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RAPID IDENTIFICATION AND GENETIC RELATIONSHIP OF LOCAL VARIETIES OF *PERUVIANA* L.

GONG YUQIAO[1], LIU CHEN[2], ZHANG XIANGYU[3], SHIGUO QIANG[4] AND QIAO HONGYU[1,5*]

[1]Modern Vegetable Industry Technology and Germplasm Resource Innovation Team, College of Horticulture, Jilin Agricultural University, Changchun 130118, China; [2]Changchun Lnstitute of Biological Products Co., Ltd. [3]Academy of Forestry of Jilin Province; [4]Changbai Mountain Academy of Sciences [5]Northeast Asia Special Germplasm Resource Conservation and Innovation Center Vegetable Breeding Technology Innovation Team, College of Horticulture, Jilin Agricultural University, Changchun 130118, China [*]Corresponding author's email: hongyu_q2023@163.com

Abstract

Plant variety and cultivar discovery is one of the most significant and tricky issue in agricultural arrangement. This study is for barcoding of 12 *Physalis peruviana* L., landraces in northeast of China with three specific primers (ITS2, matK, rbcL) and their combinations loci such as rbcL+matK, ITS2+rbcL, ITS2+matK, ITS2+rbcL+matK. Otherwise, 10 genetic difference of landraces was assess using 10 SSR markers. The phylogenetic trees of ITS2+rbcL and rbcL+matK landraces of *Physalis peruviana* L., in a separate clade demonstrating that the combinations loci ITS2+rbcL and rbcL+matK have sufficient efficiency for landraces discrimination of *Physalis peruviana*. In addition, Mantel check showed no important correlation between genetic space and geographical distance. AMOVA analysis showed that the main *Physalis peruviana* L., landraces variation of the collection existed in the within-population or cluster.

Key words: *Physalis* L. *germplasm resources*, SSR molecular markers, DNA barcode.

Introduction

Crop variety discovery amuse yourself a significant job in crop breeding, registration, grain producing, commerce and inspection, and is one of the challenges in the request of molecular markers in breeding and serious diversity diagnosis (Korir *et al*., 2013; Wang *et al*., 2020; Zhang *et al*., 2022). Abundant plant cultivars or germplasms can broaden the genetic variability and provide considerable opportunities for plant genetics and breeding research. (Li *et al*., 2021; Tran *et al*., 2009) Therefore, how to effectively identify and distinguish each plant germplasm resource in order to manage and maintain these genetic resources is crucial (Egea *et al*., 2017; Govindaraj *et al*., 2015).

At present, field phenotypic investigation based on DUS detection criteria and laboratory DNA fingerprinting are the two main methods for plant variety identification (Čurn & Žaludová, 2007; Wang *et al*., 2018). Morphological characteristics are often polygenic, not available at all stages of growth, and subject to environmental influences, difficult to evaluate quickly and objectively, requiring repeated observation. Thus, it is hardship distinguish similar varieties based on morphological characteristics alone (Kwon *et al*., 2005). DNA finger-printing is a prominent method for variety identification, which can improve the accuracy and efficiency of identification (Bhat, 2006; Xie *et al*., 2019). Nevertheless, DNA fingerprinting cannot cause referable facts, cannot reveal particular primers and polymorphic markers, and cannot be used for the separation and discovery of different kind, varieties and cultivars. A mass of varieties or landraces among crop plants need a molecular approach which have the advantages of potentially fast identification of a large numeral of individuals.

With the development of DNA barcoding in plants, the ultimate aim of DNA barcoding was to transfer from result a widespread marker to categorize plant. DNA molecular barcoding has been affirming to recognize without a doubt uncertain species in a variety of plants, such as *Dendrobium.officinale* (Chen *et al*., 2021), *lycium barbarum* (Wan *et al*., 2020) etc. Wu Yanan used ITS2 sequence for molecular identification of solanaceae hyacinus genus, which proved that ITS2 series has great potential in the identification of hyacinus species (Wu *et al*., 2016).

SSR molecular markers also are think to be one of the most principe molecular markers for plant genetic research (Feng *et al*., 2016) which are widely distributed, plentiful and diverse in eukaryotic genomes and have the benefit of excellent firmness, simple undertaking and codominance. Zhu Yujia successfully screened out 20 pairs of SSR molecular markers and analyzed the genetic relationship of 4 species of acidophytes (Zhu *et al*., 2018).

