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Assessment of genetic diversity and relationships among *Osmanthus fragrans* cultivars using AFLP markers

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Abstract This study was conducted to reveal genetic diversity among 100 *Osmanthus fragrans* cultivars using amplified fragment length polymorphism (AFLP) markers. Eight AFLP primer combinations produced a total of 443 polymorphic fragments with an average of 64 per primer combination. The percentage of polymorphic bands (86.81%), the resolving power (Rp) (32.71) and the PIC values (0.331) showed the efficiency of used primer combinations. The revealed AFLP makers were effective in distinguishing all the cultivars considered. Cluster analysis were performed to assess patterns of diversity among cultivars and showed the abundant genetic diversity. The overall distribution pattern of molecular variation suggested that 93.33% of the total genetic variance was within the identified groups and 6.67% of the genetic variation was among the identified groups. Our results showed that AFLP markers are useful for *Osmanthus fragrans* germplasm discrimination as well as for investigation of genetic diversity and variation. The information will facilitate germplasm identification, conservation and new cultivar development.

Keywords: AFLP marker, cluster analysis, genetic diversity, *Osmanthus fragrans*

INTRODUCTION

Osmanthus fragrans, a species of Oleaceae family (Ômura et al. 2000), has been cultivated for more than 2,500 years in China. As a traditional horticultural plant, *O. fragrans* is valued for both its beauty and fragrance. The fragrance of *O. fragrans* contains as many as 35 potentially exploitable chemical substances (Wang et al. 2009). This plant also has medicinal value. According to *Compendium of Materia Medica*, the root of *O. fragrans* can be used to cure toothache. *O. fragrans* flowers are extensively used in Chinese cuisine. In 2004, the ISHS (International Society for Horticultural Science) appointed the Sweet Osmanthus branch of the Chinese Flower Association as International Cultivar Registration Authority (ICRA) for the genus *Osmanthus*. There are 122 registered *O. fragrans* cultivars (Xiang and Liu, 2008) growing mainly in the northern and middle subtropics between the Qinling and Nanling Mountains. The major centers of *O. fragrans* cultivation in China are Wu County of Jiangsu Province, Hangzhou of Zhejiang Province, Xianning of Hubei Province, Chengdu of Sichuan Province, and Guilin of Guangxi Province. In recent years, global warming has allowed a northward range expansion of *O. fragrans*.

Since the mid-twentieth century, *O. fragrans* cultivars have been investigated and classified into Asiaticus and autumn divisions based on flowering season; the autumn division was further divided into three groups (Albus group, Luteus group, and Aurantiacus group) according to flower and leaf traits. Liu (1985) categorized *O. fragrans* cultivars into four groups (Asiaticus group, Albus group, Luteus group, and Aurantiacus group) on phenological and morphological traits. Liu's classification procedure is the most detailed and has gained widespread acceptance. Using this procedure, 18 cultivars of the Asiaticus group, 57 of the Albus group, 36 of the Luteus group and 43 of the Aurantiacus group were identified in a nationwide investigation. Xiang and Liu (2008) published 'An Illustrated Guide to *Osmanthus fragrans* Cultivars of China', which recorded 122 *O. fragrans* cultivars and provided detailed characteristics of these cultivars.

Table 1. Materials used in this study.

