Genetic Diversity Analysis of Youxi Bitter Tea Resources Based on ISSR Molecular Markers

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Abstract In this study, we analyzed the genetic diversity by using ISSR markers in four populations with 37 DNA samples of Youxi Kucha. In total, 66 alleles were amplified using 8 ISSR primers. A total of 66 bands were detected, of which 63 bands were polymorphic with a polymorphic proportion of 95.24 %. At the population level, the average PPL of the four populations of Youxi Kucha is 76.19 %, and the average values of Nei's gene diversity index (H) and Shannon diversity index (I) are 0.2045 and 0.3677 respectively. At the species level, the H and I are 0.3051 and 0.4650 respectively respectively. Youxi Kucha maintained a relatively high genetic variability at the population level and species level bothly. The genetic differentiation within the population was significantly higher than that between populations, but the genetic differentiation between the populations has also reached higher level (Φst = 0.17 > 0.15), the differentiation is extremely significant (P <0.01). A mantel test indicated there was no significant relationship between genetic distance and geographic distance among the populations studied. Correlation analysis shows that the diversity index has little relationship with altitude. This study carried out the molecular identification of the germplasm resources of Youxi bitter tea accurately, which can provide a theoretical basis for the protection of Youxi bitter tea and the breeding of improved tea varieties.

Keywords Youxi Kucha; ISSR; Genetic diversity; Genetic distance; Genetic differentiation

Because of its unique geographical conditions, Fujian Province is not only rich in tea tree resources, but also one of the key areas to study the origin and evolution of tea tree species in China, there have been wild tea trees in the original forests in many areas of history, with the development activities of local people and the in-depth utilization of mountain and forest resources, many wild tea tree species resources are gradually lost (Lv, 2013), Since the 1950s and 1960s, tea researchers in Fujian Province have devoted themselves to the excavation, collection and research and protection of wild tea trees, and have found large groups, small groups and single plants of wild tea trees in about 50 primitive forests and secondary forests in fujian's main tea areas, and divided into northeast and southwest distribution areas according to the native areas of wild tea trees. Wild tea tree community grows year-on-year in the natural environment far away from human activities, there are very rich trait variation and metabolic differences, which is very important for the study of tea tree evolution, innovative species, and also important materials to accelerate breeding (Qiao, 2019).

Bitter tea is a kind of unique rare tea tree species and usually found and used by the local residents in the early days in the form of wild tea trees, Yuxi County is located in the wild tea area of northeast Fujian Province, with a rare and bitter bamboo forest mixed with the original ecological bitter tea garden, which has over 200 years history. Youxi bitter tea grows at an average annual temperature of 15.5°C~16°C, an altitude of 600–1 000 m, and an area with extremely high humidity. It has strong cold and freezing resistance. The tea tastes very bitter and slightly astringent. In return to sweetness, Youxi bitter tea not only has a unique taste, but also has the effects of
reducing inflammation and relieving heat, dysentery and diarrhea. Preliminary investigations found that Youxi bitter tea populations are scattered in the growing area, mixed between tall vegetation and bitter bamboo forests. The tree type is shrubs or half trees, varying in height, and the leaves are lanceolate or oblong, with different sizes, and tender buds Mostly purple and light purple-green, mature leaves mostly turn green, and some special individual plants have dark purple leaves. After taste, the bitterness of each individual plant is different. Tang et al. (2019) determined the bitter components of some bitter tea resources in Youxi, and screened out many high-caffeine, high-theobromine, and high-EGCG specific individual plants. Some individual plants have purple buds and extremely high anthocyanin content. It also proves the richness and diversity of biological characters of Youxi bitter tea. Abundant phenotypic variation often contains rich genetic variation. To study the genetic background of Youxi bitter tea, reveal its genetic diversity and the relationship between individual plants, to protect bitter tea resources, breed specific germplasm, and improve tea varieties, etc. It is of great significance.

