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Genetic diversity analysis of *Cinnamomum cassia* Presl and *Cinnamomum cassia* Presl var. *macrophyllum* Chu based on ISSR

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ABSTRACT

ISSR technology can provide detailed data on the genetic information of species. In this study, ISSR was used to find dominant bands at the DNA level to stably identify *Cinnamomum cassia* Presl and *Cinnamomum cassia* Presl var. *macrophyllum* Chu, and *Cinnamomum cassia* Presl from different production areas. The results showed that primer UBC834 had dominant bands for *Cinnamomum cassia* Presl and *Cinnamomum cassia* Presl var. *macrophyllum* Chu samples, and primer UBC876 had dominant bands for *Cinnamomum cassia* Presl samples from different production areas. By analyzing the data of ISSR, it was found that the clustering results and the results of principal component analysis were consistent with the results of electropherograms and were divided into five categories. At the same time, the polymorphism analysis showed that *Cinnamomum cassia* Presl germplasm resources were rich in genetic diversity. In addition, the results of genetic clustering analysis showed that when the GS was higher than 0.88, it was possible to distinguish *Cinnamomum cassia* Presl and *Cinnamomum cassia* Presl var. *macrophyllum* Chu. As the genetic similarity coefficient increased, *Cinnamomum cassia* Presl from different production areas could be distinguished. The results of this study provide a scientific basis for the development and utilization of the germplasm resources of *Cinnamomum cassia* Presl and *Cinnamomum cassia* Presl var. *macrophyllum* Chu, which can meet the economic and ecological needs. It also provides a new perspective for the conservation of biodiversity and biological evolution.

1. Introduction

Cinnamomum cassia Presl, also known as Guangxi Gui, Yu Gui or Gui Shu, etc., belongs to the *Cinnamomum* Schaeff. plants. The dried bark of the *Cinnamomum cassia* Presl, a traditional Chinese medicinal herb, is extensively utilized in China due to its significant medicinal properties. *Cinnamomum cassia* Presl herb has the effect of warming the meridians, dispersing cold and relieving pain, activating blood circulation, and promoting menstruation.[1] It is mainly used to treat symptoms such as uterine cold, red eyes and sore throat, dysmenorrhea.[2] This Chinese medicine is not only used to treat arthritis[3] but also can help prevent diabetes[4] and protection against non-alcoholic fatty liver disease.[5] The main production areas of *Cinnamomum cassia* Presl are in Guangxi Province and Guangdong Province, with the distribution area in Guangxi being much larger than that in Guangdong, accounting for more than 50% of the country's total. In addition, this traditional Chinese medicine is also cultivated in small quantities in Fujian, Hainan and other provinces located at the Tropic of Cancer.[6] Through natural selection and long-term artificial cultivation, many new varieties of *Cinnamomum cassia* Presl have evolved. As a dual-purpose medicine and food, it boasts a broad spectrum of applications, spanning medicine, food, pesticides and other fields. It is one of the most pivotal medicinal plants both in China and on the global stage. Due to the gradual industrialization of its herbs, tablets, volatile oil and other related products, *Cinnamomum cassia* Presl has become an important cash crop in Guangdong Province and Guangxi Province of China, and an important pillar of the local economy.[7]

The *Cinnamomum cassia* Presl var. *macrophyllum* Chu, native to Vietnam, is the sole variant of *Cinnamomum cassia* Presl. This variant is frequently used as cinnamon and is traditionally considered superior to

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Cinnamomum cassia Presl. The Latin name for cinnamon is *Cinnamomum cassia* Presl, while *Cinnamomum cassia* Bl. is a later homonym. Consequently, the Latin name of *Cinnamomum cassia* Bl. should be *Cinnamomum cassia* Presl var. *macrophyllum* Chu.

Cinnamomum Schaeff. plants[1] are characterized by low specimen numbers, a lack of targeted systematic collections, and an absence of molecular phylogenetic evidence to support them in many genera. The limited number of specimens results in poorly recognized variants of key taxonomic characters. The taxonomic position of the *Cinnamomum* Schaeff. plants requires further investigation. The plants are extremely similar in herbal traits and even in protozoa, necessitating a combination of many aspects for comprehensive identification. *Cinnamomum cassia* Presl, with its long history of cultivation and extensive planting area, presents a challenge as the raw drug traits of camphor plants are more similar in appearance and odor, leading to a higher incidence of mixed forgeries. Therefore, there is a need for quick and accurate identification methods.

Accurate species identification forms the foundation of plant research. The detection of genetic variation through morphological or phenotypic traits presents the most direct and convenient method.[2] The close relationship between *Cinnamomum cassia* Presl and *Cinnamomum cassia* Presl var. *macrophyllum* Chu results in a striking similarity in appearance, making it challenging for non-professionals to distinguish between them directly.