Guniang is an annual horticultural plant of the *Physalis peruviana* L. *solanaceae*, which is rich in functional components, which can relieve fatigue, eliminate muscle pain, reduce blood pressure, prevent arteriosclerosis and cardiovascular disease, and protect the skin. The pulp can be used as raw material to produce all kinds of juice, canned, jelly, jam and dried fruit. (Jiang *et al*., 2019; Yan *et al*., 2020) At present, the economic status of *Physalis peruviana* L., is getting higher and higher in some cities of northeast China (Yang *et al*., 2017; Wang *et al*., 2020).

At present, the landraces of *Physalis peruviana* L., are difficult to identify, and their genetic relationship is not clear. We choose 12 usual landraces build DNA barcoding to swiftly difference and recognize the diversity and use SSR molecular markers to explain their genetic association.

According to the research of Wu Yanan and the recommendation of the plant Working Group of the

International DNA Molecular Barcoding Alliance (Wu *et al*., 2016), we selected two chloroplast gene fragments rbcL and matK and one ribosomal gene fragment ITS2 to construct. Suitable primers for landrace of *Physalis peruviana* L., from SSR primers successfully labeled by Zhu Yujia (Zhu *et al*., 2018), and 10 pairs of SSR primers with strong specificity were selected. Moreover, analyzed the genetic difference and genetic alternative of these 12 landraces by SSR molecular markers, and conducted gathering analysis and Mantel diagnosis according to the genetic space.

Material and Methods

The test substance be selected for DNA origin from the frond of 12 common landraces of *Physalis peruviana* L., in Northeast China (see Table 1 for landraces facts). After DNA quality was detected by agarose gel, SSR molecular markers and DNA molecular barcodes were constructed.

Table 1. Sample information.

Folk taxonomy abbreviation	Origin of landraces	Number of samples
MD	Mudanjiang	19
EWX	suihua	16
ZDJ	Hailun	16
ZHB	suihua	15
TBQ	Daqing	18
LDH	Jiaohe	16
DLQ	Kiamusze	20
SHZ	suihua	17
TDT	Changchun	19
JPX	Changchun	15
TBT	Harbin	17
MSJ	Kiamusze	16

DNA extraction was performed using a new quick plant genome DNA extraction equipment (radiating pillar) cause by Shanghai Sangon Biotech Co., LTD. The extraction steps were carried out in accordance with the instructions.

DNA barcodes were constructed to identify 12 landraces of *Physalis peruviana* L.: Based on the study of Wu Yanan *et al*., (Wu *et al*., 2016) and the advice of plant level barcodes, two chloroplast piece genes rbcL and matK and one ribosomal fragment ITS2 were choose in this test to build DNA molecular barcodes. Chloroplast gene fragments rbcL and matK sequences were constructed on NBCI, and 10 matK and rbcL sequences were selected according to primer design principles. matK sequences were based on *Physalis pubescens* (EF438943.1). The rbcL sequence was referenced by *Physalis philadelphica* var. (MG221696.1). The ITS2 sequence of ribosome gene was obtained by removing 5.8s and 28S segments at both ends (downloaded from GenBank).

These 20 primers were cover, and eventually one pair of rbcL primers, one pair of matK primers and one set of ITS2 primers were get. The specific sequence of primers information is indicated in Table 2.

Table 2. information of screened primers.

DNA marker	Name of primers	Primer sequences (5′–3′)
ITS2	ITS2-F	ATGCGATACTTGGTGTGAAT
	ITS2-R	GACGCTTCTCCAGACTACAAT
matk	matk-F	CGATTTTGTTGGAAAATCCAGGTT
	matk-R	CGCACACTTGAAAGATAGCC
rbcl	rbcL-F	GTTGGATTCAAGGCTGGTGT
	rbcL-R	CAGCTCTACCGTAGTTTTTAGCAG

PCR amplification was performed using 30uL reaction system: 15ul 2×EasyTaq®PCRSuperMix, 1.5ul upstream and downstream primers (10umol/L), 2uL DNA template, ddH_2O supplemented to 30uL. PCR amplification procedure: pre-denaturation for 5min at 95℃; Denaturation at 94℃ for 45s, toughen at 56℃ ~ 58℃ for 45s, extension 90s at 72℃, constant undertaking for 35 series of events; Extension at 72℃ for 10min; 4℃. The PCR products were discovered by 1.0% agarose gel electrophoresis, and the increase consequence were watch under uv gel imaging system to make certain that the mark band was comprehensible and vivid without clear particular ensemble. The PCR products were sent to Shanghai Sangon Biotech Co., LTD for sequencing.