Molecular markers can be used as an important means and entry point for researches such as exploring evolutionary relationships, genetic structure and variation characteristics, exploring functional components and functional gene verification (Agarwal et al., 2008). ISSR (Inter simple sequence repeat) molecular marker technology is due to its inability The advantages of being affected by the external environment, detecting multiple allelic variations, and not affecting the performance of target traits (Ju et al., 2019) are used in the identification of tea germplasm resources (Yu et al., 2009), genetic diversity analysis and population classification (Ji et al., 2011), genetic stability analysis (Yao et al., 2008), gene mapping and assisted breeding (Roy and Chakraborty, 2009) are widely used. Zhu et al. (2017) analyzed the genetic diversity of 53 tea tree varieties in 7 regions of Fujian Province, but there are few studies using wild tea trees in Fujian Province as materials. Due to the limitation of the growth environment, Youxi bitter tea is still in a state of allowing natural growth, sporadic distribution, and extensive harvesting. Predecessors have carried out experiments on expansion and trial planting of Youxi bitter tea but did not involve research on tea genetic variation. In this study, ISSR markers were used to analyze the genetic diversity of Youxi bitter tea resources, in order to provide theoretical support for the establishment of Youxi bitter tea resources core conservation area, breeding of specific germplasm, and enrichment of the core tea tree germplasm bank in Fujian Province.

1 Results and Analysis
1.1 Amplified polymorphism analysis of Youxi bitter tea
The OD value and brightness of the extracted Youxi bitter tea gene DNA meet the ISSR-PCR amplification requirements (Figure 1), and the DNA fragment size ranges from 250 to 2 000 bp. The 8 primers selected in the previous experiment were used to amplify 37 pieces of bitter tea DNA, and 66 clear bands with polymorphism were obtained, with an average polymorphism percentage of 99.4%, and an average of 8.25 bands per primer (Table 1) Primer U836 has the largest number of amplified bands, and primers U818 and ISSR9 have the least number of amplified bands, which are 12 and 6 respectively. The amplification polymorphism ratio of most of the primers was 100%, indicating that the 37 bitter tea resources tested had high genetic polymorphism.

Figure 1 DNA electrophoresis of some bitter tea
Note: M: 2 000 bp DNA Marker; C1~BA1: part of bitter tea resources
Figure 2 amplification map of ISSR primer of UBC854

Note: M: 2 000 bp DNA Marker; C1~BA1: part of bitter tea resources

Table 1 Polymorphism of ISSR primers and their amplified products

| Code | Sequence (5′→3′) | Annealing temperature (℃) | Number of loci | Number of polymorphic loci | Percentage of polymorphic loci (PPL) (%) |
|------|-----------------|---------------------------|----------------|---------------------------|----------------------------------------|
| U818 | (CA)₈G          | 50.3                      | 6              | 6                          | 100.00                                 |
| U824 | (TG)₈G          | 53.1                      | 7              | 7                          | 100.00                                 |
| U827 | (AC)₈G          | 54.0                      | 7              | 7                          | 100.00                                 |
| U834 | (AG)₈YT         | 57.4                      | 8              | 8                          | 100.00                                 |
| U836 | (AG)₈YA         | 57.4                      | 12             | 12                         | 100.00                                 |
| U854 | (TG)₈RG         | 55.1                      | 8              | 7                          | 87.50                                  |
| U900 | ACTTCCCCACAGGTTAACA | 53.5                  | 12             | 11                         | 91.67                                  |
| ISSR9 | (CAA)₈G        | 50.3                      | 6              | 5                          | 83.33                                  |
| Total | -               | -                         | 66             | 63                         | -                                      |
| Mean  | -               | -                         | 8.25           | 7.88                       | -                                      |

Note: R=(A, G); Y=(C, T)

1.2 Genetic diversity analysis of Youxi Kucha

Analysis of ISSR results of the 4 populations of Youxi Kucha (Table 2) shows that the population polymorphism in Qiushan Village is the highest (88.89%), and Guangming Village is the lowest (73.02%). At the population level, the average polymorphism ratio of the four populations of Youxi Kucha is 76.19%, the genetic diversity index ($H$) ranges from 0.203 7 to 0.289 9, and Shannon's information index ($I$) is 0.314 8 -0.437 9, the mean values are 0.240 5 and 0.367 7 respectively. At the species level, the polymorphism ratio of the four populations of Youxi bitter tea is 95.24%, $H$ is 0.305 1, and $I$ is 0.465 0, which is higher than the Nei gene diversity index ($H$=0.154) and Shannon's information index of Fujian tea trees. ($I$ =0.242) (Zhu Chen, 2017), among which Chimu Village (CMC) has the highest level of genetic diversity ($PPL = 88.89 \%$, $H = 0.289 9$, $I = 0.4379$), and Qiushan Village has the lowest level of genetic diversity ($PPL = 68.25 \%$, $H = 0.203 7$, $I = 0.3148$).