Plants harbor a variety of chemical compounds within their structures. When sections of plant tissue are obtained, a diverse array of chemical components can be visualized under a microscope using histological staining techniques. This process facilitates an understanding of the spatial distribution of these substances within the cells of the plant tissue, a critical aspect of histological research. Depending on the research subject and the specific chemical substances targeted for detection, paraffin sections may be employed. In some cases, fresh materials may require hand-cutting or freezing for sectioning. The judicious application of certain straightforward histochemical staining methods during the process of plant tissue sectioning can not only determine the chemical properties of cell walls and intracellular contents but also assist in the identification of tissue and cellular structures. However, the use of histological and chemical methods for plant identification demands significant time and effort. This is primarily attributed to the need for fresh plant samples and complex slicing procedures, both of which are labor-intensive and technically challenging.[8] Additionally, these methods necessitate the use of high-purity chemical reagents. ISSR is a molecular labeling technique that employs a single primer to amplify a multilocus PCR.[9] This technique is not only simple and swift but also integrates the advantages of various molecular marker methods. As a result, it is extensively utilized in the identification of different varieties, the analysis of genetic diversity within the same species, and further species-specific analyses.[10] As a result, the ISSR amplification technique is extensively utilized in the identification of different varieties, the analysis of genetic diversity within the same species, and further species-specific analyses.[11] This technique has been widely adopted in the identification of different species, genetic diversity analysis of the same species and the determination of phylogenetic relationships among species.[12] In addition to its simplicity, cost-effectiveness and time efficiency,[13] the ISSR amplification technique facilitates the rapid production of highly polymorphic markers. This is particularly useful in identifying polymorphisms in species with narrow germplasm. Furthermore, it has significant potential for use in marker-assisted breeding methods[14] and is already being employed in such methods.[9] Genetic diversity is a pivotal area of study in biodiversity research. Two plants of significant economic and ecological value, *Cinnamomum cassia* Presl and *Cinnamomum cassia* Presl var. *macrophyllum* Chu, serve as important subjects for this study. The exploration of their genetic diversity contributes greatly to our understanding of biodiversity. The accurate identification of plant sources is essential for ensuring the quality of traditional Chinese medicine. However, the classification of cinnamon currently relies primarily on morphological indicators, which are subject to subjective factors. Therefore, the use of ISSR technology to distinguish between *Cinnamomum cassia* Presl and *Cinnamomum cassia* Presl var. *macrophyllum* Chu carries practical significance. It plays a crucial role in the precise utilization of medicinal materials, the analysis of pharmacologically active ingredients, and breeding practices.

In the current study, populations of *Cinnamomum cassia* Presl and *Cinnamomum cassia* Presl var. *macrophyllum* Chu were selected for investigation. Leaf samples were collected from these populations and subsequently analyzed using ISSR technique. In this experiment, a total of 56 samples were compared and analyzed by ISSR technique, and it was found that the electrophoretic spectra, cluster analysis, and principal coordinate analysis after separation by agarose gel electrophoresis could accurately identify *Cinnamomum cassia* Presl and *Cinnamomum cassia* Presl var. *macrophyllum* Chu, and *Cinnamomum cassia* Presl from different origins. This is of some significance for distinguishing *Cinnamomum cassia* from *Cinnamomum cassia* Presl and *Cinnamomum cassia* Presl var. *macrophyllum* Chu of different origins.

2. Materials and methods

2.1. Samples used in the experiment

The samples used in the experiment were collected as in Table 1 and stored at −20 °C for subsequent experiments.

2.2. Reagents used in the experiment

TaKaRa MiniBEST Plant Genomic DNA Extraction Kit (TaKaRa, Japan) primers were synthesized by DynaTech Biotechnology Co. and sequences are shown in Table 2.

2.3. Instruments used for the experiment

PCR instrument (Bio-rad C1000), Nano-100 Nucleic Acid Analyzer.

2.4. Experimental methods

2.4.1. DNA extraction

Sample DNA was extracted using TaKaRa MiniBEST Plant Genomic DNA Extraction Kit (TaKaRa, Japan), 1.5% agarose gel electrophoresis was performed, and DNA concentration and purity were detected by Nano-100 Nucleic Acid Analyzer. The samples were stored at −20 °C in a refrigerator.

2.4.2. PCR amplification and product detection

The operation was carried out on a Bio-rad C1000 instrument using the Premix Taq ™ (Ex Taq ™ Version 2.0 plus dye) (TaKaRa, Japan) kit, which requires the reaction solution to be configured on ice. The total reaction system was 25 μL, Premix Taq 12.5 μL; primer 2.0 μL; DNA template 3.0 μL; and sterilized water 3.5 μL. PCR reaction: Stage 1 Predeformation, Repeat:1, 94 °C for 4 min; Stage 2 PCR reaction, Repeat: 36, 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 90 s Stage 3 Dissociation. The amplification products were detected by 1.5% agarose gel electrophoresis, and the primer sequences with clear bands and stable reaction were selected.

2.5. Data analysis and statistics

Sites amplified by identical primers were classified as a single band. Bands appearing at same position on the gel were marked as “1”, while absent bands were marked as “0”. This information was tabulated to form a matrix. The total number of bands and the number of polymorphic bands amplified by each primer were counted to calculate the polymorphism rate. The data was processed using NTSYSpc-2.10e software, generating an affinity dendrogram and a principal coordinate scatter

Table 1
Sample collection location, acquisition time, growth years and serial number.

