A Study on the Phenotypic Genetic Diversity of Camellia Germplasm Resources in Macheng City

Zhen SHEN *

Hubei Ecology Polytechnic College, Wuhan 430200, China

Abstract The genetic diversity analysis was done on 11 phenotypic traits of 35 camellia germplasm materials. The results showed that there were varying degrees of variation in 11 traits concerning the flowers and leaves selected in this study, and petal number had the largest coefficient of variation, followed by ovary height, and leaf length had the smallest coefficient of variation. *F* test results showed that the differences in 11 phenotypic traits reached a significant level. Through the trait correlation analysis, it was found that there was a positive or negative significant correlation between the 11 phenotypic traits. Principal component analysis results showed that the cumulative contribution rate of the first three principal components was 71.185%, the eigenvector which reflected flower pattern was largest, and flower pattern had a great impact on classification of camellia variety. Based on the genetic differences in traits between varieties, the 35 test materials were divided into three categories: Group I was characterized by large flower pattern; Group II was characterized by large leaf pattern; Group III was characterized by many petals. Flower diameter and flower height were important standards for classification of camellia variety.

Key words Camellia, Germplasm resources, Phenotypic traits, Genetic diversity

1 Introduction

Camellia is a genus of flowering plants in the family Theaceae, and it is one of China's top ten flowers. According to statistics, China now has more than 500 varieties of camellia[1], and there have been more than 15000 varieties of camellia in the world[1-2]. Since the cultivation of camellia in Macheng City from the 1980s, about 400 varieties of camellia have been collected and introduced. Long-term introduction and cultivation promotes the genetic variation of camellia while enriching the camellia germplasm resources in Macheng City. People often predict the viability and evolutionary potential of species by assessing the genetic diversity of species. In the study of genetic diversity of plant varieties, researchers often describe or measure the genetically stable traits of flowers and leaves, and use statistical methods for relational analysis, principal component analysis and cluster analysis, so as to reflect the degree of biological genetic variation. Currently, there have been mature studies on genetic diversity of agronomic traits of crop and fruit germplasm resources[3-8], however, the report on genetic diversity of traits of ornamental plant germplasm resources is quite limited. Based on principal component analysis and cluster analysis, this paper comprehensively analyzed the genetic diversity of main ornamental traits of camellia germplasm resources, which provided a reference for the collection, preservation, evaluation and utilization of camellia germplasm resources in Macheng City.

2 Materials and methods

2.1 Materials 35 representative camellia germplasm resources materials were selected from camellia germplasm resource nursery in Wunaoshan Forest Farm in Macheng City. The material name and number were listed in Table 1.

Table 1 Test camellia variety number, name and origin

Number	Name	Number	Name
1	Pisi Xiansheng	19	Aili Mudanwang
2	Yisha Anni	20	Daian Nahuanghou
3	Nanwang	21	Disi
4	Kena	22	Luanshi Jiaren
5	Huangda	23	Shizixiao
6	Nuccio's Carousel	24	Pake Sixiansheng
7	Heimofa	25	Huangjia Tianerong
8	Linbulei	26	Niuxi Aomeiyu
9	Maoyuan Heimanao	27	Sishaluo
10	Baibaota	28	Huafurong
11	Magu Xianzi	29	Zhuapolian
12	Hongshi Baxueshi	30	Liujiao Dahong
13	Sawada's Dream	31	Dakate
14	Huaheling	32	Jinpanlizhi
15	Wali Nashen	33	Nvhuang Erhao
16	Dawen Deshuguang	34	Qingrenjie
17	Hashi Weixiao	35	Dahejin
18	Shanshuo		

2.2 Research methods Based on China's camellia variety classification, testing guidelines and database of known species[9] as well as camellia germplasm resource description criterion and data standards[10], this paper tested and described 11 traits about the test camellia materials, including petal number, petal length, petal width, petal thickness, flower diameter, flower height, ovary height, leaf length, leaf width, petiole length, leaf thickness. The testing of each variety and each trait was repeated three times. Excel 2003 was used for statistical analysis of average, maximum, minimum, standard deviation and coefficient of variation of traits.