Table 2 Genetic diversity of youxi bitter tea distributed among different populations detected by ISSR

| Group      | Observed number of alleles ($Na$) | Effective number of alleles ($Ne$) | Nei's gene diversity ($H$) | Shannon's information index ($I$) | The number of polymorphic loci ($NPL$) | The percentage of polymorphic loci ($PPL$) (%) |
|------------|-----------------------------------|-----------------------------------|---------------------------|-----------------------------------|----------------------------------------|-----------------------------------------------|
| CMC        | 1.888 9                           | 1.492 7                           | 0.289 9                  | 0.437 9                           | 56                                     | 88.89                                         |
| NCMC       | 1.746 0                           | 1.349 5                           | 0.218 7                  | 0.340 7                           | 46                                     | 73.02                                         |
| GMV        | 1.730 2                           | 1.417 8                           | 0.249 7                  | 0.377 3                           | 47                                     | 74.60                                         |
| QSV        | 1.632 5                           | 1.336 7                           | 0.203 7                  | 0.314 8                           | 43                                     | 68.25                                         |
| Population | 1.749 4                           | 1.399 2                           | 0.240 5                  | 0.367 7                           | 48                                     | 76.19                                         |
| Species    | 1.952 4                           | 1.504 6                           | 0.305 1                  | 0.465 0                           | 60                                     | 95.24                                         |

Note: CMC: Chimu Cun; NCMC: Non-Chimu Cun; GMV: Guangming Village; QSV: Qiushan Village
1.3 Genetic differentiation of ISSR in Youxi Kucha

Analyzing the genetic structure of the ISSR of Youxi bitter tea (Table 3), the total genetic diversity of the four populations of Youxi bitter tea is 0.3023, and the genetic diversity within and between populations is 0.2405 and 0.0618. The gene differentiation coefficient is 0.2043, which means that the genetic differentiation between species is 20.43%, and 79.57% of the genetic differentiation occurs in the population of Youxi Kucha. Gene flow (Nm) is an important factor in the variation of species populations. Studies have shown that Nm ≥ 1, rich gene communication among populations, and Nm <1, less gene communication among populations (Wright, 1931). The gene flow among the bitter tea populations in Youxi was 1.9469 > 1. The genetic migration of individual tea plants may bring genes that were not in the original population and increase the genetic differentiation of bitter tea populations. The PHIst coefficient (Φst) can indicate the degree of genetic differentiation of the population (Buso et al., 1998). Φst between 0 and 0.05 indicates that the differentiation among populations is weak. A value between 0.05 and 0.15 indicates a moderate differentiation, and a value between 0.15 and 0.25 means high differentiation (Wright, 1949). The results of AMOVA analysis show that Youxi bitter tea PHIst coefficient (Φst) = 0.17, indicating a large degree of genetic differentiation. It shows that in the total genetic variation, 83% of the variation occurred within the population, and 17% of the variation occurred between the populations, but the genetic variation between the populations reached a very significant level (P <0.01).

Table 3 Nei's analysis of Youxi kucha population

| Item     | Ht   | Hs   | Gst  | Nm   |
|----------|------|------|------|------|
| Mean     | 0.3023 | 0.2405 | 0.2043 | 1.9469 |

Table 4 The Pearson test between genetic index and geographic distribution

| Diversity index | Longitude | Latitude | Altitude |
|-----------------|-----------|----------|----------|
| Na              | 0.768 (0.116) | -0.300 (0.350) | -0.185 (0.407) |
| Ne              | 0.500 (0.250) | -0.487 (0.256) | 0.031 (0.484) |
| H               | 0.530 (0.235) | -0.499 (0.250) | 0.034 (0.483) |
| I               | 0.570 (0.215) | -0.473 (0.263) | 0.000 (0.500) |
| PPL             | -0.464 (0.268) | 0.632 (0.184) | -0.225 (0.387) |