Collection location	Coordinates	Acquisition time	Growth years	Serial number	Sample information
Dabingkeng Village Pingshui Village Committee Lubu Town Gaoyao City Zhaoqing City Guangdong Province	23°15′59″N, 112°19′53″E	2019.06	5 years old	1–8	*Cinnamomum cassia* Presl
Siwang Village Ziliang Town Rong County Yulin City Guangxi Province	22°64′N, 111°14′E	2019.12	5 years old	9–14	*Cinnamomum cassia* Presl
Meizhu Village Yanbin Town Luoding City Guangdong Province	22°51′00″N, 111°21′18″E	2019.03	4 years old	15–17	*Cinnamomum cassia* Presl
Songnan Village Silun Town Luoding City Guangdong Province	22°42′20″N,	2019.03	6 years old	18–25	*Cinnamomum cassia* Presl
Huangshakou Village Lishao Town Luoding City Guangdong Province	111°15′54″E 22°45′19″N, 111°22′57″E	2019.03	5 years old	26–35	*Cinnamomum cassia* Presl
Hongguan Town Maoming City Guangdong Province	22°28′29″N, 111°07′14″E	2019.08	6 years old	36–41	*Cinnamomum cassia* Presl var. *macrophyllum* Chu
County Road 203 near Yangtouhua in Rong County Yulin City Guangxi Province	22°36′09″N, 110°47′07″E	2020.06	6 years old	42–47	*Cinnamomum cassia* Presl
Xindong Village Luoxiu Town Guiping City Guangxi Province	23°04′01″N, 110°17′20″E	2020.04	6 years old	48–53	*Cinnamomum cassia* Presl
Hantang Village Wuli Town Lingshan County Qinzhou City Guangxi Province	22°24′59″N, 109°17′27″E	2020.08	5 years old	54–56	*Cinnamomum cassia* Presl

Table 2
ISSR-PCR primer sequences.

Primer No	Sequence (5′–3′)	Academic	Suitable
UBC811	GAGAGAGAGAGAGAGAC	52 °C	51 °C
UBC824	TCTCTCTCTCTCTCTCG	50 °C	50 °C
UBC825	ACACACACACACACACT	52 °C	51 °C
UBC834	AGAGAGAGAGAGAGAGYT	50 °C	51 °C
UBC835	AGAGAGAGAGAGAGAGYC	50 °C	50 °C
UBC836	AGAGAGAGAGAGAGAGYA	52 °C	52 °C
UBC848	CACACACACACACACARG	52 °C	54 °C
UBC855	ACACACACACACACACYT	52 °C	53 °C
UBC856	ACACACACACACACACYA	50 °C	53 °C
UBC857	ACACACACACACACACYG	51 °C	52 °C
UBC868	GAAGAAGAAGAAGAAGAA	51 °C	51 °C
UBC876	GATAGATAGACAGACA	50 °C	51 °C

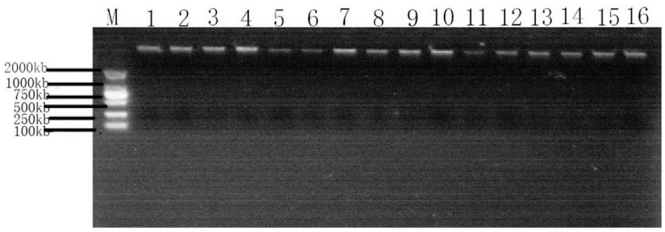

Fig. 1. Test result of DNA gel electrophoresis of *Cinnamomum cassia* Presl and *Cinnamomum cassia* Presl var. *macrophyllum* Chu.
M: Marker, 1–6: Cinnamomum cassia Presl var. macrophyllum Chu samples, 7–16: Cinnamomum cassia Presl samples.

plot. The quality of the clustering results was evaluated using a Cophenetic correlation test, with the results analyzed after calculating the obtained r-value. Using the binary matrix, PopGene software was employed to compute the count of polymorphic loci, the percentage of polymorphic loci, the number of effective alleles, the Nei genetic diversity index, and the Shannon information index. The NTSYS software was used to calculate the genetic similarity coefficient matrix of the test materials. This was then analyzed using the UPGMA method for clustering and subjected to PCA. The UPGMA clustering results were further validated using a cophenetic correlation test via the COPH method.

3. Results

3.1. DNA extraction quality testing

The extracted DNA underwent electrophoresis at 130 V for a duration of 13 min. The electrophoretic bands of the sample DNA were observed using a gel imager, which displayed clear and bright electrophoretic bands without any diffusion (Fig. 1). This observation suggested that the extracted DNA was not degraded. The absorbance ratio OD260/280, measured by the Nano-100 ultraviolet spectrophotometer, ranged between 1.6 and 1.8. This indicated that the DNA samples were of high purity, with minimal or no other residues present, thereby meeting the requirements for subsequent experiments. All remaining DNA samples also satisfied the experimental requirements.

3.2. PCR amplification results

Twelve ISSR primers were employed to amplify the DNA of 50 samples of *Cinnamomum cassia* Presl and 6 samples of *Cinnamomum cassia* Presl var. *macrophyllum Chu*. The PCR products were separated using 1.2% gel electrophoresis and analyzed through gel imaging. This process yielded a fingerprint with overall bright and clear bands.

In Fig. 2, compared with the amplification results of *Cinnamomum cassia* samples with primer UBC834, *Cinnamomum cassia* has specific bands, which can distinguish *Cinnamomum cassia* Presl samples and *Cinnamomum cassia* Presl var. *macrophyllum Chu*. In Fig. 3, the amplification results of *Cinnamomum cassia* Presl samples numbered 26–35 with primer UBC855 have four bands, whereas the amplification results of the other samples have three bands only. The 29–35 samples are from Huangshakou Village, Lishao Town, Luoding City, Guangdong Province, but their amplification results do not have the bands marked in the Fig. 3, which may be due to the difference in seed sources. In Fig. 4, primer UBC876 was used to amplify *Cinnamomum cassia* Presl samples numbered 9–14, which were taken from Siwang Village, Ziliang Town, Rong County, Yulin City, Guangxi Province, and the amplification results of the other samples had specific bands, which could distinguish *Cinnamomum cassia* Presl in this production area from other samples.

