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Genetic Structure and Diversity of Common Wild Rice of Different Populations in Hainan

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Abstract This study mainly explored the genetic diversity and structure of common wild rice (*Oryza rufipogon* Griff.) of Sanya population and Qionghai population in Hainan of China. By analyzing a total of 37 SSR markers in two populations, significant differences in genetic diversity and differentiation were revealed between these two populations. The results showed that the polymorphism rate of SSR loci in the Sanya population could reach 91.8919%, while the polymorphism rate in the Qionghai population was 81.0811%, indicating that the genetic variation level of the Sanya population was higher than that of the Qionghai population. By calculating the direct count heterozygosity, expected heterozygosity, and unbiased heterozygosity at each SSR locus, it was further confirmed that the Sanya population has a higher genetic variation. By calculating the genetic differentiation index (FST), the study found that the overall FST value of the two populations was 0.3909, indicating significant genetic structural differences between the two populations. The findings of this study can provide new insights into the genetic resources of common wild rice in Hainan and scientific basis for the conservation strategies of common wild rice in Hainan.

Keywords Ordinary wild rice (*Oryza rufipogon* Griff.); Genetic diversity; Genetic structure; SSR marking

Wild rice (*Oryza rufipogon* Griff.), a close ancestor of cultivated rice (*Oryza sativa* L.), is a significant genetic resource with important ecological and genetic value (Ding et al., 2022). It serves as a key species for studying rice evolution and is a crucial component of biodiversity conservation and genetic resources. Native to tropical and subtropical regions of Asia, particularly in southern China, the populations of wild rice on Hainan Island are especially rich. They thrive in diverse ecological environments, including riversides, wetlands, and forest undergrowth.

As a wild species, wild rice can survive and reproduce under natural conditions, displaying high adaptability and environmental tolerance. This adaptability is evident not only in its physiological traits but also in its rich genetic diversity (Tiwari et al., 2020). Compared to cultivated rice, wild rice retains more genetic variation, providing valuable genetic resources for rice cultivation. In recent years, with the rapid development of biotechnology, research on the genetic structure and diversity of wild rice has become a hotspot in genetics and agricultural science (Lam et al., 2019; Xie et al., 2020). These studies are of great scientific and practical significance for enhancing the adaptability and productivity of cultivated rice, as well as for understanding the ecological adaptability and evolutionary processes of plants.

Despite a certain research foundation on the genetic diversity of wild rice, our understanding of genetic differences among wild rice populations remains insufficient. Wild rice populations from different geographical locations may exhibit distinct genetic characteristics due to environmental selection or genetic drift. The specific differences and adaptive traits of these characteristics have not been fully studied. Particularly in Hainan Island, research on the genetic differences among wild rice populations and their adaptability to environmental changes is relatively scarce. This limits our comprehensive understanding and effective utilization of their genetic resources.

Moreover, due to agricultural expansion, urbanization, and climate change, the natural habitats of Hainan wild rice are rapidly shrinking, directly threatening the preservation of the genetic diversity of Hainan wild rice (Singh et al., 2018; Shan and Li, 2023). Therefore, in-depth research on the genetic structure and diversity of different wild rice populations in Hainan is not only crucial for revealing the mechanisms of their adaptive evolution but also provides scientific basis for the conservation and rational utilization of this precious genetic resource.

This study employs simple sequence repeat (SSR) marker technology to systematically analyze the wild rice populations in Sanya and Qionghai, Hainan. Given the increasing genetic homogenization of modern rice varieties, research on the genetic differences of wild rice populations holds great potential value for enhancing the genetic diversity, stress resistance, and adaptability of rice. Through this study, we aim to provide new insights into the genetic resources of wild rice in Hainan Island and offer scientific basis for conservation strategies of wild rice in Hainan Island.

1 Results and Analysis

1.1 Allele richness and polymorphism

In the genetic diversity study of different wild rice populations, this study utilized 37 pairs of SSR primers located on 12 chromosomes of the rice genome to perform PCR amplification on the genomic DNA of 11 wild rice samples from Sanya and 11 from Qionghai. On average, there were about three pairs of SSR primers per chromosome. Each pair of SSR primers successfully amplified clear and distinguishable DNA bands (Figure 1). Each primer pair corresponds to a specific gene locus on a chromosome. These loci exhibited various characteristics in genetic diversity, mainly reflected in the number of alleles.