1.4 Genetic distance among populations of Kucha Youxi

The genetic identity among the 4 populations of Youxi Kucha ranged from 0.7061 to 0.9689, the genetic distance ranged from 0.0740 to 0.1682 (Table 5), and the mean values were 0.8294 and 0.1142 respectively. Among them, Qiushan Village (QSV) population and Chimu Village Non-Protection Area (NCMC) population have the highest genetic agreement (0.9287) and the smallest genetic distance (0.0740); Chimu Village (CMC) and Guangming Village (GMV) have the highest genetic identity. The degree of agreement was the smallest (0.8452), and the genetic distance was the largest (0.1682). In the UPGMA cluster map (Figure 3), the Qiushan Village and Chimu Village non-protected areas cluster together, the Guangming Village population is the farthest from them, and the Chimu Village population is in the middle position. The PCA results of the four populations of Youxi Kucha are consistent with the UPGMA results (Figure 4). The differences represented by the first two principal components PC1 and PC2 are 11.85% and 21.06%, respectively. Among them, there are some samples in
Guangming Village and Qiushan Village not completely separated, and there may be reasons such as artificial introduction that cause them to be similar in genetic background.

Table 5 Nei’s genetic identity (above diagonal) and genetic distance (below diagonal) between populations of Youxi Kucha

| Village          | Guangming Village | Chimu Village (Non-protected areas) | Chimu Village | Qiushan Village |
|------------------|-------------------|-------------------------------------|---------------|-----------------|
| Guangming Village| ****              | 0.889 3                             | 0.845 2       | 0.894 1         |
| Chimu Village (Non-0.117 3 protected areas) | ****              | 0.896 7                             | 0.928 7       |                 |
| Chimu Village    | 0.168 2           | 0.109                               | ****          | 0.900 4         |
| Qiushan Village  | 0.112             | 0.074                               | 0.104 9       | ****            |

Figure 3 Dendrogram of UPGMA cluster analysis based on Nei’s genetic distances among 4 populations of Youxi Kucha

Figure 4 Principal coordinates analysis (PCA) on the individuals of 4 populations in Youxi Kucha

1.5 Cluster analysis based on ISSR molecular markers

The UPGMA cluster analysis was performed on 37 samples of Youxi bitter tea from 4 populations (Figure 5). When the similarity coefficient is 0.31, bitter tea can be roughly divided into 2 categories. There are 7 samples in the first group, which are red. The samples E2, E3, E4, E5, E6, E7 from the non-protected area of the tomb village, and the second group are all the remaining samples. Group II can be divided into 3 subgroups. Group II-1 has 7 samples, including all samples from Guangming Village and 1 sample from Chimu Village non-protected area. Group II-2 contains 12 samples, including most of the red samples. Samples from the Tomb Village Reserve and one sample from the Chi Tomb Village non-protected area E1. There are 12 samples in group II-3, including all...
samples from Qiushan Village and 5 samples from Chimu Village Reserve. Among them, BY26 and BY33 have the same genetic distance, which suggests that their genetic backgrounds are extremely similar or the same.

1.6 Correlation analysis of geographic distance and genetic distance of Youxi bitter tea resources

In this experiment, Genalex software was used to perform Mantel test on the genetic distance and geographic distance matrix of 4 populations of Youxi Kucha resources, and P=0.13> 0.05. If P> 0.05, there is no correlation between the two matrices, P < There is a correlation at 0.05 (Burns et al., 2004). It shows that the genetic distance of Youxi bitter tea resource samples has little correlation with geographic distance (Figure 6), and geographic distance is not the dominant factor in the genetic differentiation of Youxi bitter tea.