3.3. Polymorphism analysis

The PCR amplification was performed on 50 samples of *Cinnamomum cassia* Presl and 6 samples of *Cinnamomum cassia* Presl var. *macrophyllum Chu* using 12 primers. The PCR amplification results for primers UBC834, UBC835, and UBC836 were unsatisfactory, but successful fingerprints were established for the 9 primers. These 9 fingerprints amplified a total of 107 bands, with 54 bands accounting for 50.5% of the polymorphisms (Table 3). The number of bands amplified by each primer varied, ranging from 8 to 16. Primer UBC824 amplified the highest total number of bands

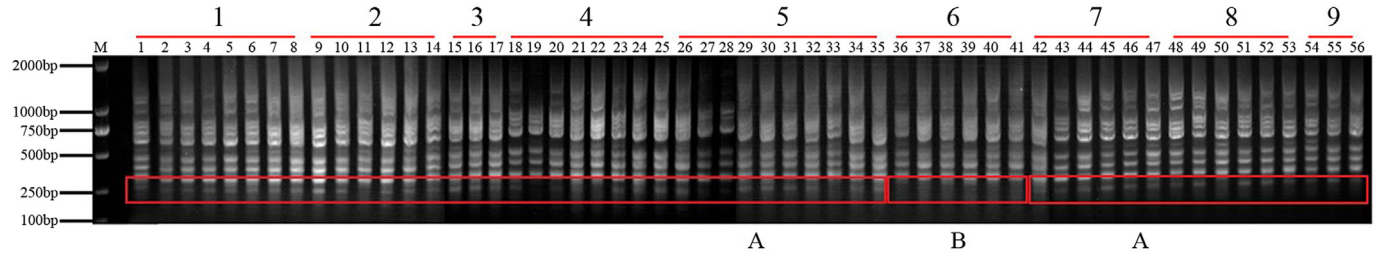

Fig. 2. Amplification results of primer UBC825 on 50 *Cinnamomum cassia* Presl samples and 6 *Cinnamomum cassia* Presl var. *macrophyllum Chu* samples.
M: Marker, A: *Cinnamomum cassia* Presl, B: *Cinnamomum cassia* Presl var. *macrophyllum Chu*.
1. Dabingkeng Village Pingshui Village Committee, Lubu Town Gaoyao City Zhaoqing City Guangdong Province 2. Siwang Village Ziliang Town Rong County Yulin City Guangxi Province 3. Meizhu Village Yanbin Town Luoding City Guangdong Province 4. Songnan Village Silun Town Luoding City Guangdong Province 5. Huangshakou Village Lishao Town Luoding City Guangdong Province 6. Hongguan Town Maoming City Guangdong Province 7. County Road 203 near Yangtouhua in Rong County Yulin City Guangxi Province 8. Xindong Village Luoxiu Town Guiping City Guangxi Province 9. Hantang Village Wuli Town Lingshan County Qinzhou City Guangxi Province.

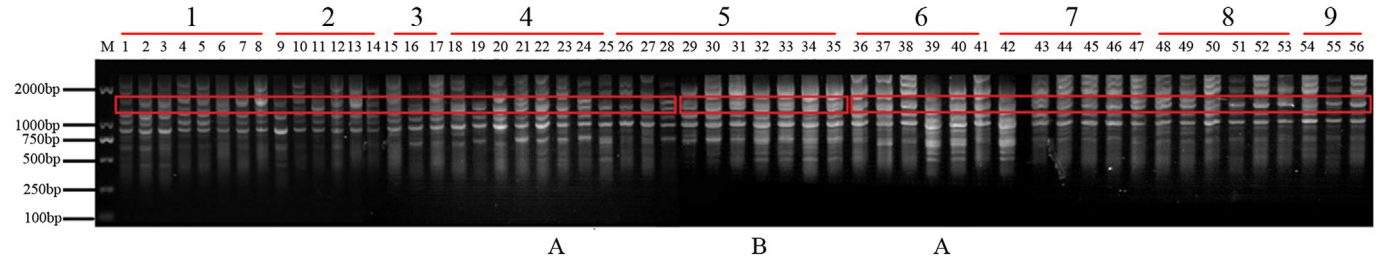

Fig. 3. Amplification results of primer UBC855 on 50 *Cinnamomum cassia* Presl samples and 6 *Cinnamomum cassia* Presl var. *macrophyllum Chu* samples.
Sample identification is the same as Fig. 2.

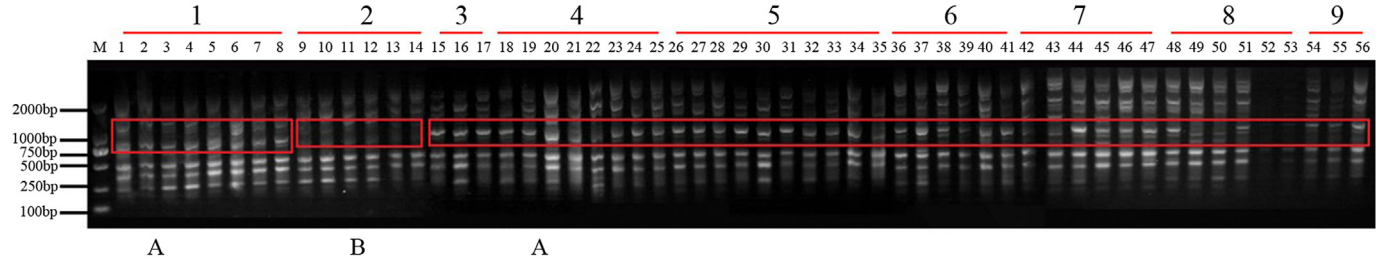

Fig. 4. Amplification results of primer UBC876 on 50 *Cinnamomum cassia* Presl samples and 6 *Cinnamomum cassia* Presl var. *macrophyllum Chu* samples.
Sample identification is the same as Fig. 2.