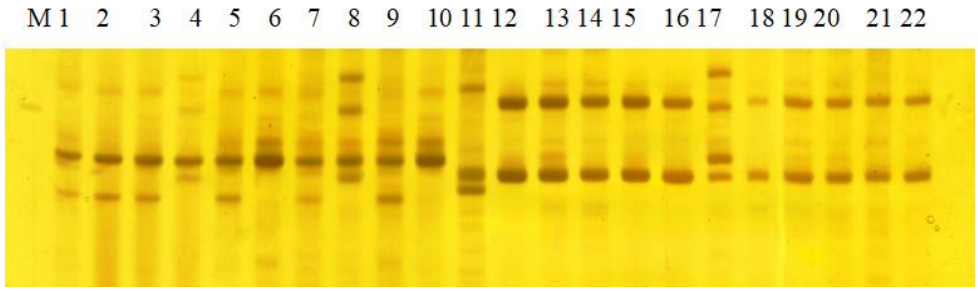

Figure 1 The result of PCR with RM42 in Sanya and Qionghai common wild rice

Note: M: DNA Marker; 1-11: Common wild rice from Sanya; 12-22: Common wild rice from Qionghai

The data shows that among these 37 SSR loci, the number of alleles ranged from 2 to 7, with a total of 107 alleles identified, averaging 3 alleles per locus. Specifically, loci with 2 alleles were the most common, with a total of 15 loci, accounting for 40.5405% of the total. This was followed by loci with 3 alleles, totaling 12 loci, making up 32.4324% of the total. Additionally, there were 3 loci with 4 alleles and 5 loci with 5 alleles, accounting for 8.1081% and 13.5135% respectively. Loci with 6 and 7 alleles each had 1 occurrence, representing only 2.7027% each (Figure 2).

From the perspective of genetic analysis, the distribution of allele numbers reflects the differences in genetic diversity at different gene loci. A higher number of alleles indicates greater genetic diversity. By analyzing allelic richness, we can better understand the current state of genetic diversity within the rice genome and the genetic structure within rice populations.

By assessing the polymorphism of SSR loci in the two populations, this study found that the polymorphism rate of SSR loci in the Sanya population reached 91.8919%, while the polymorphism rate in the Qionghai population was 81.0811%. These data indicate that the genetic variation level in the Sanya population is higher than that in the Qionghai population.

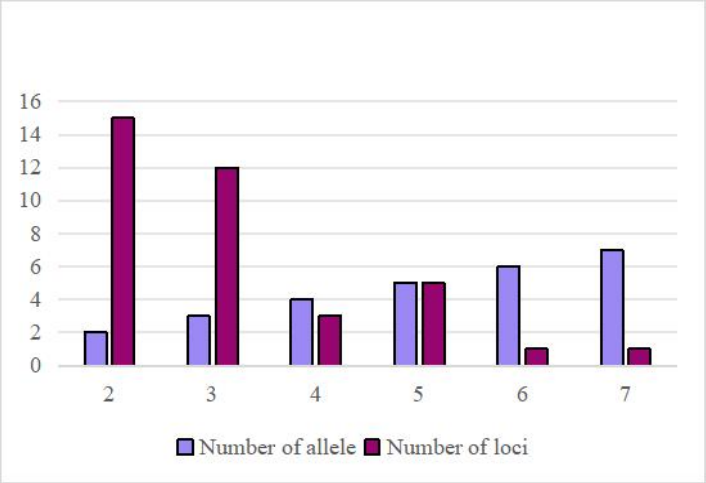

Figure 2 The distribution of alleles in SSR locus

1.2 Distribution of allele frequencies in two populations

By calculating allele frequencies (P=n/22), the study observed inconsistent distribution patterns of allele frequencies between the two populations, revealing significant genetic structure differences. Among the 107 alleles identified at 37 SSR loci, specific alleles on different chromosomes showed varying frequency distribution patterns between the two populations (Figure 3).

Fifty-three alleles on chromosomes 1, 2, 4, 5, 6, 7, 8, 9, and 11 exhibited significantly different distribution frequencies between the two populations. Among them, eight key alleles, RM5-4, RM18-2, RM11-2, RM42-2, RM206-2, RM17-3, RM20-1, RM11-1, showed highly dispersed frequency distributions between the two populations. The first seven alleles had very low frequencies in the Sanya population, nearly zero, while their frequencies in the Qionghai population were close to 1.0000. Conversely, RM11-1 had a high frequency (0.9091) in the Sanya population and was undetected in the Qionghai population, indicating clear population specificity.