Code	Cultivar name	Collection site	Code	Cultivar name	Collection site
1	Sijigui	Shanghai	51	Moye Jingui	Guilin, Guangxi
2	Yueyuegui	Shanghai	52	Jinqiugui	Nanjing, Jiangsu
3	Rixianggui	Shanghai	53	Chenghuan Jingui	Nanjing, Jiangsu
4	Daye Sijigui	Shanghai	54	Zaojingui	Xianning, Hubei
5	Fodingzhu	Shanghai	55	Heishan Jingui	Guilin, Guangxi
6	Jinyetianxiang	Jinhua, Zhejiang	56	Dahua Jingui	Hangzhou, Zhejiang
7	Xiaoye Sijigui	Shanghai	57	Yuanban Jingui	Guilin, Guangxi
8	Tianxiangtaige	Shanghai	58	Huangchuan Jingui	Xianning, Hubei
9	Huangjin	Nanjing, Jiangsu	59	Taoyehuang	Hangzhou, Zhejiang
10	Dongxianghong	Hangzhou, Zhejiang	60	Chuizhihuang	Hangzhou, Zhejiang
11	Xiaoronghuang	Nanjing, Jiangsu	61	Hangzhouhuang	Hangzhou, Zhejiang
12	Tiannvsanhua	Hangzhou, Zhejiang	62	Lianye Jingui	Hangzhou, Zhejiang
13	Xiaoyefodingzhu	Nanjing, Jiangsu	63	Wanjingui	Hangzhou, Zhejiang
14	Boye Yingui	Hangzhou, Zhejiang	64	Wanxin	Nanjing, Jiangsu
15	Mihua Ziyingui	Nanjing, Jiangsu	65	Ziyun	Guilin, Guangxi
16	Yinxing	Xianning, Hubei	66	Baozhu	Guilin, Guangxi
17	Changgengbai	Xianning, Hubei	67	Zhuguan Zijingui	Nanjing, Jiangsu
18	Jiangnanliren	Xianning, Hubei	68	Liuyesugui	Hangzhou, Zhejiang
19	Dahua Zaoyingui	Nanjing, Jiangsu	69	Duoya Jingui	Shanghai
20	Zigeng Ziyingui	Nanjing, Jiangsu	70	Dayehuang	Nanjing, Jiangsu
21	Yulinglong	Nanjing, Jiangsu	71	Yuhuahuang	Nanjing, Jiangsu
22	Xiaye Wanyingui	Nanjing, Jiangsu	72	Boye Jingui	Hangzhou, Zhejiang
23	Zaoyingui	Shanghai	73	Wandianjin	Hangzhou, Zhejiang
24	Liuyegui	Shanghai	74	Congzhongxiao	Hangzhou, Zhejiang
25	Baijie	Shanghai	75	Zuiyun	Xianning, Hubei
26	Jiulonggui	Shanghai	76	Mantiaohong	Hangzhou, Zhejiang
27	Wanyingui	Shanghai	77	Gecheng Dangui	Xianning, Hubei
28	Yulianyinsi	Hangzhou, Zhejiang	78	Chiye Dangui	Xianning, Hubei
29	Changyebizhu	Hangzhou, Zhejiang	79	Zaohong	Chengdu, Sichuan
30	Daye Yinggui	Hangzhou, Zhejiang	80	Honghai	Chengdu, Sichuan
31	Juanban Ziyingui	Hangzhou, Zhejiang	81	Xionghuanggui	Nanjing, Jiangsu
32	Duoban Ziyingui	Nanjing, Jiangsu	82	Yingye Dangui	Shanghai
33	Ziyingui	Shanghai	83	Shanghai Dangui	Shanghai
34	Yangmeiye Yingui	Hangzhou, Zhejiang	84	Jiaorong	Hangzhou, Zhejiang
35	Ehuang	Hangzhou, Zhejiang	85	Xiaoyan	Hangzhou, Zhejiang
36	Xiangyun	Hangzhou, Zhejiang	86	Zuijihong	Guilin, Guangxi
37	Kuoye Zaoyingui	Nanjing, Jiangsu	87	Suzhouhong	Nanjing, Jiangsu
38	Chuanyinqiu	Nanjing, Jiangsu	88	Zhusha Dangui	Shanghai
39	Xizi Yingui	Nanjing, Jiangsu	89	Suzhouqiancheng	Nanjing, Jiangsu
40	Zaohuang	Shanghai	90	Yuhuahong	Nanjing, Jiangsu
41	Mantianxing	Nanjing, Jiangsu	91	Danhongcuizhou	Nanjing, Jiangsu
42	Xiaojiabiyu	Guilin, Guangxi	92	Taoye Dangui	Nanjing, Jiangsu
43	Zie	Guilin, Guangxi	93	Hangzhou Dangui	Nanjing, Jiangsu
44	Xiaoye Yingui	Guilin, Guangxi	94	Pucheng Dangui	Nanjing, Jiangsu
45	Susheng Jingui	Chengdu, Sichuan	95	Zhuangyuanhong	Hangzhou, Zhejiang
46	Daye Zijingui	Nanjing, Jiangsu	96	Hongyanningxiang	Hangzhou, Zhejiang
47	Qiugui	Xianning, Huibei	97	Xiaoye Dangui	Hangzhou, Zhejiang
48	Jinhuataige	Xianning, Huibei	98	Chenghong Dangui	Hangzhou, Zhejiang
49	Zaozihuang	Hangzhou, Zhejiang	99	Zidangui	Hangzhou, Zhejiang
50	Jinshigui	Nanjing, Jiangsu	100	Yunnan Dangui	Kunming, Yunnan

1～13, Asiaticus group; 14～44, Albus group; 45～75, Luteus group; 76～100, Aurantiacus group (Xiang and Liu, 2008).