The data processing: Sequencing peak figure using software CodonCode Aligner 3.7.1 proof-read, take out small standard sequence and primers area, the sequence and the NCBI (https://www.ncbi.nlm.nih.gov) Blast compared to the corresponding sequence. The PhyloSuite was used to cascade different genes. The nucleotide diversity of ITS2, rbcL and matK sequences of 12 germplasm resources of *Physalis peruviana* L., were analyzed using Dnasp5 software. MEGA5.0 computer program K2P space model (Kimura2-parameter Distance) was used to work out genetic distance, and MEGA5.0 software was used to calculate interspecific and intraspecific genetic distance, mutation locus and mutation rate, etc. Phylogenetic trees were constructed by neighbor-joining tree (NJ) using MEGA5.0 software.

SSR markers were used to identify 12 species: We screened 20 pairs of SSR primers used by Zhu Yujia. (Zhu *et al*., 2018) to study the genetic relationship of acidophytes, and found 10 pairs of SSR primers suitable for 12 common landraces of *Physalis peruviana* L., in this study. See Supplementary Table 1 for SSR primer information.

25uL reaction system was used for PCR amplification: 12.5ul 2×EasyTaq®PCRSuperMix, 1.5ul upstream and downstream primers (10umol/L), 1uL DNA template, ddH_2O supplemented to 25uL, PCR amplification procedure: 94℃ pre-denaturation for 4min; 94℃ denaturation 30s, 10℃ + fading temperature 30s, 72℃ extension 1min, continuous running 20 cycles; 94℃ denaturation 30s, fading temperature 30s, 72℃ extension 1min, continuous running 25 cycles; Extension at 72℃ for 10min; 4℃. The PCR products were discovered by 1.0% agarose gel electrophoresis, and the increase consequence were watch under uv gel imaging system to discover the ensemble standard. Electrophoresis was performed using 6% polypropylene gel.

The data processing: Strip statistics were carried out on electrophoresis images of SSR products by using Quantity One software, and a binary matrix was established. GenAlex6.3 was used for statistical analysis of binary matrix to get a diversity of polymorphic facts sign, genetic distance and main organize (PCA) analysis consequence, genetic space and geographical distance correspondence test (Mantel test). Based on genetic distance, MEGA5.0 software was used for cluster analysis of germplasm resources in *Physalis peruviana* L., cultivation. Use R language to draw circle tree graph.

Supplementary Table 1. SSR primer information.

Primer	Sequence(5'to3')	Tm (℃)
SSR9F	TGCTCCGAGTTTTAGGGTTC	60
SSR9R	GCAGTTGGTAAAGTTGAGAGACG	60
SSR10F	GCTTCCTATTGTGTTGCCTGA	58
SSR10R	ACTTTGGGTTTCGGGAATTG	58
SSR11F	CAGCTGAAATAAGAGAGTGATTGG	57
SSR11R	CCCTCTTTTTCTCCTCCGAGT	57
SSR13F	GCGGAATCCATTGTTTTTCA	58
SSR13R	CCGATGGAGTATAGTCACGCAAA	58
SSR15F	GCTTGTTGATCAGCTTTCTTTG	57
SSR15R	TGGATCATAACCTTGCTAATGC	57
SSR36F	ATGAACCACATGTCGGAGGA	58
SSR36R	GGGGATCCAAACGAAGTGTA	58
SSR54F	CGGCTGGTATGCTTACAAAGAT	58
SSR54R	GCACTTCCACTGTTTTTAACTTCC	58
SSR55F	CACCTAVATAGGCAGCCAAAA	58
SSR55R	ATTTGTGGGCGGAGGAAG	58
SSR112F	CTACGCCTACCACTTGCACA	60
SSR112R	CAGTGGAAGCCTCAAGATCC	60
SSR118F	AATCAAGGGTCAGAAGAAATGG	58
SSR118R	GCAAGAATGGATGTGGGTGT	58