2 Discussion

2.1 Genetic diversity of Youxi bitter tea

Species genetic diversity is the prerequisite for their survival and development and evolution (Chen et al., 2017). Accurate evaluation of the genetic diversity of bitter tea resources is of great guiding significance for the analysis of tea tree evolutionary potential, protection and innovative utilization of tea germplasm resources. The average
Nei's genetic diversity index ($H$) of Youxi bitter tea population level is 0.2405, and the species-level Nei's genetic diversity index ($H$) is 0.3051, which is higher than the plant population counted by Nybom (2004) The average genetic diversity (0.22~0.23) and the total Nei's genetic diversity index (0.224) of tea trees in Fujian Province (Zhu et al., 2017) indicate that Youxi bitter tea has high genetic diversity at both the population and species level. The factors that affect the genetic diversity of tea populations are diverse, such as internal causes such as gene mutation, genetic drift, and gene exchange, as well as external causes such as population isolation and habitat fragmentation caused by changes in the growth environment and intervention by human activities,(Wen et al., 2010). Previous studies have shown that population reduction and isolation will lead to the loss of genetic diversity (Chen, 2017). Most of the wild resource growth areas of Youxi bitter tea have been destroyed or reclaimed for agricultural land. Most of the plants have been distributed sporadically. The areas are separated by mountains. It is inferred that the Youxi bitter tea resource population originally had a relatively rich genetic basis. At the species level, bitter tea retains a high overall genetic diversity ($H = 0.3051$), and the genetic diversity at the population level increases with the population level of bitter tea. The number of individuals and their distribution range became smaller and gradually decreased ($H = 0.2405$).

2.2 Genetic structure and differentiation of Youxi bitter tea population

The degree of genetic differentiation among populations of Youxi bitter tea ($G_{st} = 0.2043$, $\Phi_{st} = 0.17$) is lower than the total genetic differentiation coefficient of tea trees in Fujian Province ($G_{st} = 0.332$) studied by Zhu Chen et al. (2017), the results showed that the genetic variation of Youxi Kucha was mainly within populations rather than between populations. The gene flow ($Nm$) is 1.9469 which indicates that there is a certain degree of information exchange and genetic drift among the four populations of Youxi bitter tea, which is consistent with the fact that the tea trees in Fujian Province have more frequent genetic information exchanges (Irwin et al., 2005). The range of differentiation index $\Phi_{st}$ between 0.15 and 0.25 indicates a greater degree of differentiation among plant populations. The differentiation index $\Phi_{st}$ of Youxi bitter tea is 0.17, indicating that although there is a certain degree of information exchange among the 4 populations of Youxi bitter tea, however, the degree of genetic differentiation is also relatively large, which is speculated to be related to the mountain isolation among the Youxi bitter tea populations, and the results are consistent with the results of Cozzolino et al. (2003) that physical isolation such as high mountains can increase the genetic differentiation among plant populations. The level of genetic differentiation among populations of Youxi bitter tea is also related to its reproduction mode. Most bitter tea plants in the wild reproduce through seeds. The genetic differentiation level of Kucha population in Youxi is also related to its propagation mode. Most of the wild Kucha plants propagate through seeds. The original terrain of Youxi Kucha is relatively steep, and the geographical isolation, population number and scale reduction are not easy for gene exchange among populations, which leads to the increase of genetic differentiation level among populations.

Mantel test results show that geographic distance is not the dominant reason for the genetic diversity of Youxi bitter tea populations, and there may also be artificial introductions among tea populations that lead to frequent gene exchanges of tea plants in different habitats, regions, and traits. The genetic variation and environmental factors of tea plants will lead to differences in phenotypic traits. Youxi bitter tea resources are extremely rich in phenotypic variation. Both bitter tea BY3 and BY25 are located in Chimu Village, but BY3 has oblong leaves with purple buds. BY25 has lanceolate leaves, with green buds and leaves. Different populations of bitter tea have different leaf types, leaf colors, and leaf sizes, indicating that although the physical isolation of mountains and hills and the reduction of population size are limited to some extent The genetic information exchange between populations has been improved, but due to the tea plant’s own reproductive system and artificial introduction and other reasons, there is also a certain degree of gene exchange, which leads to relatively rich morphological variation.
2.3 Protection and utilization of Youxi bitter tea

