GENETIC DIVERSITY ANALYSIS OF 20 ABIU GERMPLASM BASED ON SCoT MOLECULAR MARKERS

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Abstract. Abiu is a new, excellent, and rare fruit. The genetic diversity of 20 Abiu germplasm resources was determined using SCoT molecular marker technology. A total of 361 bands were amplified using 24 selected primers, including 309 polymorphic bands, with an average polymorphism ratio (PPB) of 85.60%. The number of alleles (*Na*) in the population was 1.9501, number of effective alleles (*Ne*) was 1.4659, gene diversity index (*H*) was 0.2806, and Shannon diversity index (*I*) was 0.4314, indicating high genetic diversity among the materials. The results of cluster analysis showed that the inter-specific genetic coefficient of 20 varieties of Abiu was between 0.53-0.76, and the results of principal component analysis were consistent with those of cluster analysis. The analysis of the population structure revealed that the 20 Abiu samples could be categorized into seven distinct groups. Of all the tested materials, 14 samples had diverse genetic backgrounds and complex genetic compositions, accounting for 70% of the tested materials, suggesting that the population genetics of the 20 samples had a rich genetic basis and complex and diverse sources. SCoT, which is an effective molecular marker, can be used to analyze the genetic diversity of the germplasm resources of Abiu. This study will be useful for the identification, collection, classification, protection, and evaluation of Abiu germplasm resources.
Keywords: *rare fruits, molecular marker technology, population structure, genetic composition, identification*

Introduction

Pouteria caimito, a member of the Sapotaceae family, is commonly referred to as yellow star apple, honey egg yolk fruit, or golden fruit. They are also known as Abiu and Caimito. This tropical evergreen fruit tree is native to the upper Amazon River region and thrives in warm and humid climates. In 1987, the Taiwan Fengshan Tropical Horticulture Research Institute introduced Abiu from Singapore to Taiwan, China.

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Later, Taiwan Pingtung University of Science and Technology introduced Abiu from the University of the Philippines and Hawaii for trial planting and selected Abiu superior line (Yan, 2002; Wang et al., 2020). Abiu is mainly distributed in Venezuela, Peru, Ecuador, Trinidad, Tobago, and Brazil, and planted in the Philippines, Malaysia, and Hawaii in the United States. The introduction of Abiu in China has been limited to a few areas, such as Hainan, Guangxi, Guangdong, Yunnan, and Fujian, although the scale of economic planting development is increasing rapidly. Nonetheless, the development of commercial and economically viable cultivation methods has not yet been established (He et al., 2012; Wang et al., 2020; Zhou et al., 2020).

Superior strains of Abiu, originally from Taiwan, have been introduced to various Chinese provinces, including Hainan, Guangxi, Guangdong, Yunnan, and Fujian. These strains have been successfully cultivated, and after a period of growth, they display normal blossoming and fruiting behaviors and excellent biological characteristics. By measuring and determining a number of economic traits of Abiu, it was shown that the Abiu introduced in Hainan and Guangxi has good fruit economic traits (He et al., 2012; Wang et al., 2020), which proves that the introduction of Abiu in China has great potential and development space, and can provide a reference for the planting of Abiu in tropical and subtropical countries. Abiu is a fruit distributed in various countries and regions, and owing to its introduction, there is also a mix of varieties. The Abiu introduced in China has been planted in different places and sold in the market without standardization, leading to confusion in the seedling market and the serious phenomenon of synonyms. The next steps in variety classification and naming must be on the agenda.

The appearance of the Abiu is bright and yellow-like crystals, the sweetness is high, the flesh is soft, plant diseases and insect pests are few, and the perennial fruit tree can pick fruit all year round and is very suitable for cultivation in tropical and partial subtropical regions. Abiu is a fruit with a high edible value and is packed with essential nutrients such as protein, ash powder, vitamin B2, tryptophan, and lysine. It is also rich in fibre and pectin, which can prevent and alleviate constipation, aid detoxification, and promote skin health when consumed regularly. In addition, Abiu has been recognized for its medicinal properties. The derivative products of Abiu, such as Abiu juice, mixed fruit, cocktail, fruit salad, sauce, and yoghurt, also have broad market prospects (Lim et al., 1992; Long et al., 2020), and the economic opportunities to plant Abiu are promising (Yan et al., 2002; Long et al., 2020).

The evaluation of molecular markers represents a breakthrough in the identification of genetic variation (Nadeem et al., 2021). The application of molecular markers plays a vital role in the identification of unique genotypes or varieties of plants, genetic improvement, evaluation of germplasm resources, and selection of effective breeding strategies (Igwe et al., 2017). In recent years, with the development of Inter simple sequence repeat (ISSR) molecular markers, ISSR technology has been increasingly used in the analysis of the genetic diversity of fruits and vegetables, such as banana (*Musa nana* Lour.) (Lu et al., 2020) and papaya (*Pseudocydonia sinensis* (Thouin) C. K. Schneid.) (Jiang et al., 2020), lute (*Eriobotrya japonica*) (Liu et al., 2021a), onion (*Allium cepa* L.) (Liu et al., 2021b). However, compared with the target initiation codon polymorphism molecular marker (start codon targeted polymorphism, SCoT), the latter is simpler and more universal, has lower cost, and has the advantages of high repeatability and rich polymorphism (Xiong et al., 2009). Collard and Machill (2009) developed SCoT molecular markers and designed 36 SCoT single primers from a short-conserved sequence in the ATG start codon region of a plant gene. The principle of