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* Corresponding author. E-mail: 498766886@qq.com

SPSS 22.0 software was used for relational analysis and principal component analysis of phenotypic traits[11]. NTsys-pc 2.1 software was used for cluster analysis on genetic diversity of phenotypic traits of test camellia variety. After normalizing raw data about 11 phenotypic traits of 35 materials, the material was used to calculate chi-squared distance between materials. Based on normalized data about 11 phenotypic traits, UPGMA was used for clustering of different camellia germplasm resource materials[12-13] and generating dendrogram.

3 Results and analysis

3.1 Genetic variability analysis of phenotypic traits of camellia germplasm resources The genetic variability analysis on 11 phenotypic traits of 35 test camellia germplasm resource materials could be shown in Table 2, and the variation amplitude reflected the degree of extreme variation of one trait variable in different test camellia germplasm resources. The standard deviation reflected the degree of discrepancy between trait variable and mean. The coefficient of variation was the ratio of standard deviation to mean, which reflected the degree of variation between germplasm resource materials. The greater the coefficient of variation, the larger the genetic differences among varieties. From Table 2, it was found that there were varying degrees of variations in 11 traits about flowers and leaves, and the petal number had the maximum variation range and highest standard deviation, indicating that the trait had high degree of dispersion. Leaf thickness and petal thickness had low degree of variation, small standard deviation and low degree of dispersion. The coefficient of variation of 11 traits was in the order of petal number > ovary height > petal thickness > petal width > flower height > leaf width > petiole length > leaf thickness > flower diameter > petal length > leaf length. Petal number had the largest coefficient of variation (61.33%), while leaf length had the smallest coefficient of variation (18.38%), indicating that the leaf length was relatively stable and there was highest degree of variation in petal number. F test was used for differences among varieties, and the results (Table 2) indicated that there were significant differences in 11 phenotypic traits among 35 camellia varieties.

Table 2 Genetic variation analysis on phenotypic traits of the test camellia varieties

Traits	Variation range//mm	Mean//mm	Coefficient of variation//%	*F* value
Petal number	8.00 - 182.00	57.03 ± 34.98	61.33	31.821**
Petal length	27.96 - 78.00	44.43 ± 9.16	20.62	8.253**
Petal width	21.18 - 63.14	40.29 ± 10.29	25.54	20.090**
Petal thickness	0.46 - 1.86	0.78 ± 0.24	30.77	2.791**
Flower diameter	61.46 - 134.35	89.50 ± 18.54	20.72	18.399**
Flower height	4.97 - 80.01	47.92 ± 12.19	25.44	12.253**
Ovary height	2.38 - 12.44	6.59 ± 2.46	37.33	13.195**
Leaf length	47.43 - 115.06	81.56 ± 14.99	18.38	11.521**
Leaf width	26.26 - 99.00	42.11 ± 10.37	24.63	3.502**
Petiole length	0.69 - 15.80	10.55 ± 2.41	22.84	5.330**
Leaf thickness	0.39 - 1.51	0.89 ± 0.19	21.35	5.323**

Note: ** indicated a significant difference at the 0.01 level.

Table 3 Relational analysis on 11 phenotypic traits of camellia

Traits	1	2	3	4	5	6	7	8	9	10	11
1	1.000										
2	-0.316	1.000									
3	-0.491**	0.824**	1.000								
4	-0.199	0.430**	0.300	1.000							
5	-0.288	0.802**	0.724**	0.435**	1.000						
6	-0.438**	0.733**	0.809**	0.279	0.734**	1.000					
7	0.210	-0.298	-0.331	-0.436**	-0.267	-0.325	1.000				
8	0.016	0.208	0.137	0.018	0.325	0.124	0.053	1.000			
9	-0.103	0.176	0.108	-0.006	0.228	0.036	0.091	0.743**	1.000		
10	0.136	0.048	0.011	-0.056	0.250	0.034	0.143	0.689**	0.563**	1.000	
11	-0.109	0.248	0.229	0.226	0.278	0.260	-0.194	0.485**	0.500**	0.231	1.000

Note: ** indicated significant correlation at the 0.01 level; 1 (petal number), 2 (petal length), 3 (petal width), 4 (petal thickness), 5 (flower diameter), 6 (flower height), 7 (ovary height), 8 (leaf length), 9 (leaf width), 10 (petiole length), 11 (leaf thickness).

