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Genetic Diversity Analysis of *Cotoneaster schantungensis* Klotz. Using SRAP Marker

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Abstract

In order to detect the genetic relationship of different *Cotoneaster schantungensis* natural populations, 16 samples were selected for genetic diversity analysis based on SRAP markers. Twelve pairs primers were selected out, 93 bands were amplified, of which 91 bands (97.85%) were polymorphic. At species level, the average value of Nei's genetic diversity (H) was 0.2213, Shannon's information index (I) was 0.3596, whereas at population level, they were 0.1955, 0.3121, the percentage of polymorphic loci was 75.27%. The genetic differentiation coefficient in populations (Gst) was 0.0788, gene flow Nm was 5.8467, indicating that there was 7.88% of the variation among populations and 92.12% of the variance within populations and the variation within populations was relatively high. These results indicate that *C. schantungensis* has high genetic diversity and low differentiation among populations. Although most Cotoneaster species are apomictic, *C. schantungensis*, however, may be an exception.

Keywords

***Cotoneaster schantungensis* Klotz., Apomictic, SRAP, Genetic Diversity, Population**

1. Introduction

Cotoneaster schantungensis Klotz. is a unique species of Shandong, the distribution of it is limited in southern mountain area of Jinan, and it is scattered under *Platycladus orientalis* and *Cotinus coggygria* plantation sporadic sporadically, with very few numbers. It is in state of extreme endangerment [1]-[3]. Genetic diversities of endangered plants can be tested effectively with molecular marker technology, thus the threatening mechanism can be grasped and the corresponding protection measures can be formulated [4]-[14]. Sequence-related ampli-

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fied polymorphism (SRAP) is a kind of new molecular marker technology based on PCR, it is more convenience, high co-dominance, easy for separation of strips and sequencing, and it doesn't need to know sequence information of species in advance, and is richer in information provided in the aspect of morphologic variation and evolutionary history when compared with AFLP, so it has been widely used at present [15]-[17]. As a kind of extremely small population species, the previous research has been studies only on its living environment and community structure and there is no report on its genetic diversity [18]. Our research makes genetic diversity study on 16 samples of *C. schantungensis* with SRAP marker technology, the result shows that the genetic diversity level of intrapopulational of *C. schantungensis* is high, genetic differentiation is large, which provides scientific basis for the formulation of reasonable and efficient protection measures and enhances the protection of endangered species.

2. Materials and Methods

2.1. Plant Materials

C. schantungensis distributes in low altitude mountain area of limestone in southern Jinan, at present, two distributions, "Fo Yu" and "Red Leaves Valley" are found, and there are only about 300 clumps. In these 16 samples of *C. schantungensis* materials are used for experiment, No. 1 - No. 11 are derived from "Fo Yu" (36˚35'N 117˚06'E, elevation 300 - 350 m), No. 12 - No. 16 are derived from "Red Leaves Valley" (36˚28'N 117˚09'E, elevation 500 - 600 m). The sampling interval of individuals is more than 30 meters, and normal growing healthy without diseases and insect pests are selected, the young fresh leaves on the top of it are collected to be put in quick-seal bags with allachroic silica gel, the air in bags is squeezed out, which makes it rapid drying.

2.2. Research Methods

2.2.1. Genome DNA Extraction

Total DNA was obtained from young leaves using improved CTAB method [19]. The quality and concentration of DNA was tested with 0.8% agarose gel electrophoresis and ultraviolet spectrophotometer, then diluted it to 50 $ng \cdot \mu L^{-1}$ and stored it in −20˚C for standby application.

2.2.2. SRAP-PCR Amplification Analysis

SRAP-PCRs were run in 25 μl volumes containing 2.5 μl 10× PCR buffer (5.0 mM $MgCl_2$), 30 ng genomic DNA, 0.3 μM of each primer, 7.5 mM dNTPs and 2 Unit of Taq DNA polymerase. PCR amplifications were performed in Bio-Rad PCR amplifier Cycler using the following cycling parameters: 5 min denaturing at 94˚C; 5 cycles of 94˚C for 1 min; 35˚C for 1 min and 72˚C for 1 min; 30 cycles of 94˚C for 1 min, 50˚C for 1 min and 72˚C for 1 min; followed by a final extension of 7 min at 72˚C, then store at 4˚C.