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SCoT molecular markers is to design a single primer based on the conservation of sequences flanking the ATG translation start site in plant genes to amplify the genome and generate dominant polymorphic markers biased towards candidate functional gene regions (Xiong et al., 2009). SCoT markers have been used in population genetics and genetic diversity analysis of various plants. Such as cereals (Feng et al., 2021), legumes (Yeken et al., 2022), fruits (Qi et al., 2022) and vegetable crops (Pi et al., 2020), medicinal plants (Shekhawat et al., 2018), forest trees (Alikhani et al.2014), ornamental plants (Mostafavi et al., 2021) and other economically important plants (Arya et al., 2014; Heikrujam et al., 2015; Chhajer et al., 2017; Golkar and Nourbakhsh, 2019; Liu and Esfandani, 2022). Much progress has been made in genetic diversity analysis among species and varieties and genetic analysis of hybrid offspring. Yeken et al. (2022) used SCoT molecular marker technology to analyze the genetic diversity of 53 common bean (*Glycine max* (Linn.) Merr.) varieties, 22 Turkish-registered bean varieties, and 12 genotypic bean varieties were registered by the United States Department of Agriculture. Finally, the genetic diversity of common bean varieties was determined, and the role of SCoT molecular markers in the genetic population structure was confirmed. This increases the efficiency and accuracy of genetic breeding; Pi et al. (2020) used SCoT molecular markers to identify 15 sugar beet (*Beta vulgaris* L.) varieties and used the unique characteristics of SCoT to rapidly identify the authenticity of sugar beet varieties using fewer primers, which promoted the rapid development of the sugar beet industry; Alikhani et al. (2014) analyzed the genetic variation and structure of 125 Quercus fabri (*Quercus acutissima*) resources in different geographical locations using SCoT, ISSR, and Irap molecular markers. The results showed that SCoT molecular markers could better reflect the genetic diversity among individuals. SCoT molecular markers can be used to further understand the genetic relationship of Quercus fabri and provide a more solid molecular biological basis for protecting wild forest resources; Arya et al. (2014) Used SCoT molecular marker technology to analyze the genetic structure and population structure of 20 germplasm resources of Maoni fruit (*Morinda tomentose* Heyne) to evaluate its genetic variation, thus achieving the purpose of further collection, protection, and utilization of the germplasm resources of Nona marina in India and worldwide. Therefore, SCoT molecular markers are used in many plant species and play different roles; however, SCoT molecular markers have not been reported in the genetic diversity analysis of Abiu. Based on previous studies of SCoT markers, the SCoT analysis system of Abiu can be established. A study was conducted to analyze the genetic diversity of 20 Abiu germplasm resources by using DNA-level genetic material. This study aimed to investigate the genetic relationship, population structure, and complexity of the genetic background among the tested materials. This study aimed to address the issue of the same Abiu varieties being marketed under different names, and to improve the development, utilization, and protection of Abiu germplasm resources. Abiu is a rare fruit with a high economic and nutritional value. In this study, the genetic diversity of 20 Abiu germplasm resources was analyzed using the SCoT molecular marker, and the genetic relationship and genetic background of the tested materials were analyzed at the molecular genetic level, which filled the gap in the SCoT molecular marker of Abiu at home and abroad. This research can facilitate the establishment of an Abiu germplasm resource nursery and standardize the breeding process for Abiu. Furthermore, it can improve the utilization and protection of Abiu germplasm resources, ultimately enhancing the market quality of Abiu. In doing so, it can promote economic cultivation and further develop the economic market of Abiu.

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Materials and methods

Test materials

The 20 fruit samples for the analysis were obtained from Jiangmen, Zhanjiang, and Guangzhou test bases (*Table 1*). 20 samples of fresh and tender leaves were collected. Each sample was mixed with five trees of the same age. When sampling from a single tree, the samples were mixed from the top, bottom, middle, inside, and outside, and then the collected samples were placed in the corresponding sample bags for storage, immediately stored in liquid nitrogen, taken back to the laboratory, and placed in a refrigerator at - 80°C for standby.

Table 1. *20 germplasm resources of Abiu fruit*

Sample No	Sample name	Sampling place
1	Golden Peach	Jiangmen, China
2	Kim Mi	Jiangmen, China
3	Jin Xue	Jiangmen, China
4	Jin Duo	Jiangmen, China
5	Gold No.6	Zhanjiang, China
6	Jin Le	Guangzhou, China
7	Jin Yu	Jiangmen, China
8	Jin Jiu	Jiangmen, China
9	King Long	Jiangmen, China
10	Jin Ding	Jiangmen, China
11	Golden Fruit	Jiangmen, China
12	Dajinsha	Zhanjiang, China
13	Abiu Dong Mi	Zhanjiang, China
14	White Crystal	Zhanjiang, China
15	Jiuda No.1	Zhanjiang, China
16	Shengda No.1	Zhanjiang, China
17	Topaz	Zhanjiang, China
18	Abiu Pount	Zhanjiang, China
19	Golden Bay	Jiangmen, China
20	Abiu Sweet	Zhanjiang, China

The base sequences of 80 primers were designed according to the primers designed by Qin and Hu (2016), and were synthesized by Sangon Biological (Shanghai) Co., Ltd. The DNA marker and 2 × Ex Taq Master Mix (containing dye) were purchased from Yisheng Biotechnology (Shanghai) Co., Ltd., and Ethidium Bromide (EB) was purchased from Sangon Biological (Shanghai) Co., Ltd.

Extraction and detection of total DNA from leaves

According to Doyle (Doyle and Doyle, 1987), DNA was extracted from the leaves of 20 kinds of Abiu in 100-200 mg samples; there were many polysaccharides and phenols in the leaves of Abiu. Therefore, in the process of extracting DNA from Abiu, attention should be paid to removing polysaccharides and phenols from leaves. Polyvinylpyrrolidone (PVP) was added during blade grinding to prevent oxidative

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browning of the material, the quality of the extracted DNA was detected by 1% agarose gel electrophoresis, and a microplate reader was used to detect its purity and concentration. Finally, the extracted DNA was diluted to 50 ng/μL with TE buffer and stored in a refrigerator at -20°C for later use.

Establishment of SCoT-PCR reaction system for Abiu

The SCoT-PCR method, described by Han et al. (2011), Huang et al. (2013) and Chen et al. (2010), the Abiu SCoT-PCR reaction system was established and optimized. The reaction mixture consisted of 2 × Taq PCR Mix 10 μl, DNA 1 μl, primer 0.6 μl, and ddH_2O 8.4 μl. Reaction system: pre-deformation at 94°C for 5 min, deformation at 94°C for 1 min, annealing at 50°C for 1 min, extension at 72°C for 2 min for a total of 35 cycles, and a final extension at 72°C for 8 min.