Table 3
Polymorphism analysis.

Primer number	Number of polymorphic bands	Total number of amplified bands	Polymorphism
UBC811	3	10	30.0%
UBC824	14	16	87.5%
UBC825	5	14	35.7%
UBC848	4	8	50.0%
UBC855	6	16	37.5%
UBC856	5	8	62.5%
UBC857	6	10	60.0%
UBC868	3	11	27.3%
UBC876	7	14	50.0%

with 16, while primer UBC848 amplified the fewest with only 8. The polymorphism rates of the bands amplified by the 12 primers varied among the 56 samples. For instance, the bands amplified by primer UBC824 contained 14 polymorphic bands, resulting in a polymorphism rate of 87.5%, whereas only 3 out of the 11 bands amplified by primer UBC868 were polymorphic bands with a polymorphism rate of 27.3%.

This indicates that cinnamon germplasm resources are abundant in genetic diversity, and molecular markers can be employed to discern the kinship relationship within *Cinnamomum cassia* Presl germplasm. The high ratio of polymorphism signifies the complexity of the genetic material, and results of the amplification results can furnish a wealth of genetic information.

3.4. Genetic clustering analysis

A dendrogram illustrating the genetic relatedness of 50 *Cinnamomum cassia* Presl samples and 6 *Cinnamomum cassia* Presl var. *macrophyllum Chu* samples was constructed using the UPGMA method with the software NTSYSpc-2.10e (Fig. 5). The numbers 1–35, 42–56 indicated *Cinnamomum cassia* Presl, while the numbers 36–41 indicated *Cinnamomum cassia* Presl var. *macrophyllum Chu*. The results demonstrated that the 56 cinnamon samples could be classified into two primary groups when the GS was 0.88. A1 included cinnamon samples numbered 1–35 and 42–56, and A2 included cinnamon samples numbered 36–41. Since the samples in the first category are all *Cinnamomum cassia* Presl and the samples in the second category are all *Cinnamomum cassia* Presl var. *macrophyllum*

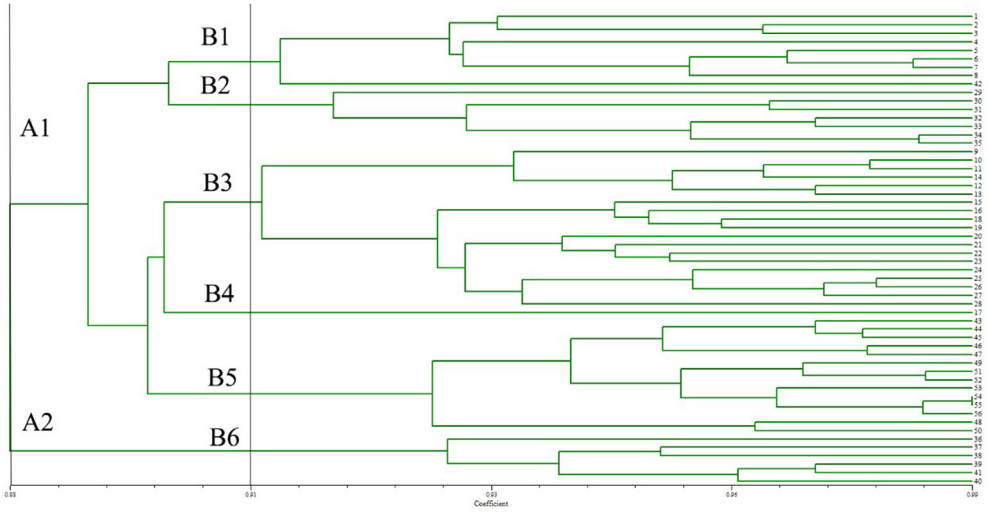

Fig. 5. ISSR cluster analysis of 56 samples. GS = 0.88, A1(1–35,42-56): Cinnamomum cassia Presl samples, A2(36–41): Cinnamomum cassia Presl var. macrophyllum Chu; GS = 0.91, B1(1–8):Zhaoqing City Guangdong Province Cinnamomum cassia Presl samples, B2(29–35):Yunfu City Guangdong Province Cinnamomum cassia Presl samples, B3(9–28) 9-14:Yulin City Guangxi Province Cinnamomum cassia Presl samples, 15–28: Luoding City Guangdong Province Cinnamomum cassia Presl samples, B4(17): Luoding City Guangdong Province Cinnamomum cassia Presl samples, B5(43–56): Guangxi Province Cinnamomum cassia Presl samples, B6(36–41):Maoming City, Guangdong Province Cinnamomum cassia Presl var. macrophyllum Chu samples.

Chu, it becomes feasible to differentiate between *Cinnamomum cassia* Presl and *Cinnamomum cassia* Presl var. *macrophyllum Chu*.