Genetic diversity study of *O. fragrans* on the molecular level has advanced significantly due to the rapid development of molecular biology. The results of a RAPD analysis of some Wuhan cultivars appeared to support the traditional morphology-based classification criteria, demonstrating high levels of genetic diversity of *O. fragrans*. Employing RAPD and ISSR-PCR techniques, several recent studies presented similar results. For example, Li et al. (2009) used the SRAP technique to analyze *O. fragrans* cultivars; the results indicate that many *O. fragrans* Albus cultivars are highly mutated, and some Luteus cultivars with deeper-colored flowers are closely related to certain members of the Aurantiacus group. Using Amplified fragment length polymorphism (AFLP) markers, Yan et al. (2009) analyzed some *O. fragrans* cultivars. Because of less cultivars used and only a few certified by International Cultivar Registration Authority (ICRA), the results of the previous studies are limited in reveal the genetic diversity of *O. fragrans*.

We report here the genetic diversity of *O. fragrans* cultivars based on fluoresence-based AFLP makers. The two main objectives for germplasm management are: (1) to explore an effective mean of *O. fragrans* cultivars identification, (2) to establish of genetic database, which could provide molecular evidence for selection and breeding of the cultivars and genetic relationship in *O. fragrans.*

MATERIALS AND METHODS

Plant materials and DNA extraction

This study used a total of 100 samples collected from the main cultivated land in China. The samples consisted of 13 cultivars in Asiaticus group, 31 cultivars in Albus group, 31 cultivars in Luteus group, and 25 cultivars in Aurantiacus group (Table 1). Total cellular DNA was extracted from frozen leaves sampled from each accession following the procedure described by Elameen et al. (2008). After purification, the DNA concentration was estimated using the spectrophotometer, then diluted to 100 ng/µl before used.

AFLP analysis

AFLP analysis was carried out based on the protocol described by Bonants et al. (2000). Genomic DNA (250 ng) was digested with PstI and MseI (2.5 U each) (Fermentas) in a final volume of 20 ml for 4 hrs at 37ºC and then the adapters were ligated to the digested DNA fragments. A preselective amplification using the primer pair PstI+G/MseI+C was performed using the following cycling parameters: 94ºC for 4 min; 30 cycles of 94ºC for 30 sec, 56ºC for 30 sec, 72ºC for 60 sec and 72ºC for 10 min. The 40-fold diluted preamplified DNA was amplified using the PstI+3 and MseI+3 primer pairs. The MseI+3 primers were labelled with a 6-FAM fluorescent dye. Eight primer combinations were used for the selective amplifications (Table 2). Amplification was conducted for 1 cycle of 95ºC for 30 sec, 65ºC for 40 sec and 72ºC for 60 sec, then lowering the annealing temperature by 0.7ºC decrements for 12 cycles; and then 23 cycles of 95ºC for 30 sec, 56ºC for 40 sec and 72ºC for 60 sec.

The amplified products from selective amplifications were loaded and run on the ABI PRISM 377 DNA Sequencer (Applied Biosystems). Data were analyzed using GENESCAN 3.1 software.

Data analysis

The efficiency of each primer combinations was estimated by the number of bands, and the polymorphic bands were used to calculate the percentage of polymorphic bands (PPB). The estimation of the resolving power (Rp) allowed the evaluation of the ability of the most informative primers to differentiate between cultivars, Rp = ∑Ib; where: Ib=1-(2 x | 0.5-*p* |) and *p* is the accessions proportion containing the I band (Ben Tamarzizt et al. 2009). Moreover, the discriminating power of derived markers was calculated by the assessment of the polymorphism information content (PIC) using the following formula: $\mathrm{PIC} = 1 - \sum_{i=1}^{k} p_i^2$, where P_i is the frequency of the *i*th allele in the set of genotypes investigated (Muthusamy et al. 2008; Uzun et al. 2009).