Polymorphic primer screening and PCR amplification detection

80 primers were diluted to 10 μmol/L with TE buffer, and 20 kinds of DNA samples were mixed with 80 primers for PCR amplification.80 primers were screened, and the PCR products were detected by 2% agarose gel electrophoresis, and the primers that could be successfully amplified were selected by referring to Zhang et al. (2015). Three kinds of Abiu ('Golden Peach', 'King Long', and 'Jin Ding') with large leaf phenotypic differences (*Figs. 1a-l* and *2*; *Table 2*), and they were selected for secondary screening of the primers obtained after the primary screening, and the PCR products were electrophoresed with 2% agarose gel to select the primers with clear bands and good polymorphism. Primers with good polymorphism selected after the secondary screening were used to carry out PCR amplification on all Abiu fruit DNAs. All PCR products were detected using 2% agarose gel electrophoresis, and an electrophoretogram was generated by photographing the gel imaging system.

Data processing and analysis

According to the electrophoresis results of the PCR amplification products, the "0/1" system was used to record the position of the bands, and the "0/1" matrix of SCoT markers was established to count the polymorphic bands and calculate the polymorphism ratio (Zhao et al., 2012). Polymorphic bands refer to bands that exist in some materials but not in other materials for a certain amplification band in the tested materials. Polymorphism ratio (%) = number of polymorphic bands/total number of bands × 100% (Chen et al., 2018). The number of polymorphic loci, percentage of polymorphic loci, number of alleles (*Na*), number of effective alleles (*Ne*), gene diversity index (*H*), and Shannon diversity index (*I*) was calculated using software POPGENE 1.32 (Yeh et al., 1999; Li et al., 2021).

NTSYS-pc2.10e software was used to calculate the similarity coefficient between samples, and a similarity coefficient heat map was constructed using the unweighted pairwise arithmetic average (UPGMA) method for cluster analysis (Zhao et al., 2012). Principal component analysis (PCA) was performed based on the genetic similarity coefficient.

Software Structure 2. 3. 4 was used to analyze the population structure of the Abiu varieties. The K value of the number of groups was set to 2-10, each parameter was run 20 times, the burn-in time of each run was set to 50000, and the number of repetitions was 50000. The calculation results were packaged and uploaded to

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Structure Harvester (https://taylor0.biology.ucla.edu/structureHarvester/), determining the optimal cluster number K according to the ΔK value in the analysis results, and finally constructing a population structure diagram of 20 Abiu fruit germplasms (Li et al., 2021).

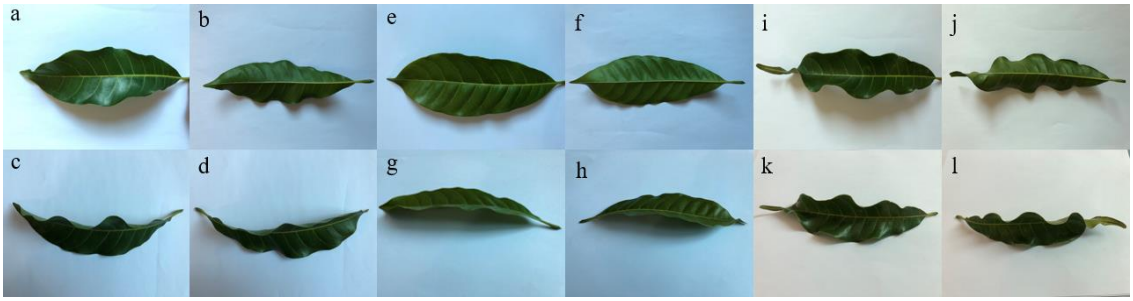

Figure 1. *(a-d) Respectively the upper, lower, left, and right parts of the leaves of the 'Golden Peach'; (e-h) Respectively the upper, lower, left, and right parts of the leaves of the 'King Long';(i-l) Respectively the upper, lower, left, and right parts of the leaves of the 'Jin Ding'*

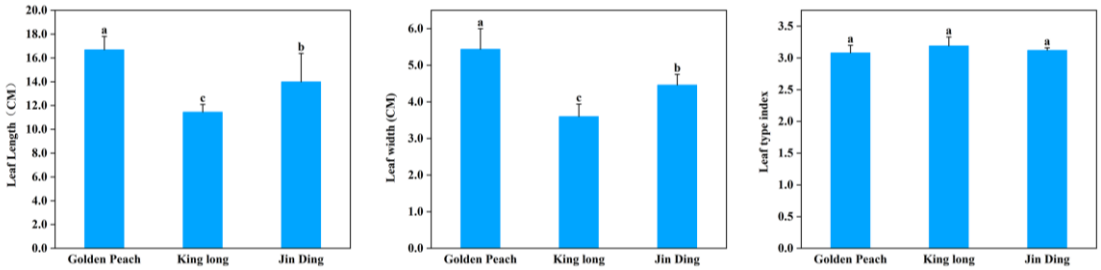

Figure 2. *Leaf length (a), leaf width (b), and leaf type index (c) of three types of Abiu with significant differences. Values represent the mean ± SD of 20 independent biological replicates. Lowercase letters indicate significant differences among different leaves at the same time point ($p < 0.05$)*

Table 2. *Leaf related indicators of three types of Abiu with significant differences in leaf phenotype*

Leaves related indicators	Sample name		
	Golden Peach	**King Long**	**Jin Ding**
Leaf shape	Lanceolate	Lanceolate	Lanceolate
Leaf tip shape	Sharp pointed shape	Sharp pointed shape	Sharp pointed shape
Leaf base shape	Wedge	Wedge	Wedge
Leaf edge shape	Shallow wavy	Full edge or shallow wavy	Wavy
Leaf edge waviness	Mild	Near smooth	Moderate
Leaf length (cm)	16.69 ± 1.12a	11.45 ± 0.64c	14.00 ± 2.38b
Leaf width (cm)	5.44 ± 0.56a	3.6 ± 0.34c	4.46 ± 0.29b
Leaf type index	3.08 ± 0.12a	3.19 ± 0.14a	3.12 ± 0.04a
Foliage color	Dark green	Yellow green	Dark green
Vein	Pinnate vein	Pinnate vein	Pinnate vein

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Results

Extraction and detection of genomic DNA from leaves

The extracted DNA was detected by 1% agarose gel electrophoresis, and the results showed that the extracted DNA bands were clear and obvious, with no apparent tailing phenomenon. A microplate reader was used to detect the purity, and the results showed that the A260/A280 ratio of the extracted DNA samples was between 1.8 and 1.9, indicating that the purity of the extracted DNA was high. The concentrations were all > 100 ng/μL. DNA was of good quality and could be used in subsequent experiments.