Results

Amplification and sequence analysis of ITS2, rbcL and matK: The three barcodes, ITS2, rbcL, and matK indicate 100% victory speed for PCR increase. The succession feature of the three area are current in Table 3. The ITS2 sequences had nine changeable area, the rbcL sequences had three changeable sites and the matK sequences had six variable sites among the twelve *Physalis peruviana* L., landraces (Table S2). The genetic distances for the ITS2 sequence ranged from 0 to 0.02883, the rbcL sequence ranged from 0 to 0.00279 and the matK sequence ranged from 0 to 0.00307 (Tables S3, S4, S5). ITS2 sequence had two haplotypes, haplotype diversity index was 0.142, nucleotide diversity index is 0.00596. There were three haplotypes in rbcL sequence, the haplotype difference was 0.107, and the nucleotide diversity list was 0.005178. There were 8 haplotypes in matK sequence, haplotype diversity was 0.450, nucleotide diversity index was 0.0009. matK had the highest numeral of haplotypes and the tall difference of haplotypes. The nucleotide diversity of ITS2 sequence was the highest.

Table 3. Nucleotide diversity of ITS2, rbcL, matk sequences.

DNA marker	h	Hd	Pi
ITS2	2	0.142	0.00596
rbcL	3	0.107	0.005178
matk	8	0.45	0.0009

Phylogenetic analysis: The effectiveness of one gene DNA molecular barcoding for the descovery of 12 agreement was small. The identification efficiency of double gene DNA barcode ITS2+rbcL was 100% (Fig. 1a), double gene DNA barcode rbcL+matk was 100% (Fig. 1b), and triple gene DNA barcode ITS2+rbcL+matk was 83.4% (Fig. 1c).