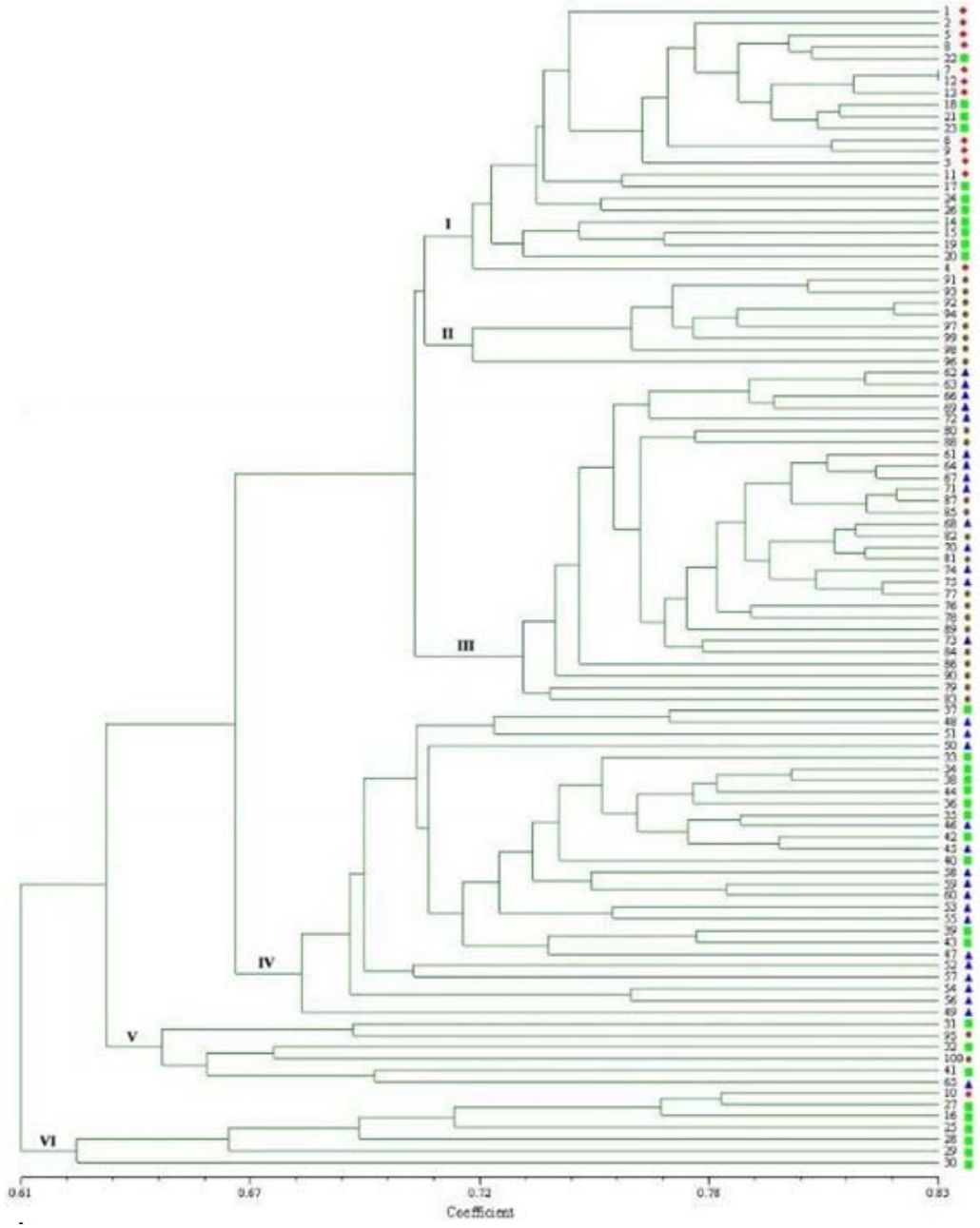

Fig. 1 Dendrogram of 100 *O. fragrans* cultivars resulting from UPGMA cluster analysis based on 443 AFLP makers. ● Asiaticus group, ■ Albus group, ▲ Luteus group, ● Aurantiacus group.

AFLP amplified fragments were scored as present (1) or absent (0) and entered into a binary qualitative data matrix. The presence/absence data matrix of the AFLP phenotypes was analyzed using the NTSYS-pc software package (Rohlf, 2000). A similarity matrix was constructed based on Dice's coefficient. The similarity matrix was used to construct a dendrogram using the unweighted pair group method arithmetic average (UPGMA) to determine genetic relationships among the germplasm studied.