These results reveal significant differences in genetic diversity and genetic structure between the two populations. These differences may be related to geographical isolation, ecological environment differences, or historical genetic drift. By comparing the distribution of allele frequencies, we can gain a deeper understanding of the genetic differences and potential evolutionary processes between the two populations.

1.3 Observed heterozygosity in two populations

By analyzing the SSR loci in the two populations, this study obtained detailed data on direct count heterozygosity, expected heterozygosity, and unbiased heterozygosity. The observed, expected, and unbiased heterozygosity at each SSR locus in the Sanya and Qionghai populations exhibited different characteristics.

Regarding observed heterozygosity, the SSR loci in the Sanya population ranged from 0.0000 to 1.0000. RM169 was completely homozygous with an observed heterozygosity of 0.0000, while RM5, RM280, and RM293 were completely heterozygous with an observed heterozygosity of 1.0000. Similarly, in the Qionghai population, observed heterozygosity ranged from 0.0000 to 1.0000. RM80 and RM127 were completely homozygous with observed heterozygosity of 0.0000, while RM293, RM185, RM162, RM19, RM202, RM229, RM295, and RM242 were completely heterozygous with observed heterozygosity of 1.0000.

In the Sanya population, the range of expected heterozygosity for SSR loci was 0.1653 to 0.7397. In comparison, in the Qionghai population, it ranged from 0.0868 to 0.5000. Additionally, the range of unbiased heterozygosity in the Sanya population was 0.1732 to 0.7749, whereas in the Qionghai population, it ranged from 0.0909 to 0.5584. On average, the observed heterozygosity, expected heterozygosity, and unbiased heterozygosity in the Sanya population were higher than those in the Qionghai population, being 0.5651, 0.4449, and 0.4661, respectively, compared to 0.4097, 0.2057, and 0.2670 in the Qionghai population. These data reveal high genetic variation in SSR loci in both populations, with the Sanya population showing significantly higher variation than the Qionghai population.

Figure 3 The comparison of frequencies of alleles at 37 SSR loci in Sanya and Qionghai populations

Note: The X-axis represents alleles; The Y-axis represents the frequency of alleles

By calculating the fixation index (F), this study also observed that the heterozygosity ratios of most SSR loci were significantly higher than expected under Hardy-Weinberg equilibrium, particularly in the Sanya population, where many loci had negative F values ranging from -0.9091 to -0.0449, indicating higher actual heterozygosity than theoretically expected (Table 1). Although the Qionghai population had 12 loci showing heterozygosity consistent with Hardy-Weinberg equilibrium, the remaining 15 loci also exhibited similar negative deviations, ranging from -0.9091 to -0.1665, with only one locus showing lower heterozygosity than expected under Hardy-Weinberg equilibrium, with an F value of 1. Overall, the fixation index (F) for SSR loci in the Sanya population was -0.2124, while in the Qionghai population, it was -0.5277.

Table 1 The value of heterozygosity fixation index and their mean value in two populations