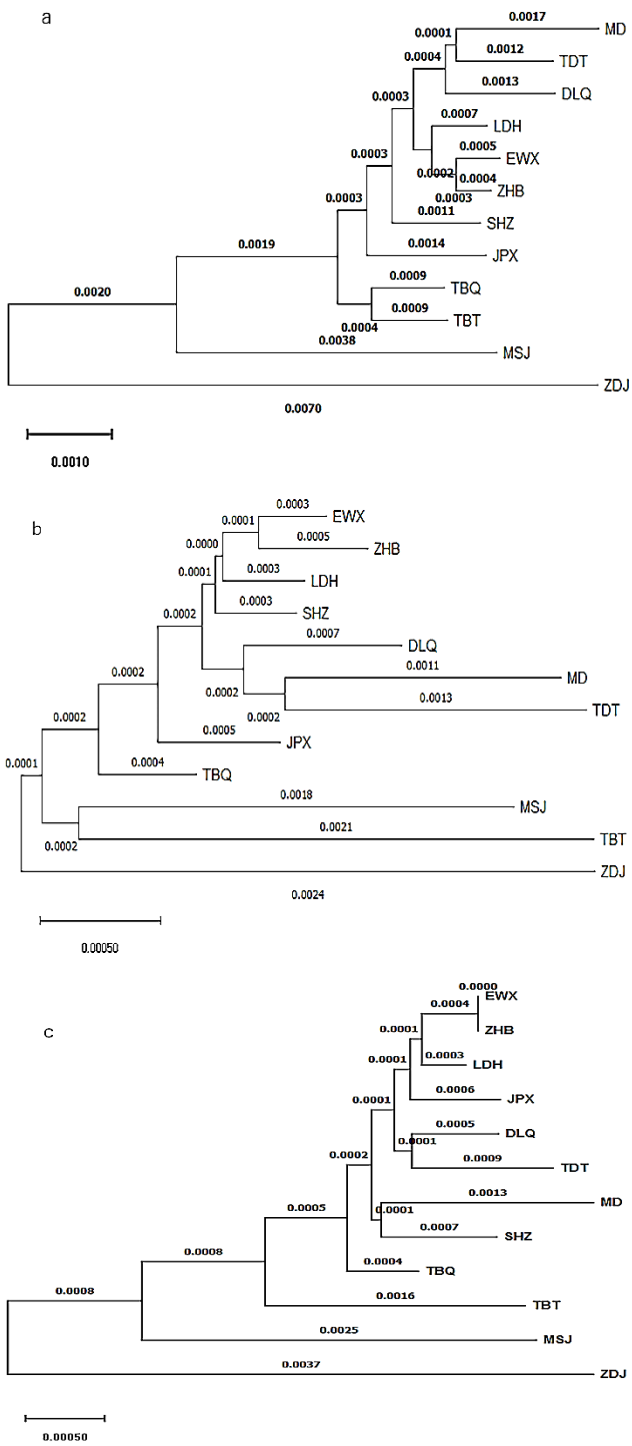

Fig. 1. NJ clustering tree.

Supplementary Table 2. ITS2, matk, rbcL variation sites.

Sample	ITS2.var									sample	Matk.var						sample	rbcL.var		
Var. point	29	31	39	147	156	160	171	184	185	Var. point	1	2	3	791	873	874	Var. point	33	34	480
Conserved base	T	G	G	T	A	G	G	C	G	Conserved base	A	A	T	G	G	C	Conserved base	G	T	C
MD	*	*	*	*	*	*	*	*	*	MD	*	*	*	*	*	A	MD	*	*	*
EWX	*	*	*	*	*	*	*	*	*	EWX	*	*	*	*	*	*	EWX	*	*	*
ZDJ	G	C	T	G	T	C	C	A	C	ZDJ	T	*	*	*	T	*	ZDJ	*	*	—
ZHB	*	*	*	*	*	*	*	*	*	ZHB	*	*	*	*	*	*	ZHB	*	*	*
TBQ	*	*	*	*	*	*	*	*	*	TBQ	*	*	*	*	*	*	TBQ	*	*	T
LDH	*	*	*	*	*	*	*	*	*	LDH	*	*	*	*	*	*	LDH	*	*	*
DLQ	*	*	*	*	*	*	*	*	*	DLQ	*	*	*	*	*	*	DLQ	*	*	*
SHZ	*	*	*	*	*	*	*	*	*	SHZ	*	*	*	*	*	*	SHZ	T	G	—
TDT	*	*	*	*	*	*	*	*	*	TDT	G	*	*	*	*	*	TDT	*	*	*
JPX	*	*	*	*	*	*	*	*	*	JPX	*	*	*	*	*	*	JPX	*	*	T
TBT	*	*	*	*	*	*	*	*	*	TBT	T	*	A	*	C	*	TBT	*	*	—
MSJ	*	*	*	G	T	C	C	A	C	MSJ	T	*	*	*	T	T	MSJ	*	*	T

Supplementary Table 3. ITS2 genetic distance table.

landrace	MD	EWX	ZDJ	ZHB	TBQ	LDH	DLQ	SHZ	TDT	JPX	TBT	MSJ
MD												
EWX	0											
ZDJ	0.02883	0.02883										
ZHB	0	0	0.02883									
TBQ	0	0	0.02883	0								
LDH	0	0	0.02883	0	0							
DLQ	0	0	0.02883	0	0	0						
SHZ	0	0	0.02883	0	0	0	0					
TDT	0	0	0.02883	0	0	0	0	0				
JPX	0	0	0.02883	0	0	0	0	0	0			
TBT	0	0	0.02883	0	0	0	0	0	0	0		
MSJ	0.01441	0.01441	0.02402	0.01441	0.01441	0.01441	0.01441	0.01441	0.01441	0.01441	0.01441	
Genetic distance within germplasm resources	0	0	0.02883	0	0	0	0	0	0	0	0	0.02883

Supplementary Table 4. matk genetic distance table.