Polymorphism analysis of SCoT-PCR amplified products

Through primary and secondary screening of primers, 24 primers with clear amplification bands and good polymorphism were screened from 80 primers that were used for SCoT-PCR amplification of 20 Abiu DNAs (*Table 3*). The results showed that 361 bands were amplified using the 24 primers, with an average of 15.04 primers per primer, of which 309 bands were polymorphic, with an average of 12.88 bands and the average percentage of polymorphism ratio (PPB) was 85. 60%. The amplification results of primers S11, S43, S52, and S69 on the 20 Abiu germplasms are shown in *Figure 3a-d*. The amplified electrophoretic bands were clear, polymorphic, and specific, and SCoT markers were abundant.

Table 3. *SCoT primer amplification sequence and amplification results*

Primer number	Primer sequences (5'~3')	Total number of strips/strip	Number of polymorphic bands/band	Polymorphism ratio/%
S11	AAGCAATGGCTACCACCA	12	10	83.3
S18	ACCATGGCTACCACCGCC	20	17	85.0
S21	ACGACATGGCGACCCACA	19	16	84.2
S22	AACCATGGCTACCACCAC	11	6	54.5
S26	ACCATGGCTACCACCGTC	16	12	75.0
S28	CCATGGCTACCACCGCCA	16	16	100.0
S30	CCATGGCTACCACCGGCG	17	14	82.4
S33	CCATGGCTACCACCGCAG	15	14	93.3
S34	ACCATGGCTACCACCGCA	14	13	92.9
S37	ACGACATGGCGACCAGCG	17	14	82.4
S38	AAGCAATGGCTACCACCG	11	9	81.8
S43	CAATGGCTACCACCGCAG	17	17	100.0
S46	CCATGGCTACCACCGGCA	13	11	84.6
S47	ACAATGGCTACCACTGCC	16	12	75.0
S48	ACAATGGCTACCACTGGC	15	14	93.3
S52	ACAATGGCTACCACTGCA	14	10	71.4
S61	CAACAATGGCTACCACCG	11	10	90.9
S67	ACCATGGCTACCAGCGGC	17	17	100.0
S69	ACCATGGCTACCAGCGCA	14	10	71.4
S71	CCATGGCTACCACCGCCG	14	11	78.6
S72	CCATGGCTACCACCGCCC	13	12	92.3
S74	CCATGGCTACCACCGGCA	16	16	100.0
S75	CCATGGCTACCACCGGAG	17	14	82.4
S77	CCATGGCTACCACTACCC	15	14	93.3

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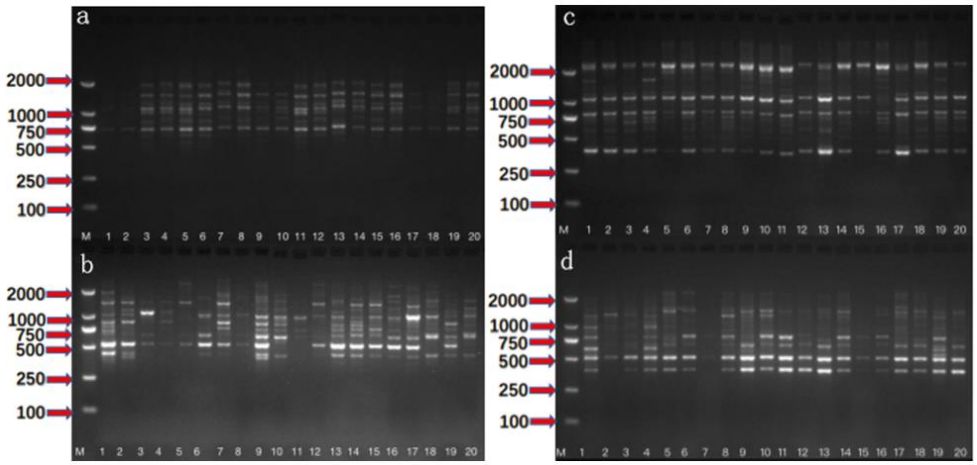

Figure 3. *(a) The amplification results of primer S11 on 20 germplasm resources of Abiu; (b) The amplification results of primer S43 on 20 germplasm resources of Abiu; (c) The amplification results of primer S52 on 20 germplasm resources of Abiu; (d) The amplification results of primer S69 on 20 germplasm resources of Abiu. M:2000-bp DNA Marker; 1:'Golden Peach'; 2:'Kim Mi'; 3:'Jin Xue'; 4:'Jin Duo'; 5:'Gold No.6'; 6:'Jin Le'; 7:'Jin Yu'; 8:'Jin Jiu'; 9:'King Long'; 10:' Jin Ding'; 11:'Golden Fruit'; 12:'Dajinsha'; 13:'Abiu Dong Mi'; 14:'White Crystal'; 15:'Jiuda No.1'; 16:'Shengda No.1'; 17:'Topaz'; 18:'Abiu Point'; 19:'Golden Bay'; 20:'Abiu Sweet'*

Analysis of population diversity

POPGENE1.32 software was used to analyze the amplification results of 24 primers, and the results showed that the number of polymorphic loci was 343, and the percentage of polymorphic loci was 95. 01%. The Number of alleles (*Na*) was 1.9501, the number of effective alleles (*Ne*) was 1.4659, Nei's genetic diversity index (*H*) was 0.2806, and Shannon diversity index (*I*) was 0.4314 (*Table 4*). The results showed rich genetic diversity among the 20 Abiu germplasms.