SSR	Sanya population				Qionghai population			
locus	Direct count	Expected	Unbiased	F	Direct count	Expected	Unbiased	F
RM140	0.6364	0.6653	0.6970	0.0870	0.0909	0.0868	0.0909	0
RM48	0.2727	0.3512	0.3680	0.2590	0.0000	0.0000	0.0000	-
RM114	0.4545	0.3512	0.3680	-0.2351	0.7273	0.4628	0.4848	-0.5002
RM237	0.9091	0.5785	0.6061	-0.5000	0.0909	0.0868	0.0909	0
RM143	0.2727	0.5000	0.5238	0.4794	0.7273	0.4959	0.5195	-0.4000
RM127	0.8182	0.7397	0.7749	-0.0559	0.0000	0.1653	0.1732	1
RM131	0.4545	0.3512	0.3680	-0.2351	0.0909	0.0868	0.0909	0
RM293	1.0000	0.5000	0.5238	-0.9091	1.0000	0.5000	0.5238	-0.9091
RM280	1.0000	0.5413	0.5671	-0.7637	0.0000	0.0000	0.0000	-
RM185	0.8182	0.4835	0.5065	-0.6154	1.0000	0.5000	0.5238	-0.9091
RM11	0.1818	0.1653	0.1732	-0.0497	0.0909	0.0868	0.0909	0
RM111	0.5455	0.4959	0.5195	-0.0449	0.0909	0.0868	0.0909	0
RM162	0.6364	0.4339	0.4545	-0.4002	1.0000	0.5000	0.5238	-0.9091
RM126	0.1818	0.1653	0.1732	-0.0497	0.0000	0.0000	0.0000	-
RM171	0.4545	0.3512	0.3680	-0.2351	0.0000	0.0000	0.0000	-
RM321	0.4545	0.3512	0.3680	-0.2351	0.0000	0.0000	0.0000	-
RM19	0.9091	0.5620	0.5887	-0.5443	1.0000	0.5000	0.5238	-0.9091
RM202	1.0000	0.5000	0.5238	-0.9091	1.0000	0.5000	0.5238	-0.9091
RM206	0.6364	0.5413	0.5671	-0.1222	0.0909	0.0868	0.0909	0
RM222	0.0000	0.0000	0.0000	-	0.0909	0.0868	0.0909	0
RM229	0.7273	0.5826	0.6104	-0.1915	1.0000	0.5413	0.5671	-0.7634
RM239	0.7273	0.4628	0.4848	-0.5002	0.0909	0.0868	0.0909	0
RM3	0.8182	0.5413	0.5671	-0.4228	0.0000	0.0000	0.0000	-
RM160	0.0000	0.0000	0.0000	-	0.3636	0.2975	0.3117	-0.1665
RM163	0.4545	0.3512	0.3680	-0.2351	0.4545	0.3512	0.3680	-0.2351
RM295	0.7273	0.5620	0.5887	-0.2354	1.0000	0.5000	0.5238	-0.9091
RM20	0.5455	0.3967	0.4156	-0.3126	0.0909	0.0868	0.0909	0
RM5	1.0000	0.6198	0.6494	-0.5399	0.0000	0.0000	0.0000	-
RM17	0.5455	0.5785	0.6061	0.1000	0.0909	0.0868	0.0909	0
RM42	0.8182	0.6074	0.6364	-0.2857	0.0909	0.0868	0.0909	0
RM26	0.8182	0.5744	0.6017	-0.3598	0.9091	0.5331	0.5584	-0.6280
RM80	0.4545	0.7314	0.7662	0.4068	0.0000	0.3967	0.4156	1
RM242	0.9091	0.6198	0.6494	-0.4000	1.0000	0.5000	0.5238	-0.9091
RM169	0.0000	0.5195	0.4959	1	0.9091	0.4959	0.5195	-0.7500
RM166	0.0000	0.0000	0.0000	-	0.9091	0.4959	0.5195	-0.7500
RM2	0.5455	0.3967	0.4156	-0.3126	1.0000	0.5000	0.5238	-0.9091
RM18	0.1818	0.3140	0.3290	-0.4474	0.0909	0.0868	0.0909	0
Mean	0.5651	0.4449	0.4661	-0.2124	0.4079	0.2057	0.2627	-0.5527

1.4 Genetic Differentiation Between the Two Populations

Using Wright's F_{ST} method, this study analyzed the genetic differentiation of different SSR loci between the two populations. The results showed varying degrees of genetic differences at certain SSR loci (Table 2). Specifically, there was no genetic differentiation between the SSR loci RM222 and RM293, both with an F_{ST} value of 0.0000, indicating that these loci had identical genetic compositions in both populations. On the other hand, 10 SSR loci, including RM295, RM185, RM19, RM114, RM26, RM143, RM126, RM162, RM163, and RM131, showed minor genetic differentiation, with F_{ST} values ranging from 0.0083 to 0.0984. Additionally, 25 SSR loci, including RM160, RM148, RM171, RM321, RM239, RM242, RM2, RM3, RM202, RM111, RM237, RM127, RM229, RM80, RM140, RM280, RM169, RM166, RM42, RM206, RM17, RM5, RM20, RM18, and RM11, displayed significant genetic differentiation, with F_{ST} values ranging from 0.1500 to 0.8676, indicating substantial genetic differences between the two populations at these loci.

Overall, the global F_{ST} value was 0.3909, indicating that approximately 39.09% of the genetic variation was attributed to differences between the Sanya and Qionghai wild rice populations, highlighting significant genetic structure differences between them. These findings are crucial for understanding the genetic background and evolutionary history of these two populations.