Youxi bitter tea resources have undergone a long genetic evolution and natural selection, and maintain high genetic diversity at the population level. However, its habitat has been destroyed, the distribution area is gradually scattered, and the population is facing degradation and disappearance. Therefore, the protection and utilization of Youxi bitter tea are very urgent. The genetic diversity of tea plants is the basic condition for innovative germplasm and improved varieties. In terms of protection strategies, try to treat each population as a whole, and establish a protected area in the native place of bitter tea to protect the genetic diversity of the Youxi bitter tea population to the greatest extent. Therefore, priority should be given to protecting bitter tea from Chimu Village and Guangming Village, which have high genetic diversity. Secondly, it is possible to establish wild tea tree resource nurseries for ex situ preservation by combining the distance of genetic relationship and the phenotypic traits of tea trees (Liu et al., 2020). Due to the special quality and scarcity of wild bitter tea resources, the cuttings should be preserved as much as possible to include different types of wild bitter tea trees. In the analysis of the genetic relationship of the ISSR molecular markers of Youxi bitter tea, it was found that genetic variation occurred within the population. For example, B1 and B3 in Chimu Village were clustered together, and the relationship was relatively close, but the color of buds and leaves of B1 plant was observed purple, while B3 is green leaves, forming the diversity and complexity of genetic relationship. Therefore, the next step can be the research on parent screening, introduction and domestication of the Youxi bitter tea resource population, new varieties selection and other aspects. Artificial large-scale planting of Youxi bitter tea provides materials for the extraction and utilization of a large number of functional ingredients.

3 Materials and Methods

3.1 Experimental materials

The investigation found that the individual wild Youxi bitter tea plants are scattered and mixed in the forests of Youxi County in the Daiyun Mountains. They are roughly divided into four populations according to the growth area. The growing well bitter tea individual plants were collected in the spring of 2016. There are 37 samples (Table 6), including 16 samples from Chimu Village (CMC), 8 samples from CMC Non-Conservation Area (NCMC), 6 samples from Guangming Village (GMV), and 7 samples from Qiushan Village (QSV) 7, Fuyun 6 was used as a conventional control variety. With the assistance of Tangchuan Gaoshan Tea Cooperative in Youxi County, the picking standard is one bud and two leaves, the sample is solidified in liquid nitrogen, and stored in a refrigerator at -80 °C.

3.2 Main equipment

- Micro grinder PULVERISETTE23 (German Flying Spur);
- Eppendorf AG 22331 Hamburg centrifuge (Germany, Eppendorf GmbH);
- Eppendorf research pipette (Germany, Eppendorf GmbH);
- DYY-6D electrophoresis instrument (Beijing Liu Yi Instrument Factory);
- Syngene G:BOX F3 gel imaging (British SYNGENE company);
- IMS-40 automatic snowflake ice maker (Changshu Xueke Electric Co., Ltd.);
- HWS-12 electric heating constant temperature water bath (Shanghai Yiheng Scientific Instrument Co., Ltd.);
- 2× Easy Taq Super Mix (Beijing Quanshijin Biotechnology Co., Ltd.);
- DL2000 DNA Marker (Japan, TaKaRa);
- GelRed nucleic acid gel dye (Biotium, USA);
- Thermo NanoDrop2000C UV/Vis Spectrophotometer (The United States, Thermo Scientific);
- Biospin Plant Genomic DNA Extraction Kit (Bioflux, Hangzhou Bori Technology Co., Ltd.);
- GI54DS autoclave (American Micro), T100 Thermal Cycler PCR instrument (United States Bio-Rad).

3.3 DNA extraction and quality testing

Extract the DNA of bitter tea samples with the complete gold plant DNA extraction kit. The DNA quality was detected by 1% agarose gel electrophoresis. The DNA concentration and purity were detected by UV-visible spectrophotometer. The extracted DNA was diluted to 50 ng/μL. Store in the refrigerator at -20°C for later use. The DNA template was detected by 1.0% agarose gel electrophoresis. After completion, it was observed and photographed under ultraviolet light.
3.4 Primer screening, ISSR-PCR reaction system and amplification program

