Diversity analysis of leaf phenotypic characters of Pyracantha fortunaeana

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Abstract. The leaf phenotypic characters of wild *Pyracantha fortunaeana* germplasm resources were investigated, from Sichuan and Yunnan Province in southwest China. The results indicated that there were abundant diversity in leaf length, leaf width, leaf index, petiole length and leaf area. The leaf phenotypic characters of *Pyracantha fortunaeana* germplasm resources from different source were significant different. The average coefficient of variation on leaf length, leaf width, leaf index, petiole length and leaf area of the four wild populations were more than 20%, where the average variation coefficient being the largest (48.62%), and the average coefficient of variation in leaf index was the smallest (22.60%). Among the populations, the average coefficient on variation of SCYA population was the biggest (45.97%), and the average coefficient of variation of YNZT population was the lowest (25.53%). Correlation analysis among phenotypic traits showed that there were significant or extremely significant positive correlations between the leaf traits of *Pyracantha fortunaeana*.

1.Introduction

Pyracantha fortuneana is a wild evergreen shrub which belongs to the *Pyracantha Roem.* of Maloideae from the family Rosaceae. There are 7 kinds of germplasm resources of *Pyracantha Roem.* in China, among which the most widely distributed and largest reserves is *Pyracantha fortunaeana* [1]. At present, there are quite a few reports on the application of *P. fortunaeana* fruit at home and abroad [2], *Pyracantha* fruit contains a variety of essential amino acids, vitamins, mineral elements and other nutrients, which are used to make fruit juice drinks, jams, fruit vinegar, fruit wine and other foods [3-5]. In addition, *Pyracantha* plants are also good for landscaping and bonsai trees [6-7].

China has vast territory and abundant plant resources. But the plant resources were damaged seriously due to the missing of protection measures. The research on diversity is an important prerequisite for preserving plant resources and breeding excellent germplasm. The collection and preservation of plant germplasm resources are based on the genetic diversity of plant species [8], while the intraspecific genetic diversity is the sum of genetic variation within individuals and population, which includes the level of phenotypic, biochemical, chromosome, protein, nucleic acid etc. Among them, phenotype is a combination of various morphological features, is one of the important means to study genetic diversity, and is also the most direct embodiment of plant adaptation environment [9]. Leaf morphology is an important morphological feature, which is closely related to plant nutrition and ecological factors as well as plant reproduction [10].

In the past 20 years, scholars have reported on the biology, ecology, introduction and cultivation, and fruit quality of *P. fortunaeana* [2, 11], while few reports on the genetic diversity of *P. fortunaeana* especially on the diversity of phenotypic. In this paper, we studied the genetic diversity of four wild populations in southwest China from phenotypic level. The main objective of this study was to compare genetic diversity of wild *P. fortunaeana* and provide a scientific basis for the conservation and sustainable utilization for *P. fortunaeana* wild germplasm resources in southwest China.

2.Materials and Method

2.1 Plant materials

A total of 51 leaf materials were analysed in this study (Table 1). Among these, 29 leaf materials were collected from Ya'an (SCYA), Mianyang (SCMY), Sichuan Province, another 22 leaf materials were sampled from Kunming (YNKM), Zhaotong (YNZT) in Yunnan Province from October 2016 to November 2017. Investigation sites (2-5) were selected in each population, each site was more than 10 km apart, and 4-15 plants were randomly selected from each survey site, and 50 mature leaves of the middle spring shoot of the current year were harvested from the periphery of the tree crown, respectively.

Table 1. Sample information of four *P.fortunaeana* populations

Population	Locations	Numbers	Altitude/m	Longitude latitude
SCYA	Jinwo Village, Caoke Township, Shimian County	5	1858	102°02′E 29°22′N
	Lianhe Village, Huilong Township, Shimian County	5	1964	102°25′E 29°08′N
	Zhuma Village, Huilong Township, Shimian County	4	1342	102°29′E 29°04′N
SCMY	Youxian Town, Youxian District	5	542	104°46′E 31°32′N
	Shen Kang Town, Youxian District	4	498	104°44′E 31°32′N
	Southwest University of Science and Technology Farm	6	483	104°41′E 31°32′N
YNZT	Zhaoyang District Phoenix Mountain Forest Park	4	1950	103°41′E 27°18′N
	Qinggangling Township, Zhaoyang District	5	2090	103°43′E 27°28′N
YNKM	Kunming Botanical Garden	4	1960	102°44′E 25°08′N
	Qinglong Mountain, Xishan District	5	2150	102°37′E 25°02′N
	Heilong Pool, Panlong District	4	1915	102°44′E 25°08′N

2.2 Leaf trait measurement methods

The leaf length, leaf width and petiole length of *P. fortunaeana* leaf were measured by vernier calipers, leaf area was measured by YMJ-B leaf area measuring instrument (Top Instrument, China), leaf index = leaf length/leaf width [12]. Not less than 50 leaves were measured for each trait of each survey site.