Table 4. *Information of population genetic diversity*

Number of alleles (*Na*)	Number of effective alleles (*Ne*)	Nei's genetic diversity index (*H*)	Shannon diversity index (*I*)
1.9501	1.4659	0.2806	0.4314

Analysis of genetic diversity

The NTSY-PC2.10 software was used to analyze the genetic similarity coefficient of the experimental data, and a heat map of the genetic similarity coefficient was created (*Fig. 4*). The results showed that the genetic similarity coefficients of 20 Abiu germplasm resources ranged from 0.53 to 0.76, among which 'Gold No.6' and 'Jin Le' had the highest genetic similarity coefficients of 0.6096. The results showed that The genetic relationship between 'Gold No.6' and 'Jin Le' was the closest among the 20 germplasms. The genetic similarity coefficient between 'Golden Peach' and 'Golden Fruit' was the smallest, at 0. 2832, indicating that their genetic relationships were the strongest. Genetic similarity coefficient analysis showed noticeable genetic differences among the 20 Abiu germplasm resources, and they had rich genetic diversity.

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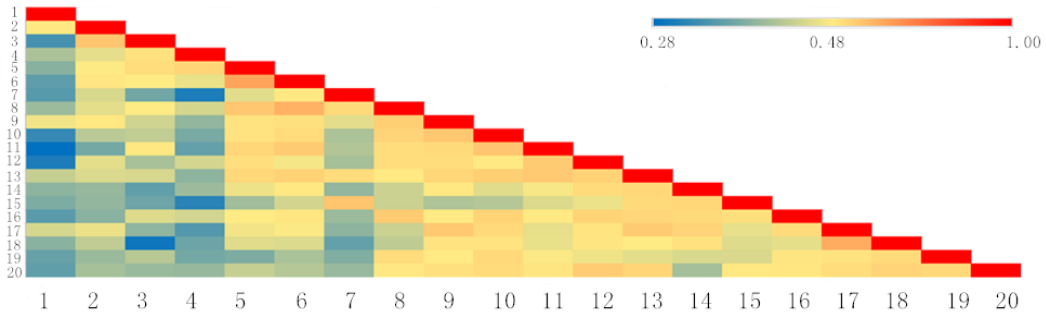

Figure 4. *Heat map of genetic similarity coefficient of 20 Abiu fruits materials*

Cluster analysis

Based on the genetic similarity coefficient, the UPGMA method and NTSY-PC2.10 software were used for cluster analysis of the 20 Abiu germplasm resources, and the results are shown in *Figure 5*. It can be seen from the dendrogram that when the genetic similarity coefficient was 0. 53, the 20 Abiu fruits were clustered into two groups: one was the Abiu fruit of 'Golden Peach', and the remaining 19 Abiu fruits were clustered into one group. When the genetic similarity coefficient was 0. 59, 20 Abiu fruits were clustered into four groups. When the genetic coefficient was 0.64, the 20 Abiu fruits were clustered into nine groups, which were as follows: the first: 'Golden Peach'; Second: 'Kim Mi', 'Jin Xue'; Third: 'Jin Duo'; Fourth: 'Gold No.6', 'Jin Le', 'Jin Jiu', 'King Long', 'Jin Ding', 'Golden Fruit', 'Abiu Dong Mi'; Fifth: 'White Crystal', 'Shengda No.1'; Sixth: 'Dajinsha', 'Abiu Sweet'; Seventh: 'Topaz', 'Abiu Point'; Eighth: 'Golden Bay'; Ninth: 'Jin Yu', 'Jiuda No.1'.

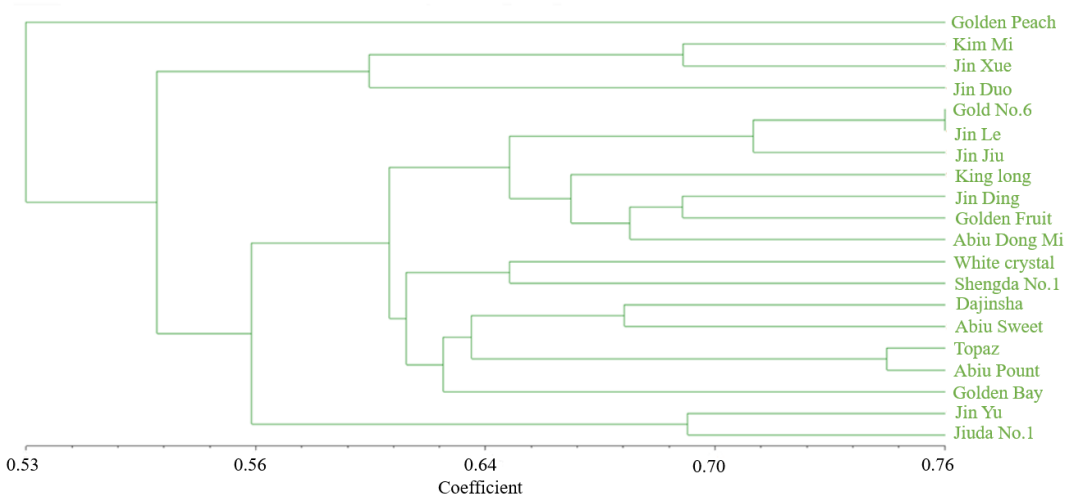

Figure 5. *Genetic cluster diagram of 20 Abiu fruits based on SCoT molecular markers*

Principal component analysis

The principal component analysis was performed according to the genetic similarity coefficient, and a two-dimensional scatter plane distribution map was drawn. The results are shown in *Figure 6*. The first and second principal coordinates are explained

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as 16. 64%, and 13. 38% of the intervarietal correlation and the cumulative contribution rate was 30. 02%, representing the primary information of the original data. In the principal coordinate analysis diagram, the closer the distance between germplasm materials, the closer the genetic relationship, and vice versa (Li et al., 2020). The results showed that the results of principal component analysis and cluster analysis were highly similar, and the genetic relationship among germplasms could be seen more intuitively, such as the distance of No.1 'Golden Peach' was far, the distance of No.2 'Kim Mi', No.3 'Jin Xue' and No.4 'Jin Duo' was close, and the distance of No.5 'Gold No.6', No.6 'Jin Le' and No.8 'Jin Jiu' was close. The combination of cluster analysis and principal component analysis could be more effective in analyzing the genetic relationships among the 20 Abiu germplasms.