Table 2 The value of F_{ST} for all loci and the mean of it

SSR locus	F_{ST}	SSR locus	F_{ST}
RM140	0.4182	RM222	0.0000
RM48	0.1800	RM229	0.3870
RM114	0.0175	RM239	0.2505
RM237	0.3750	RM3	0.3222
RM143	-0.0455	RM160	0.1500
RM127	0.3757	RM163	0.0974
RM131	0.0984	RM295	0.0083
RM293	0.0000	RM20	0.7469
RM280	0.4545	RM5	0.6690
RM185	0.0091	RM17	0.6403
RM11	0.8676	RM42	0.5812
RM111	0.3360	RM26	-0.0182
RM162	0.0545	RM80	0.3909
RM126	0.0500	RM242	0.2857
RM171	0.2000	RM169	0.4773
RM321	0.2000	RM166	0.5417
RM19	0.0167	RM2	0.2857
RM202	0.3333	RM18	0.7864
RM206	0.6238	Mean	0.3909

2 Discussion

Through genetic analysis of the Sanya and Qionghai populations of common wild rice, this study observed significant genetic differentiation between the two populations. Using simple sequence repeat (SSR) markers, we conducted a detailed analysis of the genetic diversity and structure of these populations. SSR markers were chosen for their high polymorphism and extensive application in population genetic structure analysis (Lade et al., 2020). The study found that although both populations maintained high genetic diversity, the Sanya population exhibited significantly higher genetic diversity than the Qionghai population. By calculating the observed heterozygosity, expected heterozygosity, and unbiased heterozygosity at each SSR locus, this study further confirmed the richness of genetic diversity in the Sanya population.

The calculation of Wright's FST value indicated a genetic differentiation of 39.09% between the Sanya and Qionghai populations of common wild rice. This value exceeds the average for wind-pollinated plants (G_{ST}=0.099) and the genetic differentiation average for perennial herbaceous plants (G_{ST}=0.227) (Gamba and Muchhala, 2020). However, this value is lower than that found in two other wild rice species in China—Oryza granulata and Oryza officinalis, with FST values of 0.8700 and 0.788, respectively (Lan et al., 2006). This difference in differentiation levels may be due to geographical isolation and different environmental pressures, or it may reflect the impact of human activities on population genetic structure.

The genetic research of the Sanya and Qionghai populations of common wild rice not only revealed their rich genetic diversity but also emphasized the importance of conserving these genetic resources. Genetic diversity is crucial for species to adapt to environmental changes, especially in the context of current global climate change. Maintaining genetic diversity is essential for the long-term survival of species (Lubis and Iksan, 2023). Moreover, since common wild rice contains many important genes that can enhance the disease resistance and adaptability of cultivated rice, conserving these wild resources is invaluable for maintaining agricultural biodiversity and crop improvement.

In the future, we should strengthen the protection of the natural habitats of common wild rice to maintain its high genetic variation. Additionally, future research should more extensively use high-throughput genetic marker technologies, such as single nucleotide polymorphism (SNP) markers, to obtain more detailed genetic information (Morales et al., 2020). Furthermore, research should be expanded to more geographical locations and larger populations to comprehensively assess the genetic diversity and structure of common wild rice globally. By thoroughly analyzing the genetic resources of common wild rice, we can not only enhance our understanding of its genetic structure but also better utilize these resources to promote sustainable agricultural development and biodiversity conservation.

3 Materials and Methods

3.1 Study area and sample collection

This study selected Qionghai and Sanya regions on Hainan Island as sample collection sites for common wild rice. In the Qionghai region, samples were collected from 11 points centered around Fenglou Village. In the Sanya region, samples were collected around the Hainan Institute of Tropical Agricultural Resources Development and Utilization. Eleven samples were collected from each region using a random sampling method to ensure the representativeness and reliability of the data. During sample collection, fresh leaves were collected using sterile tools, immediately frozen in liquid nitrogen, and then transferred to a -80°C freezer to ensure the integrity and viability of the DNA.