3.2 Relational analysis on phenotypic traits of camellia germplasm resources The relational analysis results of phenotypic traits (Table 3) showed that there was significant correlation among 11 phenotypic traits of camellia materials; there was significant positive or negative correlation among 7 phenotypic traits of flowers; there was also significant positive or negative correlation among 4 phenotypic traits of leaves. There was significant negative correlation between petal number and petal width or flower height; ovary height was significantly negatively correlated with petal thickness; petal length was significantly positively correlated with petal width, flower diameter and flower height; petal thickness was significantly positively correlated with petal length and flower diameter; leaf length was significantly positively correlated with leaf width and leaf thickness; petiole length was significantly positively correlated with leaf length and width.

3.3 Principal component analysis on phenotypic traits of camellia germplasm resources The principal component analysis results were shown in Table 4, and the cumulative contribution rate of the first three principal components reached 71.185%. The first principal component contributed 38.309%, and the traits with large absolute value of eigenvector included flower diameter (0.875), petal length (0.870), petal width (0.860) and flower height (0.830). These traits were the main factors influencing camellia pattern, indicating that camellia pattern was one of the main criteria for camellia variety classification. The second principal component contributed 22.858%, and the leaf length had the largest absolute value (0.8) of eigenvector while leaf width and petiole length had the value of 0.7, indicating that the traits of leaves had also an important impact on the camellia variety classification. The third principal component contributed 10.017%, and ovary height had the largest eigenvector (0.607), reflecting that ovary development affected the camellia variety classification to a certain extent.

Table 4 Principal component analysis of 11 phenotypic traits of camellia

Traits	Principal component		
	1	2	3
1	-0.484	0.274	-0.170
2	0.870	-0.173	0.150
3	0.860	-0.256	0.262
4	0.507	-0.250	-0.587
5	0.875	-0.022	0.137
6	0.830	-0.255	0.248
7	-0.428	0.354	0.607
8	0.413	0.820	-0.029
9	0.362	0.790	-0.039
10	0.231	0.783	0.100
11	0.484	0.426	-0.420

Note: Eigenvalues of traits 1-3 were 4.214, 2.514 and 1.102, respectively, with contribution rate of 38.309%, 22.858% and 10.017%, respectively, cumulative contribution rate of 38.309%, 61.168% and 71.185%, respectively.

3.4 Cluster analysis on phenotypic traits of camellia germplasm resources The UPGMA cluster analysis results about phenotypic traits of 35 test camellia germplasm resources could be shown in Fig. 1. At chi-square distance of 1.32, different resource materials could be divided into three groups. Group I: Nuccio's Carousel, Heimofa, Maoyuan Heimanao, Sishaluo, Wali Nashen, Dakate, Nvhuang Erhao. Compared with Group II and III, this group of resources had the maximum mean of flower diameter, petal length, petal width, petal thickness and flower height; the mean of petal number and ovary height was lowest; leaf length, width, thickness and petiole length were at the middle level. This group had the maximum coefficient of variation of petal width, petal thickness, flower diameter, leaf width and petiole length, indicating that the materials in Group I had large flower pattern, great flower height and rich flower pattern diversity, with an absolute advantage in the ornamental characteristics. Group II: Kena, Baibaota, Huafurong, Shanshuo, Hashi Weixiao, Pake Sixiansheng, Aili Mudanwang, Daian Nahuanghou, Liujiao Dahong, Qingrenjie. This group of materials had the maximum mean of leaf length, width, thickness and petiole length; the mean of petal number, petal length, petal width, petal thickness, flower diameter, flower height and ovary height were at the middle level. By comparing the coefficient of variation of traits between different groups, it was found that Group II had the maximum coefficient of variation of flower height, indicating that the varieties in Group II were the ornamental varieties with large leaf pattern and great flower height changes. Group III: Pisi Xiansheng, Yisha Anni, Hongshi Baxueshi, Huangda, Linbulei, Magu Xianzi, Dawen Deshuguang, Niuxi Aomeiyu, Luanshi Jiaren, Zhuapolian, Nanwang, Huaheling, Shizixiao, Dahejin, Disi, Sawada's Dream. The materials in this group had the greatest petal number and ovary height; the mean of other flower and leaf traits was lowest. The coefficient of variation of petal number, petal length, ovary height, leaf length and leaf thickness was largest, indicating that Group II had no advantages in the flower diameter and flower height and it took petal level and number as the main ornamental value.