landrace	MD	EWX	ZDJ	ZHB	TBQ	LDH	DLQ	SHZ	TDT	JPX	TBT	MSJ
MD												
EWX	0											
ZDJ	0	0										
ZHB	0	0	0									
TBQ	0.00139	0.00139	0.0007	0.00139								
LDH	0	0	0	0	0.00139							
DLQ	0	0	0	0	0.00139	0						
SHZ	0.0014	0.0014	0.0014	0.0014	0.00232	0.0014	0.0014					
TDT	0	0	0	0	0.00139	0	0	0.0014				
JPX	0.0007	0.0007	0.00035	0.0007	0.00116	0.0007	0.0007	0.00186	0.0007			
TBT	0	0	0	0	0.00093	0	0	0.0014	0	0.00046		
MSJ	0.0007	0.0007	0.00035	0.0007	0.00116	0.0007	0.0007	0.00186	0.0007	0.00093	0.00046	
Genetic distance within germplasm resources	0.00077	0.00000	0.00154	0.00000	0.00000	0.00000	0.00000	0.00000	0.00077	0.00000	0.00077	0.00307

Supplementary Table 5. rbcL genetic distance table.

landrace	MD	EWX	ZDJ	ZHB	TBQ	LDH	DLQ	SHZ	TDT	JPX	TBT	MSJ
MD												
EWX	0.00000											
ZDJ	0.00000	0.00000										
ZHB	0.00000	0.00000	0.00000									
TBQ	0.00139	0.00139	0.00070	0.00139								
LDH	0.00000	0.00000	0.00000	0.00000	0.00139							
DLQ	0.00000	0.00000	0.00000	0.00000	0.00139	0.00000						
SHZ	0.00140	0.00140	0.00140	0.00140	0.00232	0.00140	0.00140					
TDT	0.00000	0.00000	0.00000	0.00000	0.00139	0.00000	0.00000	0.00140				
JPX	0.00070	0.00070	0.00035	0.00070	0.00116	0.00070	0.00070	0.00186	0.00070			
TBT	0.00000	0.00000	0.00000	0.00000	0.00093	0.00000	0.00000	0.00140	0.00000	0.00046		
MSJ	0.00070	0.00070	0.00035	0.00070	0.00116	0.00070	0.00070	0.00186	0.00070	0.00093	0.00046	
Genetic distance within germplasm resources	0.00000	0.00000	0.00000	0.00000	0.00139	0.00000	0.00000	0.00279	0.00000	0.00139	0.00000	0.00139

SSR marker assay and their informativeness: All 10 SSR markers successfully amplified targeted DNA fragments among these 12 *Physalis peruviana* L., landraces. The analysis indicated a sum of 357 loci were recognize by 10 SSR primers. Furthermore, the polymorphism facts of SSR markers show by mean numeral of efficient alleles (Na), effective alleles (Ne), Shannon Wiener index(h), and Percentage of polymorphic loci (PPL%) were also calculated and listed in Table 4. For these 10 markers, the h ranged from 0.133 to 0.206 with a mean value of 0.167. The I of each marker changed from 0.208 to 0.323, with the averaged 0.257. The Na of each marker changed from 0.888 to 1.346. The Ne of each marker changed from 1.215 to 1.375. The ppl% varied from 44% to 68%, with an average of 54.34%, which indicated that these SSR markers were good enough for evaluating the genetic diversity of the 12 *Physalis peruviana* L., landraces.

The genetic diversity and genetic differentiation of the 12 *Physalis peruviana* L. landraces: Using SSR marker data, the inhabitant distinction was assessed by molecular analysis of difference (AMOVA), and the genetic alternative speed was 71%, and that of among populations was only 29% (Table 5). In addition, the overall Fst value (0.033) indicated a small position of genetic distinction among the accumulation of 12 *Physalis peruviana* L., landraces, according to (Wright., 1978) who marked the genetic distinction as small for Fst <0.05, mild for 0.05 < Fst <0.15, high for 0.15 < Fst <0.25, and very high for Fst >0.25.

The genetic space matrix of 12 *Physalis peruviana* L., landraces was deliberate based on the get data of SSR markers. The neighbor-joining tree of these accessions was constructed with the obtained genetic distances by the UPGMA method, and the dendrogram was shown in Fig. 2. These 12 landraces were mainly grouped into two clusters. No direct relations were revealed between the geographical location of each of the *Physalis peruviana* L., landraces origin area (Fig. 3).