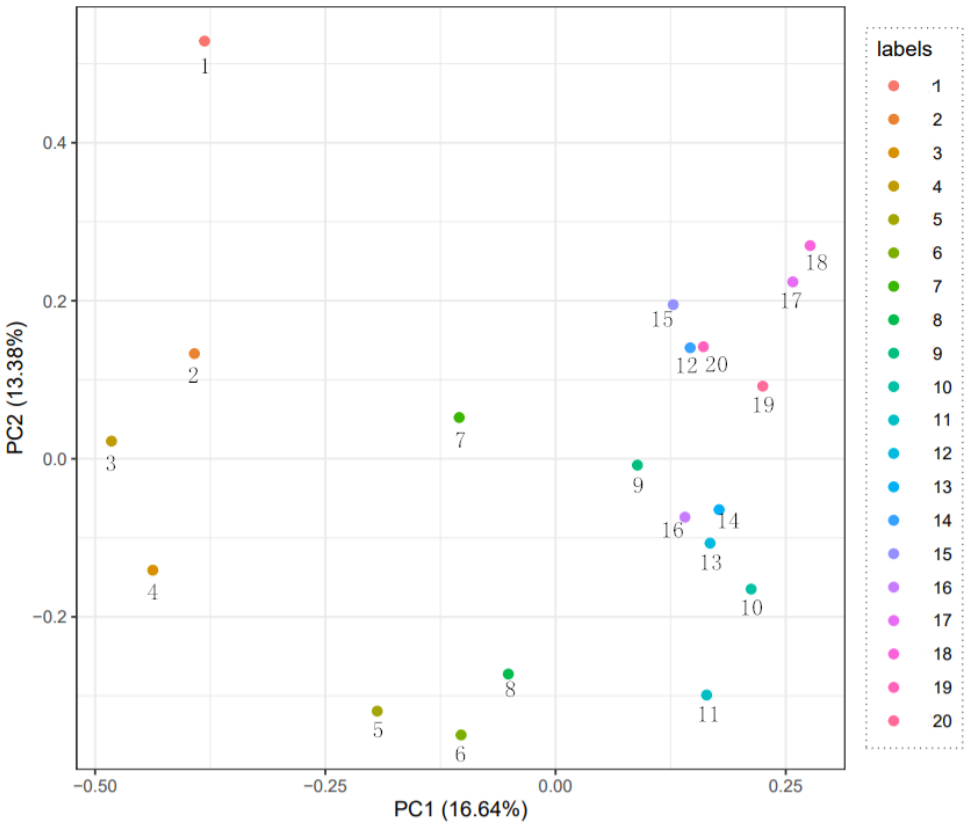

Figure 6. *Principal component analysis of 20 Abiu fruits germplasms based on SCoT marker*

Analysis of group structure

The optimum K value was determined by calculating the ΔK value using the analysis results of the Structure Harvester web page with reference to the method described by Evanno et al. (2005). Through analysis of the change in ΔK, it was found that when K = 7, the value of ΔK was the largest (*Fig. 7*). Therefore, the test materials were divided into seven groups, and the population structure of 20 germplasms of Abiu was drawn (*Fig. 8*). Different colors represent different groups, represented by S1 red, S2 green, S3 blue, S4 yellow, S5 purple, S6 light blue, and S7 brown. Among the seven groups, S7 contained the largest number of germplasms, with a total of seven germplasms accounting for 35% of the test materials. Groups S2 and S4 contained three

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germplasms, accounting for 15% of the test materials. The S3, S5, and S6 groups had two germplasms, accounting for 10% of the test materials. Finally, the S1 group includes only one germplasm, accounting for 5% of the test materials; the one germplasm is the 'White Crystal'. A dendrogram of the seven groups obtained by structural analysis is shown in *Figure 9*. From the figure, the S1, S3, and S7 groups are relatively close. In contrast, the evolutionary branches of the other groups extended in different directions, indicating that the S1, S3, and S7 groups may have a closer geographical distance.