Twelve pairs of primer combinations, selected from the initial 150 pairs of primer combinations, were used for the study (**Table 1**). Amplified products were electrophoresed on 2% agarose gel at 120 V for 2 hour, stained

Table 1. Primer sequences used for SRAP analysis.

Forward primer	Primer combination sequences	
ME4-EM11	F: 5'-TGAGTCCAAACCGGACC-3'	R: 5'-GACTGCGTACGAATTAGC-3'
ME4-EM12	F: 5-TGAGTCCAAACCGGACC-3'	R: 5'-GACTGCGTACGAATTGCC-3'
ME4-EM7	F: 5'-TGAGTCCAAACCGGACC-3'	R: 5'-GACTGCGTACGAATTCAG-3'
ME4-EM9	F: 5'-TGAGTCCAAACCGGACC-3'	R: 5'-GACTGCGTACGAATTCCA-3'
ME5-EM15	F: 5'-TGAGTCCAAACCGGAAG-3'	R: 5'-GACTGCGTACGAATTATG-3'
ME6-EM12	F: 5-TGAGTCCAAACCGGTAA-3'	R: 5'-GACTGCGTACGAATTGCC-3'
ME6-EM13	F: 5'-TGAGTCCAAACCGGTAA-3'	R: 5'-GACTGCGTACGAATTTAG-3'
ME6-EM6	F: 5'-TGAGTCCAAACCGGTAA-3'	R: 5'-GACTGCGTACGAATTGCA-3'
ME6-EM8	F: 5'-TGAGTCCAAACCGGTAA-3'	R: 5'-GACTGCGTACGAATTCCA-3'
ME6-EM9	F: 5'-TGAGTCCAAACCGGTGC-3'	R: 5'-GACTGCGTACGAATTAAC-3'
ME8-EM5	F: 5'-TGAGTCCAAACCGGTGC-3'	R: 5'-GACTGCGTACGAATTCCA-3'

with ethidium bromide (EB) and then visualized and photographed by using gel documentation system (Beijing LiuyiWD-9403CS type UV Apparatus).

2.2.3. Data Statistics and Analysis

SRAP products obtained were manually scored as present (1) or absent (0). The number and percentage of polymorphic loci, effective number of allele Ne, Nei's genetic similarity coefficient among various materials, Nei's genetic diversity index (H) and Shannon information index (I) was then analyzed by using POPGENE 1.32 [20]. Dice similarity coefficient was calculated and the corresponding cluster analysis was performed using the Unweighted Pair-Group Method with Arithmetic Average (UPGMA) [21].

3. Result and Analysis

3.1. SRAP-PCR Amplification

The selected 12 pairs of primers are used to amplify 16 pieces of materials (**Figure 1**), and then the statistics of amplified primer sites is carried on with binary character coding method (**Table 2**). It amplifies 93 sites, among them there are 91 polymorphic loci, the percentage of polymorphism is as high as 97.85%, and each primer amplifies 7.75 loci and 7.58 polymorphic loci averagely, it indicates that there are rich genetic diversities among *C. schantungensis*, and the genetic background is very complex. The number of polymorphic loci amplified by primer combination ME8-EM9 is the most, there are 12, the number of polymorphic loci amplified by primer combination ME8-EM5 is the least, and there are 3. In the 12 pairs of primers, the percentage of polymorphic loci of primer combination ME6-EM12 is the lowest, it is 75%, the percentage of polymorphic loci of the rest primer combinations are all 100%. The Effective number of alleles is 1.1788 - 1.5849, the average value is 1.3420, and the value of primer combination ME6-EM8 is the largest.

3.2. Genetic Diversity Analysis

Based on the test result of SRAP molecular marker, it shows that there is a big difference in genetic diversity of two populations of *C. schantungensis*. The percentage of polymorphic loci (86.02%), Shannon information index