30 primers with high repetition rate were screened by referring to the primer number sequences of ISSR markers of wild tea plants (Liu et al., 2014; Liu et al, In 2015, Huada gene company conducted primer synthesis and screening, and screened the high-efficiency primers with good polymorphism, clear background and high stability for Youxi Kucha resources research from 30 primers, and screened the optimal annealing temperature of each primer by gradient PCR. The ISSR-PCR system consisted of 10 μL full-length gold, 2× EasyTaq PCR Super-Mix, 1.5 μL primer, 1.5 μL DNA (50 ng/μL), 7 μL ddH2O, and the total volume was 20 μL. The samples were pre-denatured at 94 ℃ for 2 min, denatured at 94 ℃ for 30 s, annealed at 50 ℃ ~ 60 ℃ for 1 min, and extended for 2 min and 30 s at 72 ℃ for a total of 38 cycles. Finally, the samples were extended at 72 ℃ for 10 min and stored at 4 ℃.

Table 6 Materials and sources

| Code | Population       | Population code | Altitude  |
|------|------------------|-----------------|-----------|
| B1   | Chimu Village    | CMC             | 841.86    |
| B2   | Chimu Village    | CMC             | 822.86    |
| B3   | Chimu Village    | CMC             | 829.66    |
| B6   | Chimu Village    | CMC             | 766.84    |
| B7   | Chimu Village    | CMC             | 774.46    |
| B8   | Chimu Village    | CMC             | 839.12    |
| B9   | Chimu Village    | CMC             | 853.22    |
| B10  | Chimu Village    | CMC             | 823.74    |
| BY10 | Chimu Village    | CMC             | 825.02    |
| BY25 | Chimu Village    | CMC             | 841.1     |
| BY26 | Chimu Village    | CMC             | 836.76    |
| BY33 | Chimu Village    | CMC             | 835.7     |
| BA1  | Chimu Village    | CMC             | 823.7     |
| BA2  | Chimu Village    | CMC             | 846.92    |
| BA3  | Chimu Village    | CMC             | 822.46    |
| BA4  | Chimu Village    | CMC             | 823.82    |
| E1   | Chimu Village    | NCMC            | 868.9     |
| E2   | Chimu Village    | NCMC            | 863.64    |
| E3   | Chimu Village    | NCMC            | 866.62    |
| E4   | Chimu Village    | NCMC            | 862.16    |
| E5   | Chimu Village    | NCMC            | 866.14    |
| E6   | Chimu Village    | NCMC            | 892.36    |
| E7   | Chimu Village    | NCMC            | 875.96    |
| E8   | Chimu Village    | NCMC            | 882.68    |
| C1   | Guangming Village| GMV             | 1088.08   |
| C2   | Guangming Village| GMV             | 1078.56   |
| C3   | Guangming Village| GMV             | 1090.48   |
| C4   | Guangming Village| GMV             | 1064.62   |
| C5   | Guangming Village| GMV             | 913.06    |
| C6   | Guangming Village| GMV             | 908.78    |
| D1   | Qiushan Village  | QSV             | 889.66    |
| D2   | Qiushan Village  | QSV             | 828.8     |
| D3   | Qiushan Village  | QSV             | 810.08    |
| D4   | Qiushan Village  | QSV             | 860.56    |
| DY13 | Qiushan Village  | QSV             | 811.36    |
| DY14 | Qiushan Village  | QSV             | 810.7     |
| DY15 | Qiushan Village  | QSV             | 851.7     |

3.5 Data analysis

In the electrophoresis result, amplified bands in the same position are marked as 1, and no bands are marked as 0, forming the original data matrix. Use POPGENE 1.32 software to calculate genetic diversity parameters, and
genetic diversity ($D_{st}$) between populations is calculated by the formula ($D_{st} = H_{t} - H_{s}$). The R language Geosphere package was used to calculate the geographic distance between the populations, and the GenALEx 6.3 software was used for Mantel inspection. Use NTSYS software to perform UPGMA cluster analysis.

**Authors’ contributions**

Wei Shasha and Peng Jing are the experimental designers and research executives of this study; Wei Shasha, Peng Jing and Chen Zhidan completed the statistical analysis of the data and wrote the first draft of the paper; Chen Zhidan, Lin Lin, and Wu Renquan participated in the experimental design and the experimental results analysis; Sun Weijiang was the designer and director of the research and guided the experimental design, data analysis, thesis writing and revision. All authors read and approved the final manuscript.

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