*	缠绕,廊架,观叶果
千斤藤 *S. hernandifolia*	缠绕,垣,常绿	黔桂鱼藤 *D. tonkinensis*	缠绕,廊架,观叶果
华千斤藤 S. sinica	缠绕,垣,常绿		

3.10 使君子 *Quiqualis indica* L.

属使君子科使君子属落叶蔓性木质藤本。叶对生,倒卵状椭圆形,先端尖基部圆。穗状花序,悬垂状,顶生或腋生,夏季开花,初时粉白色后转桃红色,花期长达 2～3 个月。生性强健,蔓性力强,春夏枝叶浓密,为花廊、拱门、围篱或荫棚美化良材。

参考文献:

广西植物研究所. 1972. 广西植物名录[M].

广西植物研究所. 1986. 桂林植物园栽培植物名录[M].

钟济新. 1982. 广西石灰岩石山植物图谱[M]. 南宁: 广西人民出版社出版.

臧德奎, 周树军. 2000. 中国园林[M]. 北京: 中国园林期刊社出版, 79－81.

覃海宁, 方 鼎. 2003. 广西那坡县种子植物名录[M]. 北京: 中国科学技术出版社.

徐筱昌. 1999. 发展垂直绿化,增加城市绿量[J]. 中国园林, (2): 49－50.

陆明珍. 1986. 浅谈垂直绿化[J]. 园林, (2): 13.

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(5): 484－487. (in chinese)

Zang DK, Xiang QB, Liu YL. 2002. A study on the origin and evolution of Sweet *Osmanthus cultivars*[C]. Sweet Osmanthus[Ⅱ], Jilin Science and Technology Press, 1－12. (in chinese)

Lu LD, Xiong L, Gao WJ, *et al*. 2002. RAPD analysis in 12 species of Allium[J]. *Acta Bot Boreal-Occident sin*, **22**(5): 1 086－1 092.

GUIDET F. 1994. A powerful new technique to quickly prepare hundreds of plant extracts for PCR and RAPD analysis [J]. *Nucleic Acids Res*, **22**(9): 1 772.

GRAHAM GOC, MAYERS P, HENRY RJ. 1994. A simplified method for the preparation of fungal genomic DNA for PCR and RAPD analysis[J]. *Biotechniques*, **16**: 48－50.

Malyshew SV, Kartel NA. 1997. Molecular markers in mapping of plant genomes[J]. *Mol Biol*, **31**: 163－171.