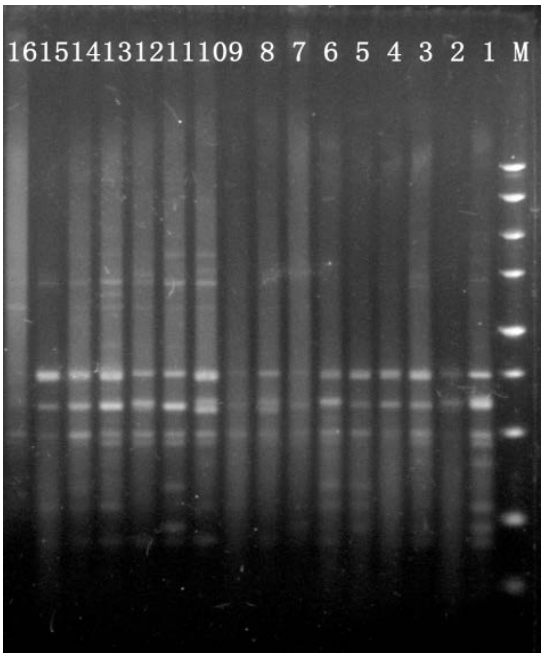

Figure 1. The amplification result of ME8-EM9. No. 1-No. 11: *C. schantungensis* derived from "Fo Yu"; No. 12-No. 16: *C. schantungensis* derived from "Red Leaves Valley" M: DNA Marker 2000.

(0.3550), Nei genetic diversity of the population in "Fo Yu" is much higher than the population in "Red Leaves Valley" (**Table 3**). Because the three parameters could uncover the genetic diversity condition of population effectively, so it indicates that the genetic diversity of population in "Fo Yu" is much higher than population in "Red Leaves Valley". The observation of two parameters of number of alleles and effective number of alleles also proves it.

In the level of species, the number of alleles and effective number of alleles is 1.9785 and 1.3405 respectively; Nei's gene diversity index H is 0.2213, Shannon polymorphic information index I is 0.3596. it indicates that the genetic diversity of *C. schantungensis* presents a high level in the level of population and species.

Table 2. Sequences of 12 pairs of SRAP primers and amplification results.

Primer combination	Number of polymorphic loci (NPL)	Percentage of polymorphic loci (PPL) (%)	Effective number of alleles (Ne)	Nei's gene diversity (H)	Shannon's information index (I)
ME8-EM5	3	100.00	1.1931	0.1585	0.2917
ME8-EM9	12	100.00	1.4581	0.2795	0.4378
ME4-EM11	8	100.00	1.1767	0.1472	0.2746
ME4-EM12	8	100.00	1.3684	0.2624	0.4276
ME4-EM9	8	100.00	1.4909	0.2944	0.4485
ME4-EM7	4	100.00	1.4950	0.3013	0.4603
ME5-EM15	11	100.00	1.2546	0.1643	0.2791
ME6-EM6	9	100.00	1.3521	0.2364	0.3848
ME6-EM8	6	100.00	1.5849	0.3380	0.5037
ME6-EM12	6	75.00	1.3277	0.2061	0.3224
ME6-EM9	10	100.00	1.2240	0.1565	0.2741
ME6-EM13	6	100.00	1.1788	0.1382	0.2528

Table 3. The genetic diversity among different populations of *C. schantungensis* based on SRAP analysis.

Populations	Percentage of polymorphic loci (PPL) (%)	Observed number of allele (Na)	Effective number of alleles (Ne)	Nei's gene diversity (H)	Shannon's information index (I)
Foyu populations	86.02	1.8602	1.3702	0.2260	0.3550
Hongyegu populations	64.52	1.6452	1.2405	0.1650	0.2691
Mean	75.27	1.7527	1.3054	0.1955	0.3121
Species level	97.85	1.9785	1.3405	0.2213	0.3596

3.3. Genetic Differentiation of Population of *C. schantungensis*

Gene differentiation analysis among populations shows that the Genetic differentiation coefficient Gst of two populations of *C. schantungensis* is 0.0788, because the Gst overall level of perennial plant, hybridization plant, insect-pollinated plant is 0.19, 0.22, 0.264 respectively [22]-[24], so the Gst of *C. schantungensis* is obvious low.

The total genetic diversity of population Ht is 0.2123, and most heritable variations exist in the population, and the genetic diversity in population Hs is 0.1955, intra-population occupies 92.1%, among populations only occupies 7.88%. The gene flow among populations estimated by Gst is 5.8467, it shows that there are frequent gene flows between the two populations.

At the same time, the UPGMA with Nei method also shows that the genetic identity of the two populations is 0.9766, the genetic distance is only 0.0236, the genetic similarity among populations is very high, and it is the same as the result that the heritable variation among populations is small.