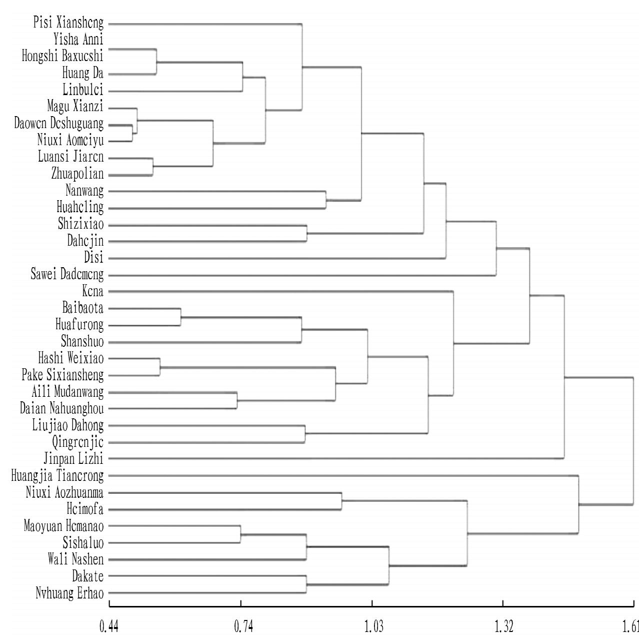

Fig. 1 UPGMA cluster analysis results about phenotypic traits of 35 test camellia germplasm resources

4 Discussions

In this paper, the genetic diversity analysis was done on 11 phenotypic traits of 35 camellia germplasm materials in Macheng City. The results showed that there were varying degrees of variation in 11 traits concerning the flowers and leaves selected in this study, and petal number had the largest coefficient of variation, followed by ovary height, and leaf length had the smallest coefficient of variation, indicating that the camellia germplasm resources in Macheng City had rich genetic diversity. Through the trait correlation analysis, it was found that there was a positive or negative significant correlation between the 11 phenotypic traits (correlation between 7 traits of flowers and correlation between 4 traits of leaves). The results showed that ovary height was negatively correlated with petal thickness, which had not been reported as yet. The internal links between ovary height and petal thickness was to be further studied. The principal component analysis results showed that the first three principal components reflected most of the information about 11 traits of all the test materials, with cumulative contribution rate of 71.185%. This might indicate that common traits made scattered contribution, and the growth of cumulative contribution rate was not obvious, thus showing the multidimensional nature of trait variation. The main traits with large eigenvector for the first principal components included flower diameter, petal length, petal width and flower height, reflecting the important indication role of flower pattern in camellia variety identification and classification. This was consistent with China's traditional variety classification method highlighting camellia pattern[14-15], and flower pattern could best reflect the evolution process and genetic relationship of varieties[16]. The second principal component reflected that leaf pattern occupied a certain position in camellia variety classification, but in the methods concerning camellia variety classification, few of them reported the variety classification according to phenotypic traits of leaves. By experiment, Wang Kuiling *et al.* pointed out that camellia leaf epidermis, stomata and other anatomical traits of leaf could be used as the basis of variety classification[17], which demonstrated the link between camellia leaf traits and camellia variety classification for the first time. The third principal component reflected the ovary developmental status, affecting the classification of camellia variety. The cluster analysis was performed on the 35 test materials and results showed that the camellia varieties with similar flower diameter, flower height, petal size and leaf size were easy to gather together, which was nothing to do with the material source but related to huge morphological differences in progeny varieties caused by long-term introduction and hybridization. The numerical traits or quality traits that reflected the phenotypic information of camellia variety, were susceptible to the interference of natural environment and human factors, so that it was difficult to grasp the evolutionary relationship and genetic relationship between camellia varieties only by traits. To further understand the genetic diversity of camellia germplasm resources, it is still necessary to use SSR, AFLP, RAPD and other molecular biology techniques for scientific evaluation of genetic relationship between camellia germplasm resources.

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