When GS reached 0.91, the 56 cinnamon samples were divided into 6 categories: B1 comprised 8 *Cinnamomum cassia* Presl samples numbered 1–8 from Zhaoqing City, Guangdong Province, B2 included 7 *Cinnamomum cassia* Presl samples numbered 29–35 from Huangshakou Village, Lishao Town, Luoding City, Yunfu City, Guangdong Province, B3 contained 20 *Cinnamomum cassia* Presl samples numbered 9–28, of which 9–14 are all from Siwang Village, Ziliang Town, Rongxian County, Yulin City, Guangxi Province, while 15–28 are all from Luoding City, Guangdong Province, B4 consisted of a single *Cinnamomum cassia* Presl sample 17 from Luoding City, Guangdong Province, B5 included *Cinnamomum cassia* Presl samples numbered 43–56 from Siwang Village, Ziliang Town, Yulin City, Rong County, Guangxi Province. B6 encompassed *Cinnamomum cassia* Presl var. *macrophyllum Chu* samples 36–41. As illustrated in Fig. 5, when the GS exceeded 0.88, *Cinnamomum cassia* Presl and *Cinnamomum cassia* Presl var. *macrophyllum Chu*. Furthermore, as the GS value increased, the samples could be further classified into different categories based on their respective collection locations.

3.5. Cophenetic correlation test results

A correlation analysis was conducted using the software Ntsys (Fig. 6). The software computed a correlation coefficient of $r = 0.75722$, signifying a robust clustering outcome for this experiment.

3.6. 2D plot of principal coordinates

In the two-dimensional coordinate map (Fig. 7), the 56 samples were organized into five distinct categories. Category A was the *Cinnamomum cassia* Presl var. *macrophyllum Chu* samples numbered 37–41. Category B was the *Cinnamomum cassia* Presl samples numbered 43–47, 49–53, and

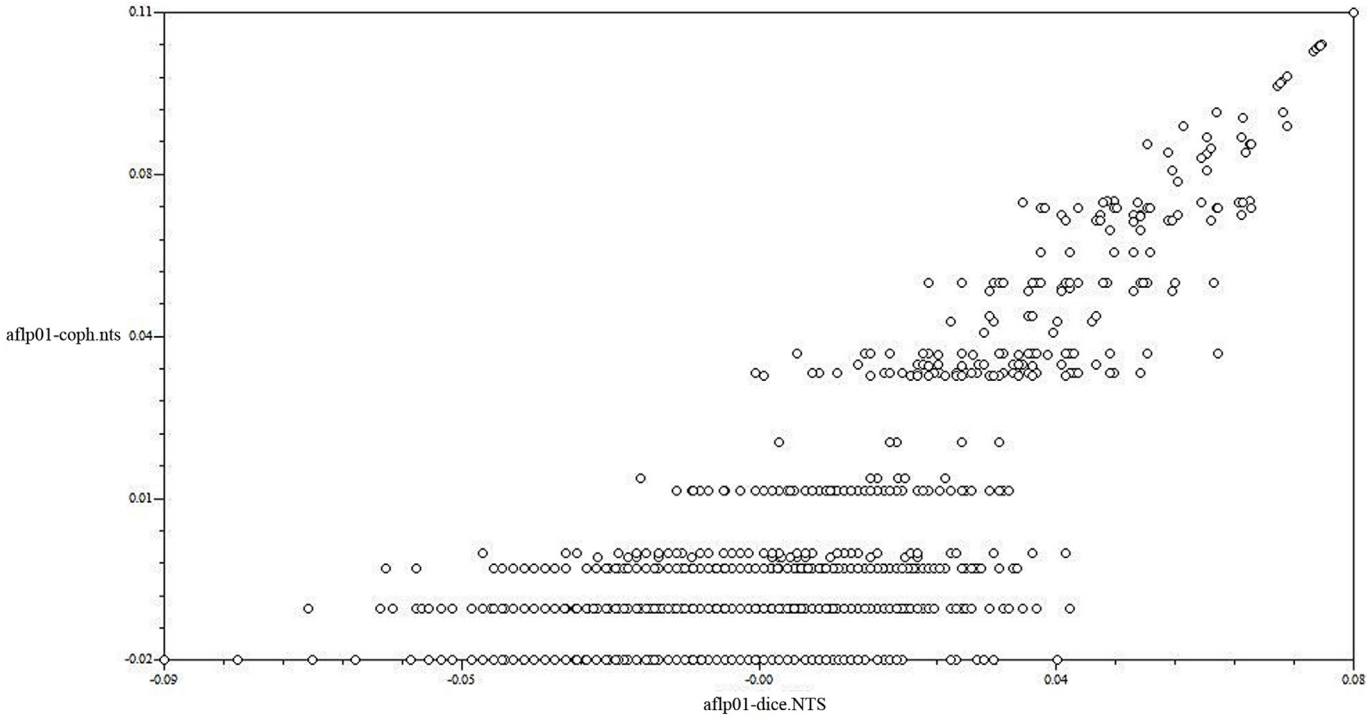

Fig. 6. Correlation test results.