3.2 DNA extraction

DNA extraction from common wild rice was performed using a modified CTAB method (Aboul-Maaty and Oraby, 2019). The specific steps are as follows: First, 2~3 g of fresh leaf samples were weighed, frozen with liquid nitrogen, and ground into a powder. The powder was transferred to a preheated 15 mL centrifuge tube containing 7 mL of preheated 1.5× CTAB extraction buffer. The mixture was incubated in a 65°C water bath for 30 minutes. It was then cooled to room temperature, and 4 mL of chloroform/isoamyl alcohol (24:1) was added. After mixing, the solution was centrifuged at 4 000 rpm at room temperature for 20 minutes. The supernatant was transferred to a new centrifuge tube, 1/10 volume of 10% CTAB and an equal volume of chloroform/isoamyl alcohol were added, mixed, and centrifuged again at 4000 rpm for 20 minutes. The supernatant was transferred to a new centrifuge tube, and an equal volume of 1% CTAB precipitation buffer was added, gently shaken until a flocculent DNA precipitate formed, and centrifuged at 3000 rpm for 10 minutes to pellet the DNA. The pellet was resuspended in 1.5~2 mL of 1N NaCl and 5 μL of RNase A, and incubated overnight at 56°C. Once the DNA was fully dissolved, 2~3 mL of 95% ice-cold ethanol (pre-cooled to 4°C) was added to precipitate the DNA, which was then collected and washed with 70% ethanol for 30 minutes, followed by a 5-minute wash with 95% ethanol, and air-dried. The air-dried DNA was dissolved in 1 mL of TE buffer, stored at 4°C for immediate use, or dried for long-term storage.

3.3 SSR primer selection and PCR reaction

In this experiment, the RM series SSR primers from the rice genome, synthesized by Shanghai Shenggong Company, were used, totaling 37 pairs (Table 3). Using the total DNA of common wild rice as the template, PCR amplification was performed with SSR primers distributed across 12 chromosomes.

The total volume of the PCR reaction was 15 μL, consisting of 9.98 μL of sterile ultrapure water, 1.5 μL of 10× PCR Buffer (500 mmol/L KCl; 100 mmol/L Tris-HCl, pH 9.0; 1% Triton X-100), 1.0 μL of 20 mmol/L MgCl2, 0.4 μL of dNTPs (dATP, dCTP, dTTP, dGTP, each at 25 mmol/L), 0.5 μL of each forward and reverse primer at 4.5 μmol/L, 0.12 μL of 5U Taqase (provided by Promega), and 1.5 μL of 20 ng template DNA.

The PCR reaction program was as follows: pre-denaturation at 94°C for 5 minutes; denaturation at 94°C for 45 seconds, annealing at 55°C for 45 seconds, and extension at 72°C for 1 minute, for 35 cycles; followed by a final extension at 72°C for 10 minutes. The PCR amplification of total DNA from common wild rice was performed using a PTC-100 PCR amplifier from MJ Research Inc. After completing the PCR amplification, the PCR products were subjected to non-denaturing polyacrylamide gel electrophoresis.

Table 3 The 37 pairs of rice SSR primers used in the experiment

Chromosome	No. of rice SSR primer
1	RM5、RM140、RM237
2	RM18、RM48、RM166
3	RM114、RM143、RM293
4	RM127、RM131、RM185、RM280
5	RM26、RM163、RM169
6	RM3、RM111、RM162
7	RM2、RM11、RM295
8	RM42、RM80、RM126
9	RM160、RM242、RM321
10	RM171、RM222、RM239
11	RM202、RM206、RM229
12	RM17、RM19、RM20

3.3 Data recording

In this study, the SSR primers used are codominant markers, with each pair of primers corresponding to a single locus. After PCR amplification using specific SSR primers, the resulting products are represented by bands on a non-denaturing polyacrylamide gel electrophoresis, which indicate different alleles. Each band position is marked by an Arabic numeral representing one allele, while another numeral represents another allele. DNA bands with the same migration rate, amplified using the same SSR primer set across all samples, are considered the same allele and are marked with the same Arabic numeral. This method allows for precise differentiation and recording of the genetic diversity at each locus in different individuals.

3.4 Statistical analysis

When analyzing the genetic data for each SSR locus, allele frequency and polymorphism rate were calculated for each locus. Additionally, the heterozygosity at each SSR locus in the population was analyzed in detail, including observed heterozygosity, expected heterozygosity, and unbiased heterozygosity estimates. Unbiased heterozygosity is adjusted based on sample size and provides a more accurate reflection of actual genetic diversity. Moreover, using Wright's F_{ST} method (Kitada et al., 2020), the degree of genetic differentiation between the two populations at different SSR loci was calculated using the appropriate formula $F_{ST} = \frac{H_T - H_S}{H_T}$.

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Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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