2.3 Statistical analysis

Statistical analysis were performed using software Microsoft Excel 2010 and IBM SPSS 22.0. Dates were analysed using a one-way analysis of variance with *Duncan's* new multiple range test at 5% and 1% confidence level. Leaf diversity index was calculated by *simpson* index. The classification method of the diversity index as follows: calculate the population average (X) and standard deviation (SD) of each trait firstly, and then divide it into 10 levels. From the first level ($X < x - 2.5 \times SD$) to the tenth level ($X > x + 2.5 \times SD$), x represents the actual measured value of each leaf, each 0.5×SD is a level and the frequency of each level was used to calculate the diversity index. Diversity index (D) = $1 - \Sigma P_i^2$, which P_i is the frequency i level of a certain trait.

3.Results and analysis

3.1 Leaf phenotypic diversity of four wild populations of P. fortunaeana

The variation range of leaf length in four wild populations from large to small was SCMY, SCYA, YNKM and YNZT. The range of leaf width varies from large to small was YNKM, SCYA, SCMY and YNZT, leaf index varies from high to low was SCMY, SCYA, YNKM and YNZT, the variation range of petiole length from high to low was SCYA, SCMY, YNZT and YNKM, leaf area varies from large to small was SCMY, YNKM, SCYA and YNZT population (Table 2). The diversity index of leaf length, leaf width, leaf index, petiole length and leaf area of each population were 0.689 - 0.862, which indicated abundant genetic diversity in leaf phenotypic characters of *P. fortunaeana.*

3.2 Multiple comparisons of leaf phenotypic characters of P. fortunaeana

Leaf length, leaf width, petiole length and leaf area of *P. fortunaeana* in SCYA populations were significantly less than the other three populations (Table 3). The leaf length, leaf index and petiole length of SCMY population were significantly lower than those of YNZT population, but the leaf width and leaf area was significantly higher than YNZT population. The values of leaf characters of YNKM population were significantly higher than the SCMY population except for the leaf width. Furthermore, the maximum of leaf length, leaf index and petiole length were YNZT population, while the leaf width and leaf area were SCMY and YNKM population, respectively.

3.3 phenotypic variation of leaf characteristics of P. fortunaeana

In different geographical environments, the coefficient of variation of each morphological feature was different, and the degree of variation among different traits of the same population was also different (Table 4). The highest coefficient of variation among the phenotypic traits was leaf area (48.62%), and the smallest was leaf index (22.60%), which indicated that the leaf index of each morphological trait was higher than other phenotypic traits. The average variability of different phenotypic characteristics of *P. fortunaeana* leaf were petiole length > leaf length > leaf width > leaf index. There were some differences in the coefficient of variation of the same traits in the four populations, which indicated that environmental heterogeneity in different regions have greet influence in the phenotypic variation of *P. fortunaeana* population. Further comparison of intra-population coefficient of variation found that the average coefficient of variation of SCYA population was the largest (45.97%), followed by SCMY population (34.22%), YNKM population (26.06%) and YNKT population (25.53%), which indicated that the leaf variability of *P. fortunaeana* of SCYA and SCMY populations in Sichuan were richer than other populations in Yunnan.

3.4 Correlation analysis between phenotypic traits of P. fortunaeana

The leaf length of *P. fortunaeana* was significantly positively correlated with leaf width, leaf index and petiole length, the correlation coefficients were 0.833, 0.473, and 0.781 (Table 5). The leaf width was correlated with petiole length and leaf area, which the correlation coefficients were 0.574 and 0.526, respectively. In addition, leaf index was significantly positively correlated with the length of petiole, and the correlation coefficient was 0.447.

Table 2. Diversity of morphological traits in four populations of *P. fortunaeana*

Populations	Trait	Mean	Maximum	Minimum	Diversity index
SCYA	Leaf length/cm	2.131	4.672	0.132	0.753
	Leaf width/cm	0.832	3.672	0.268	0.823
	Leaf index	2.514	7.882	0.290	0.823
	Petiole length/cm	0.370	1.608	0.030	0.689
	Leaf area/cm^2	1.059	4.605	0.115	0.824
SCMY	Leaf length/cm	3.098	5.998	1.430	0.853
	Leaf width/cm	1.316	2.914	0.374	0.830
	Leaf index	2.394	9.262	1.022	0.843
	Petiole length/cm	0.500	1.280	0.112	0.850
	Leaf area/cm^2	2.287	9.614	0.141	0.823
YNZT	Leaf length/cm	3.514	5.551	1.765	0.851
	Leaf width/cm	1.195	1.788	0.633	0.860