The analysis of molecular variance (AMOVA) was performed. The variance components from the analysis were used to estimate the groups' subdivisions (F-statistics) among cultivars from the four

groups (F_{ST}). $F_{ST} = V_{AP}/ (V_{AP} + V_{WP})$, where V_{AP} is the variance among groups, V_{WP} is the variance within groups (Peakall and Smouse, 2006).

RESULTS

AFLP makers and polymorphism

The 8 primer combinations, generating clear-cut AFLP profiles, resulted in a total of 514 different amplification products ranging from 50 to 500 bp in size with 443 polymorphic AFLP bands for 100 *O. fragrans* cultivars. The average of polymorphic bands per AFLP primer combination was 55.375 (Table 2) and each primer pair could distinguish all the cultivars considered. The largest number of polymorphic bands (66) was produced with primer combination P_{GAA}/M_{CTG}, and the least number of polymorphic bands (36) was detected using primer combination P_{GAC}/M_{CAG}. The percentage of polymorphic bands ranged from 80% for P_{GAC}/M_{CAG} to 95.65% for P_{GAA}/M_{CTG} primer combinations with a mean of 86.81% (Table 2). Thus, we assumed that all the tested primers were powerful to detect DNA polymophisms in *O. fragrans* cultivars. Estimates of the resolving power (Rp) showed a high rate of collective Rp, ranging from 16.18 to 39.26, with an average of 32.71 (Table 2). In addition, the polymorphism information content values varied from 0.245 to 0.393, 6 primer combinations used had a PIC exceeding 0.3, with a mean of 0.331, demonstrating a good discriminatory power of the primer combinations (Table 2). Therefore, data proved that AFLP was a powerful procedure to survey *O. fragrans* cultivars diversity.

Cluster analysis among cultivars

Based on the 443 AFLP markers, the level of similarity determined by the Dice's coefficients ranged from 0.294 to 0.780 with a mean of 0.562, suggesting that the cultivars in this study were characterized by a great similarity at DNA level. The lowest Dice's coefficient value of 0.294 has been scored between 'Changyebizhu' and 'Jiaorong'. However, 'Xiaoye Sijigui' and 'Tiannvsanhua' were most similarly, with the highest Dice's coefficient of 0.780. All the other ones have displayed different intermediate levels of genetic similarity.

Table 2. Summary of AFLP primer combination characteristics.

PC	NTF	NPF	PPB(%)	PIC	Rp
P_{GAA}/M_{CAG}	72	64	88.89	0.338	37.30
P_{GAA}/ M_{CTG}	69	66	95.65	0.365	39.26
P_{GAC}/ M_{CAC}	58	50	86.21	0.378	34.84
P_{GAC}/M_{CAG}	45	36	80	0.245	16.18
P_{GAC}/M_{CTA}	61	52	85.25	0.312	29.14
P_{GAC}/ M_{CTC}	63	58	92.06	0.393	38.06
P_{GAC}/M_{CTG}	81	65	80.25	0.269	31.44
P_{GAC}/M_{CTT}	65	56	86.15	0.350	35.44
Total	514	443			261.66
Average	64.25	55.375	86.81	0.331	32.71

*,** and ns = significantly different at $P \leq 0.05$ and 0.01, respectively and not significant at $P \leq 0.05$, according to Student's unpaired t-test.

The dendrogram constructed using UPGMA cluster analysis (Figure 1) has clustered the 100 cultivars into six major clusters. The first cluster, labeled I, was dominated with 12 Asiaticus cultivars and 11 Albus cultivars. The second and the third clusters, II and III, consisted of 22 Aurantiacus and 14 Luteus cultivars. Cluster IV, V, VI were composed of the remaining cultivars, including 20 Albus cultivars, 17 Luteus cultivars, 3 Aurantiacus cultivars and 1 Asiaticus cultivar.

Analysis of molecular variance (AMOVA)

Within and between groups of cultivars, molecular variation was estimated using the AMOVA procedure. Total variance was partitioned into components due to differentiation within and among the four *O. fragrans* cultivar groups. The overall distribution pattern of molecular variation within the cultivars suggested that approximately 93.33% of the total variance could be accounted for by the within groups component of variance. The remaining 6.67% of the variation was founded among groups. The variance component was shown to be highly significant ($P < 0.001$). The number of permutations for significance testing was set at 1000 for analysis (Table 3).