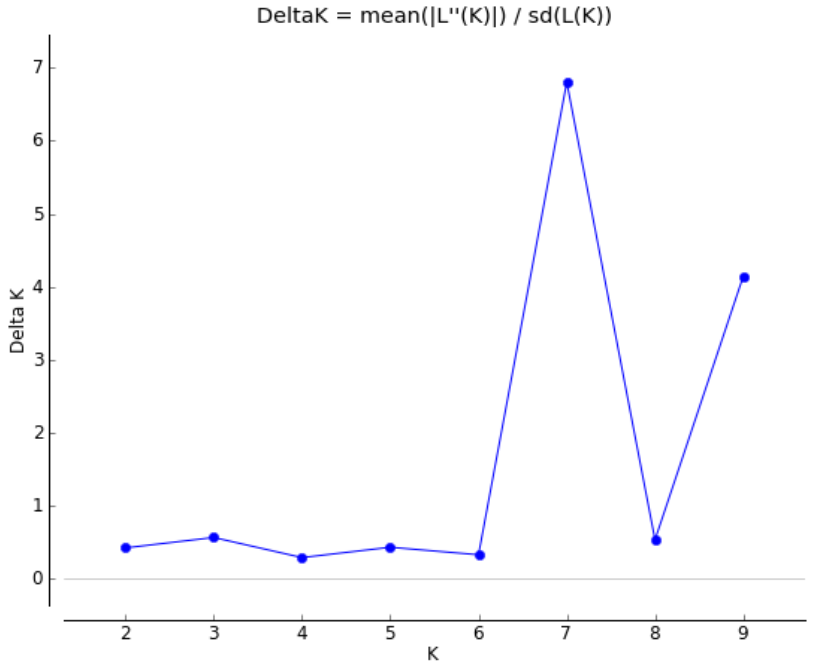

Figure 7. *Based on structure analysis Δ K value versus K value*

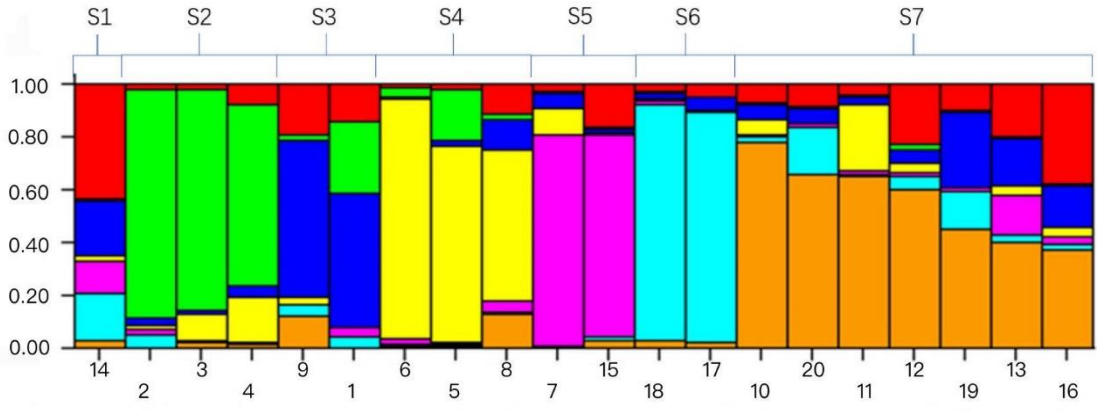

Figure 8. *Analysis of the group structure of 20 Abiu fruits materials*

Using 0.6 as the threshold to distinguish subpopulations and mixed populations, the distribution of the Q value of each tested material was analyzed. When $Q < 0.6$, it was considered to have mixed origin and complex genetic background. When $Q \geq 0.6$, it was

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considered to have a single origin and single genetic background (Su et al., 2018). The analysis results showed that the Q value of 14 of the 20 test materials was less than 0.6, accounting for 70% of the test materials, and the Q value of the six materials was more than 0.6, accounting for 30% of the test materials, indicating that the vast majority of the test materials had genetic exchange phenomena such as inter-variety hybridization, there were many blood exchanges among them, the sources were mixed and rich, and the genetic composition was relatively complex.

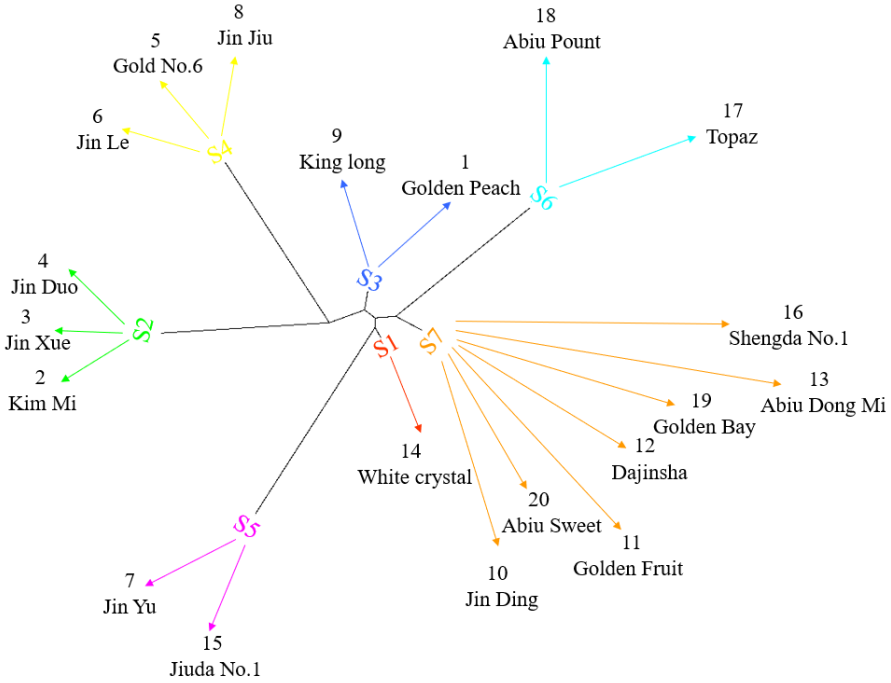

Figure 9. *The dendrogram of 7 groups of 20 Abiu fruits*

Discussion

Abiu is a rare tropical fruit that has been gaining popularity. It has golden skin, a sweet and distinctive flavor, and soft juicy flesh when fully mature. It is also rich in nutrients, such as proteins, carbohydrates, fiber, vitamins, and minerals. Abiu has also been recognized for its medicinal value. The fruit and bark are rich in triterpenoids, which generally have antitumor biological activities; fruit peel extract has anti-diarrhea and antibacterial effects and is widely used in Brazil and other places to treat respiratory diseases and diarrhea; leaf extract has α-glucosidase and α-amylase, and its inhibitory and antioxidant activities are of great significance for the prevention and treatment of diabetes and other diseases (He et al., 2023). Considering the nutritional and medicinal value of Abiu, its economic benefits should not be underestimated if it is cultivated on a large scale. SCoT is a new genotypic molecular marker closely linked to the target gene, which does not require genome sequence information in advance, has strong versatility, and can amplify repeatable polymorphic bands among closely related materials (Huang et al., 2013). At present, SCoT has been applied to plant genetic diversity exploration, fingerprint construction, differential gene expression analysis, and ecological protection (Sahu et al., 2015; Abd El-Maksoud et al., 2018; Li et al., 2019; Abd El-Moneim et al., 2021). Amom et al. (2020) used four different molecular markers to analyze the genetic