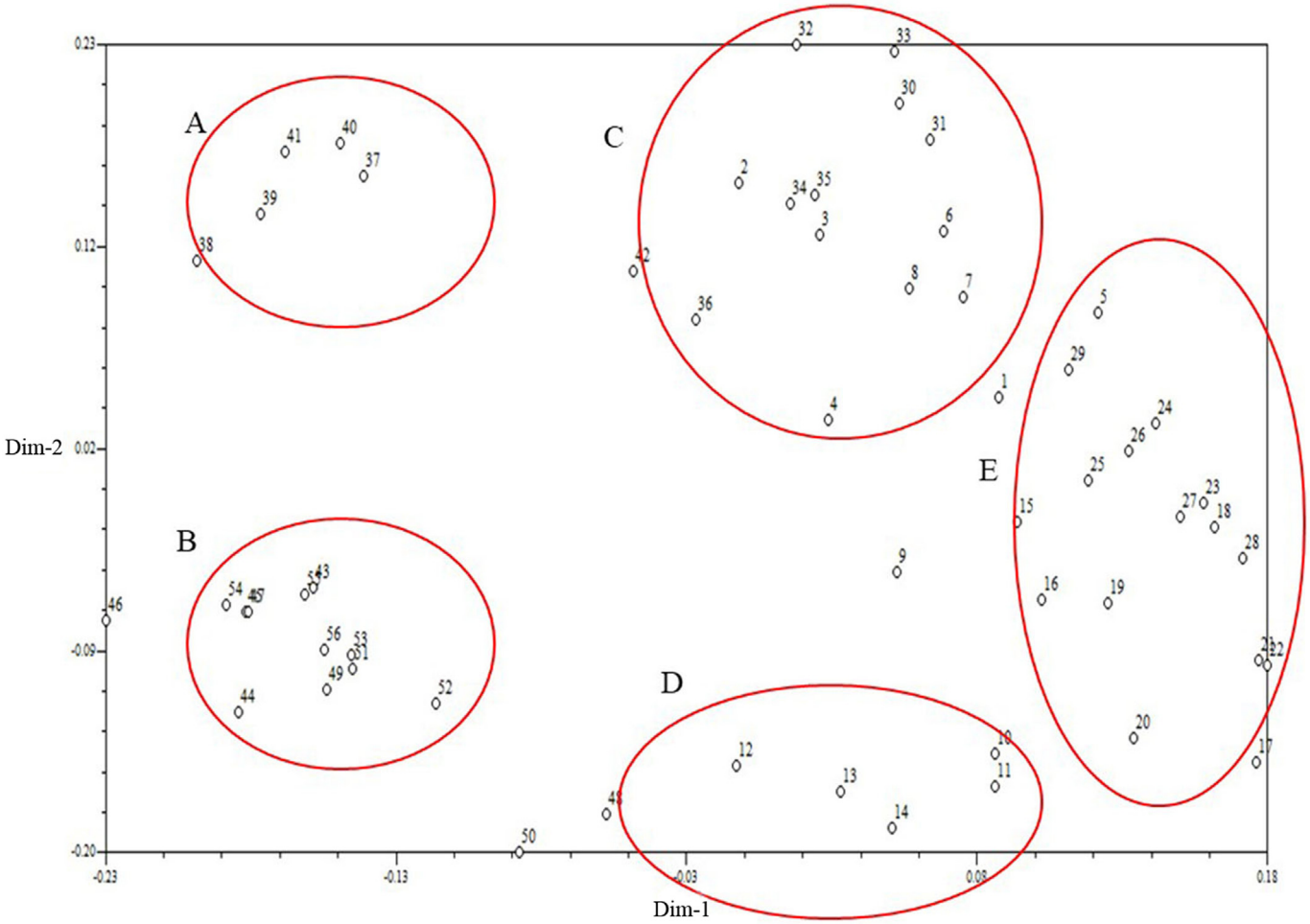

Fig. 7. Two dimensional principal coordinate scatter plots labeled with ISSR for 56 samples.
A: 37–41 Cinnamomum cassia Presl var. macrophyllum Chu samples, B: 43–47, 49–53,54-56 Cinnamomum cassia Presl samples from Guangxi Province, C: 2–4 and 6–8 Cinnamomum cassia Presl samples from Zhaoqing City Guangdong Province, 30–36 Cinnamomum cassia Presl samples from Luoding City Guangdong Province, D: 10–14 Cinnamomum cassia Presl samples from Yulin City Guangxi Province, E: 15–29 Cinnamomum cassia Presl samples from Luoding City Guangdong Province.

54–56 from Guangxi Province, of which 43–47 were taken from Yulin in Guangxi Province, 49–53 were taken from Guiping in Guangxi Province, and 54–56 were taken from Qinzhou in Guangxi Province. Category C was the *Cinnamomum cassia* Presl samples numbered 2–4, 6–8, and 30–36, respectively. *Cinnamomum cassia* Presl samples in category C were 2–4, 6–8, and 30–36, of which 2–4 and 6–8 were taken from Gaoyao, Zhaoqing, Guangdong Province, and 30–36 were taken from Huangshakou Village, Lishao Town, Luoding City, Guangdong Province, and category D was *Cinnamomum cassia* Presl samples numbered 10–14 and all from Yulin, Guangxi Province. The samples were all taken from Huangshakou Village, Lishao Town, Luoding City, Guangdong Province. Category E cinnamon samples were 15–29 and all samples were from Luoding, Guangdong. 15–17 were collected from Meizhu Village, Qianbin Town, Luoding City, Guangdong Province, 18–25 were collected from Songnan Village, Silun Town, Luoding City, Guangdong Province, and 26–29 were collected from Huangshakou Village, Lishao Town, Luoding City, Guangdong Province.

Accordingly, the 56 samples were organized into five primary categories based on their collection locations, and it was possible to accurately distinguish *Cinnamomum cassia* Presl and *Cinnamomum cassia* Presl var. *macrophyllum Chu*.