3.4. The Genetic Distance and Cluster Analysis among Individuals and Populations

Based on genetic similarity coefficient, SRAP marker genetic diversity molecular dendrogram (**Figure 2**) of tested materials is drawn with UPGMA method.

The result shows that the similarity coefficients of all materials are between 0.47 - 0.92, the average is 0.68, and it proves that the genetic relationship of tested materials is very close [25]. It can be seen from dendrogram that 16 pieces of materials can be divided into three classes at the similarity coefficients of 0.38. Individuals among populations have obvious overlaps phenomenon, and they are not classified with geographic features, three materials (15, 16, 12) of "Red Leaves Valley" and all materials of "Fo Yu" are crossed gathered as the first and second class, and No. 13 and No. 14 of "Red Leaves Valley" are solely gathered as the third class. It shows that there is no obvious correlation between genetic distance and geographic space, and it is also not obvious related to distance of two populations and genetic differentiation among populations. At the same time, the genetic distance among individuals of *C. schantungensis* in populations of "Fo Yu" is close, while the distance is far in populations of "Red Leaves Valley", it is speculate that populations in "Fo Yu" are formed by the spread distribution of populations in "Red Leaves Valley".

4. Discussion

The percentage of polymorphic loci is one of the important indexes that reflect variation level in populations [26]. At present, there is no study report of SRAP of Cotoneaster. In species levels, the genetic diversity of *C. schantungensis* is 97.85%, which is higher than *Amygdalus ledebouriana* (P = 92.1%), plants in the same family, but Nei's genetic diversity index (H = 0.2213) and Shannon information index (I = 0.3596) are a little lower than those of *A. ledebouriana* (H = 0.2809, I = 0.4327 [27]. So, although *C. schantungensis* is a unique species, it still has a high basis of genetic diversity. Previous studies also show that narrow distributed species could have higher genetic diversity [28] [29].

Gene flow is very important for evolution of plants, and it has negative correlations with population genetic differentiation [30]. For spermatophyte, the gene flow among populations is mainly produced by foreign genes brought by pollens and seeds [31]. Gene flow among population Nm is less than 1.0, various populations could differentiate intensively, when it is between 1.0 and 4.0, gene flow could give play to its homogenizing function to prevent differentiation among each population caused by genetic drift, when Nm is bigger than 4.0, the population is a random unit [32]. The Nm of *C. schantungensis* is 5.8765, it shows that gene exchange among populations is very frequent, which weakens genetic differentiation among populations, and the genetic distance between two populations is only 0.0236, the genetic identity is high, it also highly inosculates with the value of

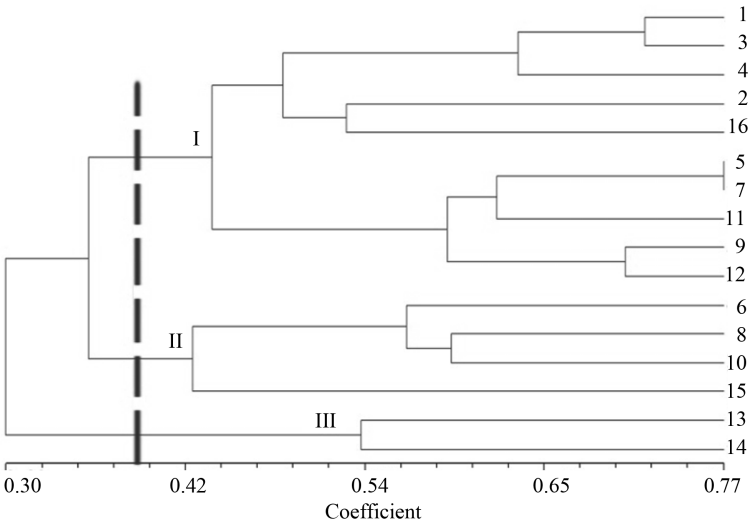

Figure 2. UPGMA cluster analysis of genetic similarity of 16 pieces of tested materials No. 1-No. 11: *C. schantungensis* derived from "Fo Yu"; No. 12-No. 16: *C. schantungensis* derived from "Red Leaves Valley".

gene flow among populations. Although most Cotoneaster are apomixes [33] [34], the research shows that *C. schantungensis* might be an exception.

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