DISCUSSION

Information on genetic diversity and variation of germplasm is important for taxonomist, germplasm identification and new cultivar development (Demir et al. 2010). Morphological characters are often susceptible to environmental influences which may result in morphological differences between the same cultivar (Sarkhosh et al. 2009). Clear and detailed assessment of diversity within cultivars can be obtained by using molecular techniques. The variety discrimination through DNA profiles is useful for the protection and conservation of the genetic resources (Piña-Escutia et al. 2010).

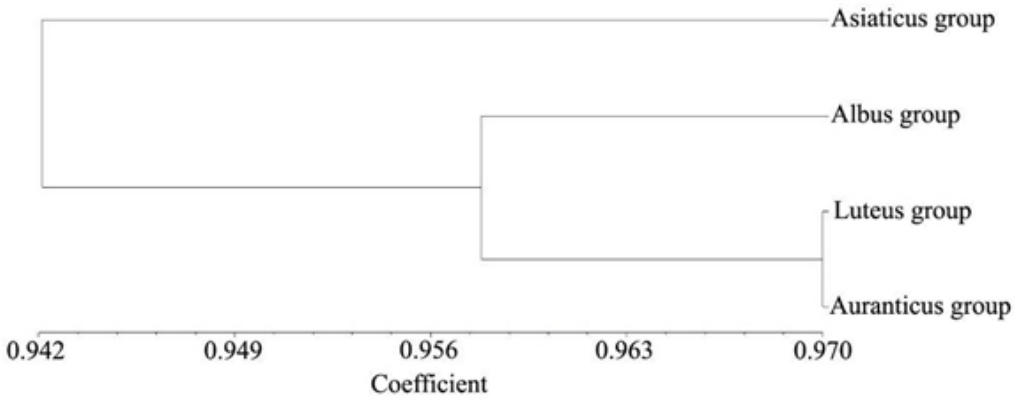

Fig. 2 Dendrogram of four *O. fragrans* cultivargroups resulting from UPGMA cluster analysis.

In the present work, AFLP technique has been used to analyze genetic diversity and relationships within the four groups. The majority of these 100 cultivars have never been considered for AFLP molecular marker studies. A total of 8 primer combinations were used in this study. Each primer combination was able to discriminate all the 100 cultivars studied. The high efficiency of primers in the genetic diversity was also supported by the measurement of resolving power and PIC value. The higher value of Rp and PIC belonged to the primer pairs P_{GAA}/ M_{CTG} and P_{GAC}/ M_{CTC} (39.26 and 0.393, respectively; Table 2), indicating a better distinction of the cultivars. These results also confirmed the utility of the Rp and PIC as a measure of the capacity of primer combination to discriminate among closely related individuals as pointed out by Prevost and Wilkinson, (1999) and Escandón et al. (2007).

According to flower and leaf traits, *O. fragrans* cutilvars can be divided into four groups, Asiaticus group, Albus group, Leutus group and Aurantiacus group (Liu, 1985). The Albus group, caespitose shrub, flower mainly in spring and autumn, with paniculiform inflorescences in spring and cymose inflorescences in autumn. The other 3 groups are big shrub or tree, with cymose inflorescences and

only flower in autumn. Flower color of the three groups differ substantially, with white, greenish white to yellowish white color (RHS-CC 1~8) in Albus group, yellow to golden yellow color (RHS-CC 9~20) in Leutus group and light orange-yellow to orange red (RHS-CC 21~30) in Aurantiacus group. In this study, the UPGMA cluster (Figure 1) showed the distribution of cultivars closeness among varieties with similar flower color. Cultivars in Albus group with light yellowish white flower color (18, 21, 22, 23, etc.) clustered together with Asiaticus group germplasms (Cluster I), showing a close genetic relationship between the two groups. Some Albus group cultivars (34, 35, 36, 38, etc.) and Luteus group cultivars (45, 46, 58, 59, etc.) clustered in cluster IV based on the similarity of the flower color (yellowish white to yellow). Cultivars in Leteus group with golden yellow flower color (61, 64, 67, 71, etc.) often clustered together with Aurantiacus group (Cluster III). Cluster V included 7 cultivars of three groups, showing the closeness among these cultivars. The other 2 clusters, II and VI were only consisted of Aurantiacus group cultivars and Albus group cultivars, respectively. The cluster analysis showed that there were close genetic relationships among cultivars with similar flower color and the flower color could be used as a good criterion of classification in discriminating *O. fragrans* germplasms. Our results are in agreement with some molecular marker study (Li et al. 2009; Yan et al. 2009), but only partially consistent with the traditional classification based on morphological characters. This might have been caused by that morphological characters are often under severe selection pressure which may result in morphological differences between the same cultivar, and AFLP markers are generally less susceptible to environmental impact.