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relationships of five important economic bamboo germplasm resources in northern India, revealing the effectiveness of each marker system in determining the genetic relationship between bamboo species and proving that SCoT molecular markers can be used for genetic diversity analysis among species and other species. Aymen et al. (2015) reported that the SCoT molecular marker showed a higher level of polymorphism than the ISSR molecular marker. Similarly, Ahmad et al. (2011) and Luo et al. (2011) confirmed that the SCoT molecular marker had higher information content and repeatability than the ISSR and RAPD molecular markers in a polymorphism test of potato (*Solanum tuberosum* L.) and mango (*Mangifera indica* L.), respectively. In addition, Zhang et al. (2023) amplified 148 polymorphic bands with 18 SCoT primers, and the percentage of polymorphic sites was 94.87%. In another study, Gupta et al. (2019) amplified 365 bands in 54 pepper (*Capsicum annuum* L.) planting resources with 36 primers, with an average of 10.43 bands per primer and an average polymorphism of 81.52%. In the analysis of genetic diversity, the number of amplified primers is an important indicator of the amount and efficiency of marker information (Amom et al., 2020). In this experiment, selected 24 primers were used to carry out SCoT molecular markers on 20 samples of Abiu. A total of 361 bands were amplified using 24 primers, including 309 polymorphic bands, with an average polymorphism ratio (PPB) of 85.60%, which proved that the selected primers had good polymorphism and could effectively distinguish the differences between 20 samples of Abiu germplasm.

Genetic diversity contains all the genetic information carried by species and is one of the most important biodiversity indicators. The SCoT molecular marker, as a dominant marker that can track target traits, can better reveal the genetic background of germplasm resources and more intuitively reflect the genetic relationship between different germplasms (Pan et al., 2022). Genetic diversity among germplasms. The number of alleles (*Na*), number of effective alleles (*Ne*), Nei's genetic diversity index (*H*), and Shannon diversity index (*I*) was 1.9501, 1.4659, 0.2806, and 0.4314, respectively. The data showed specific genetic diversity among the tested materials. This laid the foundation for analyzing the genetic relationships and population structures of 20 Abiu germplasms.

As a new and rare fruit, Abiu has been studied relatively little at home and abroad, so the genetic research background of Abiu is relatively narrow, and the use of molecular markers to analyze its genetic diversity and identify its varieties is important. In this study, the genetic similarity coefficients of 20 Abiu germplasm resources obtained using SCoT molecular markers ranged from 0.53 to 0.76. Based on the genetic similarity coefficient matrix, a heat map and cluster map of the genetic similarity coefficient were constructed, which showed that the 20 Abiu germplasm resources had significant genetic differences, especially in 'Golden Peach'. It can be seen that the 'Golden Peach' can be greatly sourced from different countries or regions and introduced to China. When the genetic coefficient was 0.64, the 20 Abiu could be divided into 9 groups, and the fourth group contained the most germplasm resources, which were 'Gold No.6', 'Jin Le', 'Jin Jiu', 'King Long', 'Jin Ding', 'Golden Fruit', 'Abiu Dong Mi', indicating that the genetic relationship of these 7 Abiu was close and had a similar or identical genetic background, Possible parents from the same region. The genetic relationship of 20 Abiu was obtained by cluster analysis, and the closer the genetic coefficient, the closer the genetic relationship. Thus, we can judge the difference in the parent source of 20 Abiu. According to the clustering results, we can preliminarily divide the source of Abiu into several regions, but the final result still

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needs to be combined with subsequent population structural analysis to obtain a more accurate genetic background and genetic relationship of these 20 Abiu.

Population structure analysis can divide the research object into different genetic groups according to the analysis results to determine the genetic background and genetic structure of all test samples. The results of this study show that most of the tested materials exhibit genetic exchange phenomena, such as inter-variety hybridization. There are many blood exchanges between them, the sources are mixed and rich, and the genetic composition is relatively complex. The results of the population structure analysis were generally consistent with those of cluster analysis. Comprehensive cluster and population structure analyzes showed that there was rich genetic diversity among the 20 varieties tested, with high genetic differences, diverse genetic backgrounds, and complex genetic composition. This indicates that the currently used Abiu in China has a rich genetic background, and the hybridization between varieties is more complex, which leads to an unclear genetic relationship of Abiu germplasm resources, and the phenomenon of synonyms in the germplasm resources market is relatively serious. Therefore, it is necessary to further promote the establishment of the Abiu germplasm resources garden and further clarify the genetic background of the common Abiu in China through this experiment, providing a basis for the rational breeding of new germplasm resources of Abiu.

Conclusions

In this study, the genetic diversity of 20 Abiu germplasm resources was analyzed using SCoT molecular markers. The results showed that the genetic diversity of the 20 germplasm resources of Abiu was high, and the distance of their genetic relationship could be determined by cluster analysis, which provided a reference for the classification and identification of Abiu varieties. The population structure analysis showed that the genetic background of 20 germplasm resources of Abiu was diverse and the genetic components were complex, and it has been proven that the genetic basis among the common germplasm resources of Abiu in China is relatively complex, which leads to confusion regarding the germplasm resources of Abiu in the market. This study provides a reference for clarifying the genetic background and population relationship of common germplasm resources of Abiu in China, and also provides a reference basis for the development and utilization of new germplasm resources of Abiu and the establishment of Abiu germplasm resource gardens. At the same time, this study provides a reference for the identification of other fruit tree germplasm resources and provides a basis for the screening and identification of seedlings of high-quality fruit at the breeding stage in terms of molecular-assisted breeding, so as to improve the overall quality of breeding fruit.

Author contributions. Conceptualization: [Yang Peng and Juan Li]; Writing and original draft preparation: [Yang Peng and Faisal Hayat]; Edited the manuscript: [Juan Li, Jiaqi Xiao and Wensheng Xuan]; Supervision, project administration, funding acquisition: [Juan Li, Panfeng Tu, Caiqin Li, Wenpei Song and Jiezhong Chen]. All the authors have read and agreed to the published version of the manuscript.

Conflict of interests. The authors declare no conflict of interests.

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