	Leaf index	2.972	4.387	1.764	0.859
	Petiole length/cm	0.575	1.236	0.192	0.845
	Leaf area/cm^2	2.100	5.533	0.321	0.855
YNKM	Leaf length/cm	3.508	6.138	1.748	0.862
	Leaf width/cm	1.279	4.984	0.724	0.836
	Leaf index	2.814	6.552	0.233	0.853
	Petiole length/cm	0.545	1.040	0.122	0.853
	Leaf area/cm^2	2.957	6.819	0.875	0.855

Table 3. Comparison of leaf traits in four populations of *P. fortunaeana*

Populations	Leaf length/cm	Leaf width/cm	Leaf index	Petiole length/cm	Leaf area/cm^2
SCYA	2.131±1.139 cC	0.832±0.382 cC	2.514±0.693 cC	0.370 ±0.187 dC	1.059 ±0.556 dC
SCMY	3.098±0.803 bB	1.316±0.312 aA	2.394±0.555 dD	0.500 ±0.191 cB	2.287 ±1.376 bB
YNZT	3.514±0.675 aA	1.195±0.230 bB	2.972±0.431 aA	0.575 ±0.172 aA	2.100 ±0.940 cB
YNKM	3.508±0.745 aA	1.279±0.271 aA	2.814±0.709 bB	0.545 ±0.140 bA	2.957 ±1.095 aA

Notes: values are 'mean ± SD', different letters within a column indicate significant difference (lowercase letters indicate $P < 0.05$, uppercase letters indicate $P < 0.01$).

Table 4. Coefficient of variation of leaf phenotypic traits in four populations of *P. fortunaeana* (%)

Populations	Leaf length	Leaf width	Leaf index	Petiole length	Leaf area	Mean
SCYA	53.43	45.95	27.57	50.36	52.53	45.97
SCMY	25.91	23.68	23.16	38.22	60.14	34.22
YNZT	19.21	19.25	14.49	29.93	44.76	25.53
YNKM	21.24	21.20	25.18	25.65	37.03	26.06
Mean	29.95	27.52	22.60	36.04	48.62	32.94

Table 5. Correlation coefficient between leaf traits of *P. fortunaeana*

Trait	Leaf length/cm	Leaf width/cm	Leaf index	Petiole length/cm	Leaf area/cm^2
Leaf length/cm	1.000				
Leaf width/cm	0.833**	1.000			
Leaf index	0.473**	-0.053	1.000		
Petiole length/cm	0.781**	0.574**	0.447**	1.000	
Leaf area/cm^2	0.394*	0.526**	-0.126	0.197	1.000

Note: *, ** indicate significant differences at the 5% level and the 1% level, respectively.

4.Discussion and conclusion

Phenotypic diversity is the elaboration of genetic diversity at the morphological level, it is the result of the interaction between the environment and the genetic factors which reflects the genetic characteristics and the adaptability to the complex environment of plant to some extent [13]. From the results of this article, the variation range of leaf area of SCMY population was largest (0.141-9.614). The genetic diversity index of leaf characters of four populations was 0.689-0.824, 0.823-0.853, 0.845-0.860, 0.836-0.862 respectively, which showed abundant genetic diversity.

Genetic variation of *Picea.spruce* [8], *Q.mongolica* [9] and almond [14] has been reported using different phenotypic traits, which indicated that phenotypic traits are an effective method to evaluate genetic diversity. In this experiment, there were significant or extremely significant differences in leaf phenotypic characters of the four wild populations of *P. fortunaeana*, the average coefficient of variation of leaf traits such as leaf length, leaf width, petiole length and leaf area was above 20%. The variation range of leaf area was the greatest and the average coefficient of variation was the largest (48.62%). The average coefficient of variation on different phenotypic characters of *P. fortunaeana* was leaf area > petiole length > leaf width > leaf index. The average coefficient of variation of phenotypic traits in four populations was SCYA (45.97%) > SCMY (34.22%) > YNKM (26.06%) > YNZT (25.53%). These variations are affected by ecological factors, but also reflect the genetic diversity, and the results of this study are similar to Wu [15].

Observing the phenotypic variation on plants in different environments is not only an important way to understand genetic variation, but also an important basis of conservation biology and genetic breeding. Plants have formed many adaptation or defense mechanisms, including morphological structure, physiological and biochemical metabolism in the process of long-term interaction with environment. These characteristics are gradually transformed into heritable traits, which is the result of plant adaptation to environmental. Morphological or phenotypic variation reflects the variation richness of genotypes, populations and ecotypes to some extent. Although only five phenotypic traits of *P. fortunaeana* leaf were discussed, the phenotype was determined by the interaction of environment and genotype, while phenotypic variation must contain genetic variation. In conclusion, according to the natural variation of *P. fortunaeana*, it could be inferred that the genetic diversity of *P. fortunaeana* is rich.

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