Table 3. Analysis of molecular variance (AMOVA) applied on molecular data.

Source of variation	Df	Sum of squares	Mean squares	Variance components	Percentage of variation	P
Among Groups	3	670.195	223.398	5.836	6.67%	<0.001
Within Groups	96	7842.805	81.696	81.696	93.33%	<0.001
Total	99	8513.000		87.532	F_{ST}=0.067	

Significance tests with 1000 permutations.

Dendrogram of four *O. fragrans* cultivar groups resulting from UPGMA cluster analysis (Figure 2), based on Dice's coefficient, showed that Luteus group and Aurantiacus group had the closest genetic relationship, and Asiaticus group had a distant relationship with the other three groups. The results were consistent with those of a previous study (Li et al. 2009).

The percentage of variations within group was, consequently, larger than that among groups. These findings suggested the presence of considerable cultivar variation among *O. fragrans* germplasm. The low divergence scored among the groups and the large variability detected within groups could be explained by the occurrence of gene flow in the natural populations from which cultivars originated and the reproduction mode. In fact, life form and breeding systems can have significant influences on the genetic variation and its partitioning (Salhi-Hannachi et al. 2005). Additionally, the F_{ST} values between cultivar groups ranged from 0.0280 to 0.1340 (Table 4). The greatest F_{ST} values were between Asiaticus and Luteus groups, and the lowest between Luteus and Aurantiacus groups. The F_{ST} value among overall groups was marginally lower (0.067), which indicated a lower degree of genetic differentiation.

Table 4. Groups pairwise F_{ST} values (below diagonal) and Coefficients values (above diagonal).

Group	Asiaticus group	Albus group	Luteus group	Aurantiacus group
Asiaticus group	****	0.9538	0.9322	0.9397
Albus group	0.0950	****	0.9676	0.9476
Luteus group	0.1340	0.0390	****	0.9698
Aurantiacus group	0.1080	0.0660	0.0280	****

The current study demonstrated that AFLP is an effective approach to assess genetic diversity and was a powerful method to discriminate *O. fragrans* cultivars. The information may be useful for future germplasm identification, conservation and new cultivar development.

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REFERENCES

BEN TAMARZIZT, H.; BARAKET, G.; BEN MUSTAPHA, S.; MARRAKCHI, M.; TRIFI, M. and SALHI-HANNACHI, A. (2009). Genetic relatedness among Tunisian plum cultivars by random amplified polymorphic DNA analysis and evaluation of phenotypic characters. *Scientia Horticulturae*, vol. 121, no. 4, p. 440-446. **[CrossRef]**

BONANTS, P.J.M.; HAGENAAR-DE WEERDT, M.; MAN IN'T VELD, W.A. and BAAYEN, R.P. (2000). Molecular characterization of natural hybrids of *Phytophthora nicotianae* and *P. cactorum*. *Phytopathology*, vol. 90, no. 8, p. 867-874. **[CrossRef]**

DEMIR, K.; BAKIR, M.; SARIKAMIŞ, G. and ACUNALP, S. (2010). Genetic diversity of eggplant (*Solanum melongena*) germplasm from Turkey assessed by SSR and RAPD markers. *Genetics and Molecular Research*, vol. 9, no. 3, p. 1568-1576. **[CrossRef]**

ELAMEEN, A.; FJELLHEIM, S.; LARSEN, A.; ROGNLI, O.A.; SUNDHEIM, L.; MSOLLA, S.; MASUMBA, E.; MTUNDA, K. and KLEMSDAL, S. (2008). Analysis of genetic diversity in a sweet potato (*Ipomoea batatas* L.) germplasm collection from Tanzania as revealed by AFLP. *Genetic Resources Crop Evolution*, vol. 55, no. 3, p. 397-408. **[CrossRef]**

ESCANDÓN, A.S.; ZELENER, N.; DE LA TORRE, M.P. and SOTO, S. (2007). Molecular identification of new varieties of *Nierembergia linariaefolia* (Graham), a native Argentinean ornamental plant. *Journal of Applied Genetics*, vol. 48, no. 2, p. 115-123.