4. Discussion

Studies have shown that ISSR is an effective means of analyzing genetic diversity. Among the many methods for genetic diversity analysis, such as AFLP, ISSR, RAPD, etc., choosing the suitable method for the experiment is the key to obtaining stable and accurate conclusions. The primary benefits of AFLP encompass: excellent repeatability, robust polymorphism, superior resolution, elimination of the necessity for Southern hybridization, broad applicability across samples and consistent heritability. However, AFLP technology necessitates stringent quality control for both the sample DNA and the endonuclease used, as well as the purity of the DNA. RAPD is a molecular marking technique that boasts key advantages such as simplicity, rapid detection and the requirement of only minimal DNA samples with relatively low purity. It is applicable for genome analysis across various organisms and is capable of detecting polymorphism throughout the entire genome. The RAPD method is capable of detecting a significant degree of DNA polymorphism with high sensitivity. The application of RAPD primers is not limited by species boundaries, which allows for the use of the same set of RAPD primers in the study of any organism, thereby showcasing its universal applicability. However, RAPD technology has limitations in the reproducibility of amplification results, which are susceptible to external environmental influences. In contrast, ISSR molecular markers exhibit outstanding polymorphism, making them ideally suited for the swift identification of species that are closely related. ISSR molecular markers have been a technique of multilocus PCR amplification using microsatellite sequences as primers. ISSR is a simple and fast technique, which combines the advantages of AFLP,[15] SSR[16] and RAPD.[17] ISSR primers are

universally applicable across various species. At present, it has been used in Morocco (*Euphorbia resinifera* O. Berg),[18] *Triticum* species,[19] wild *Rosa* species.[20]

Therefore, in this experiment, ISSR technology was used to compare and analyze 56 samples, and the electrophoretic spectra, cluster analysis, and principal coordinate analysis after separation by agarose gel electrophoresis could accurately identify *Cinnamomum cassia* Presl and *Cinnamomum cassia* Presl var. *macrophyllum Chu*. This experiment not only identified *Cinnamomum cassia* Presl and *Cinnamomum cassia* Presl var. *macrophyllum Chu*, but also identified *Cinnamomum cassia* Presl from different production areas. *Cinnamomum cassia* Presl from different production areas are very similar at the molecular level, but there are also differences, for example, *Cinnamomum cassia* Presl samples from Huangshakou Village, Lisha Town, Luoding City, Guangdong Province, China, and Siwang Village, Ziliang Town, Yulin City, Yulin City, Guangxi Province, China, are differentiated from those from other production areas. This suggests that different geographic environments have a certain effect on the genetic material and that the accumulation of genetic mutations to a certain extent and the existence of geographic isolation have led to the differentiation of the genetic material. This provides a new perspective for understanding the genetic diversity of *Cinnamomum cassia* Presl.

Cinnamomum cassia Presl, as an significant oilseed crop, has a lengthy breeding cycle.[21] This long cycle means that seed selection research is time-consuming and relatively underexplored. The theory of plant crossbreeding shows that[22] the greater the morphological difference of hybrid parents, the higher the likelihood of achieving hybrid advantage. However, a larger genetic distance reduces the success rate of crossbreeding. The polymorphism analysis in this study showed that the genetic diversity in both *Cinnamomum cassia* Presl and *Cinnamomum cassia* Presl var. *macrophyllum Chu*, providing fresh insights for the selection of superior varieties. The regions of Guangdong and Guangxi characterized by subtropical and tropical monsoon climates respectively, offer warm climates and simultaneous water and heat, conditions highly conducive to the survival of evergreen plants. In future research, *Cinnamomum cassia* Presl planting resources should be further collected and the resource base should be improved to provide basic materials for the subsequent research. This study offers guidance for further varietal selection and breeding. A more in-depth and systematic comprehensive evaluation should be conducted, combined with whole-genome sequencing and other technologies, to comprehensively reveal the overall appearance of the planting resources of *Cinnamomum cassia* Presl and *Cinnamomum cassia* Presl var. *macrophyllum Chu*. This will allow scientists to fully leverage the advantages of the resources of *Cinnamomum cassia* Presl and *Cinnamomum cassia* Presl var. *macrophyllum Chu*, and to better develop, utilize, breed new varieties.

5. Conclusion

In this study, ISSR analysis was carried out to find dominant bands at the DNA level that could stably identify *Cinnamomum cassia* Presl and *Cinnamomum cassia* Presl var. *macrophyllum Chu*, and *Cinnamomum cassia* Presl from different production areas. The clustering results, principal component analysis results, and electropherogram results were consistent, dividing the samples into five categories. The polymorphism analysis revealed that *Cinnamomum cassia* Presl germplasm resources possess a high level of genetic diversity. It was found that when the GS was higher than 0.88, it was possible to distinguish between *Cinnamomum cassia* Presl and *Cinnamomum cassia* Presl var. *macrophyllum Chu*. As the genetic similarity coefficient increased, *Cinnamomum cassia* Presl from different production areas could be distinguished.

These findings lay a solid foundation for further research on the identification methods of *Cinnamomum cassia* Presl and *Cinnamomum cassia* Presl var. *macrophyllum Chu*. Furthermore, these findings offer valuable insights that may steer the future development and utilization of the germplasm resources of these species. The richness of the genetic diversity revealed in this study emphasizes the potential for additional exploration and utilization of these resources. It also underscores the importance of conservation efforts to safeguard this diversity for future generations.

CRediT authorship contribution statement

Ziqi Zheng: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Danyun Xu:** Writing – original draft, Investigation, Data curation. **Quan Yang:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition. **Hongyang Gao:** Investigation, Funding acquisition, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abbreviations

ISSR	Inter-Simple Sequence Repeats
GS	Genetic Similarity
PCR	Polymerase Chain Reaction
SSR	Simple Sequence Repeats
DNA	Deoxyribo Nucleic Acid
PCA	Principal Component Analysis
UPGMA	Unweighted Pair-Group Method with Arithmetic Means
RAPD	Randomly Amplified Polymorphic DNA
SRAP	Sequence—Related Amplified Polymorphism
AFLP	Amplified Fragment Length Polymorphism

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