LI, M.; HOU, X.L. and HAO, R.M. (2009). Analysis of genetic relationships of *Osmanthus fragrans* based on SRAP markers. *Acta Horticulturae Sinica*, vol. 36, no. 11, p. 1667-1675.

LIU, Y.L. (1985). Preliminary study of sweet osmanthus in Nanjing area. *Journal of Nanjing Forestry University*, vol. 1, p. 30-37.

MUTHUSAMY, S.; KANAGARAJAN, S. and PONNUSAMY, S. (2008). Efficiency of RAPD and ISSR markers system in accessing genetic variation of rice bean (*Vigna umbellata*) landraces. *Electronic Journal of Biotechnology*, vol. 11, no. 3. **[CrossRef]**

ÔMURA, H.; HONDA, K. and HAYASHI, N. (2000). Floral scent of *Osmanthus fragrans* discourages foraging behavior of cabbage butterfly, *Pieris rapae*. *Journal of Chemical Ecology*, vol. 26, no. 3, p. 655-666. **[CrossRef]**

PEAKALL, R. and SMOUSE, P.E. (2006). GENALEX6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, vol. 6, no. 1, p. 288-295. **[CrossRef]**

PIÑA-ESCUTIA, J.L.; VAZQUEZ-GARCIA, L.M. and ARZATE-FERNANDEZ, A.M. (2010). Variety discrimination of *Tigridia pavonia* (L.f.) DC. assessed by different length RAPD primers. *Electronic Journal of Biotechnology*, vol. 13, no. 4. **[CrossRef]**

PREVOST, A. and WILKINSON, M.J. (1999). A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. *TAG Theoretical and Applied Genetics*, vol. 98, no. 1, p. 107-112. **[CrossRef]**

ROHLF, F.J. (2000). NTSYS-pc, Numerical Taxonomy and Multivariate Analysis System, Version 2.1 Exeter Pulications, New York, USA.

SALHI-HANNACHI, A.; CHATTI, K.; MARS, M.; MARRAKCHI, M. and TRIFI, M. (2005). Comparative analysis of genetic diversity in two Tunisian collections of fig cultivars based on random amplified polymorphic DNA and Inter simple sequence repeats fingerprints. *Genetic Resources and Crop Evolution*, vol. 52, no. 5, p. 563-573. **[CrossRef]**

SARKHOSH, A.; ZAMANI, Z.; FATAHI, R. and RANJBAR, H. (2009). Evaluation of genetic diversity among Iranian soft-seed pomegranate accessions by fruit characteristics and RAPD markers. *Scientia Horticulturae*, vol. 121, no. 3, p. 313-319. **[CrossRef]**

UZUN, A.; YESILOGLU, T.; AKA-KACAR, Y.; TUZCU, O. and GULSEN, O. (2009). Genetic diversity and relationships within *Citrus* and related genera based on sequence related amplified polymorphism markers (SRAPs). *Scientia Horticulturae*, vol. 121, no. 3, p. 306-312. **[CrossRef]**

WANG, L.M.; LI, M.T.; JIN, W.W.; LI, S.; ZHANG, S.Q. and YU, L.J. (2009). Variations in the components of *Osmanthus fragrans* Lour. essential oil at different stages of flowering. *Food Chemistry*, vol. 114, no. 1, p. 233-236. **[CrossRef]**

XIANG, Q.B. and LIU, Y.L. (2008). *An illustrated monograph of the sweet osmanthus cultivars in China*. Zhejiang Science and Technology Publishing House, 377 p. ISBN978-7-5341-3185-1.

YAN, X.Y.; XIAO, B.L.; HAN, Y.J.; YUAN, W.J. and SHANG, F.D. (2009). AFLP analysis of genetic relationships and diversity of some Chinese *Osmanthus fragrans* cultivars. *Life Science Journal*, vol. 6, no. 2, p. 11-16.

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