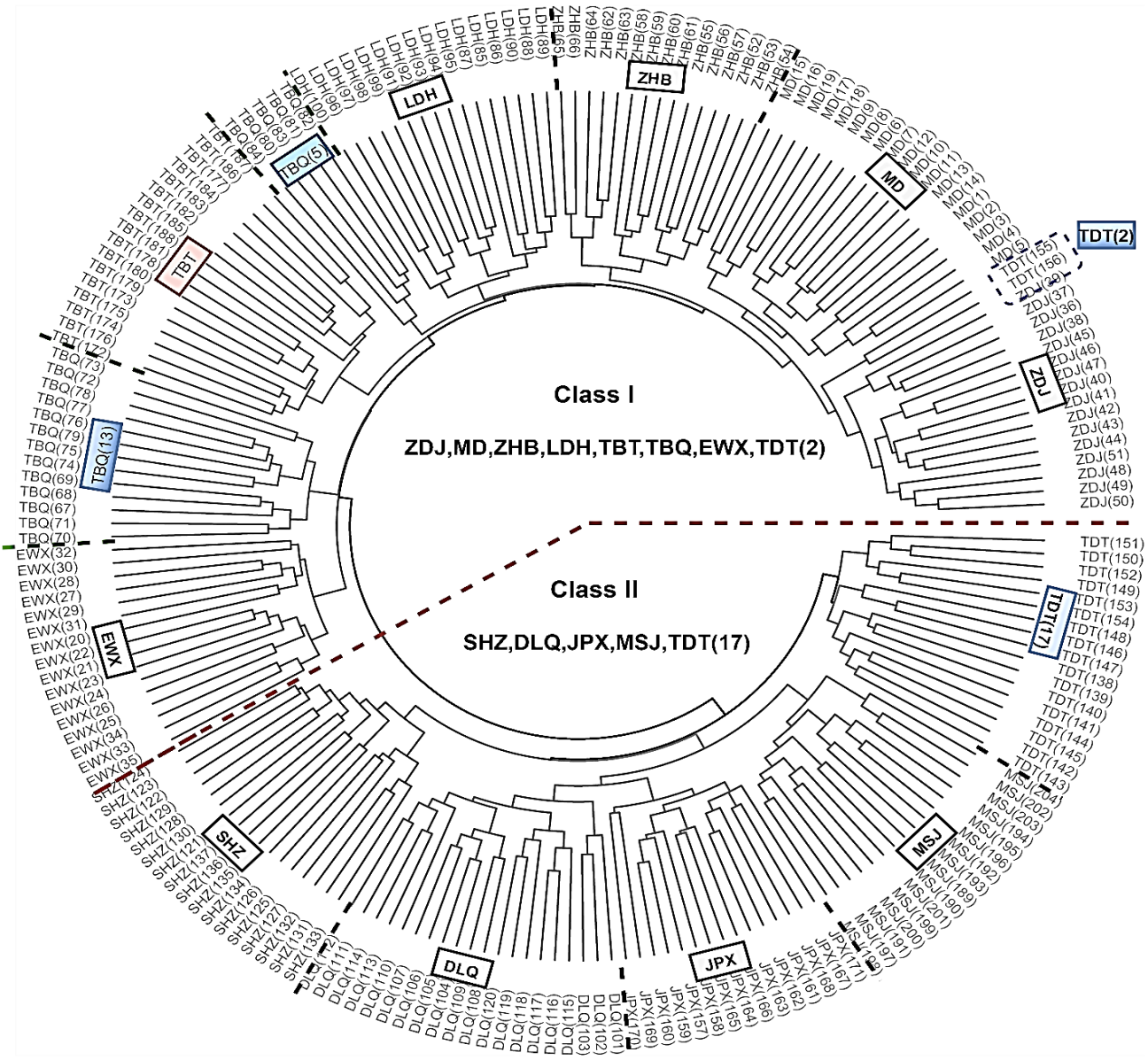

Fig. 2. Cluster analysis of 204 samples from 12 varieties of maosuan pulp.

Table 4. genetic diversity of 12 cultivated germplasm resources of different Alkekengi based on SSR.

Landrace	PPL%	Na	Ne	I	h
MD	56.58%	1.132	1.268	0.255	0.164
EWX	62.18%	1.255	1.313	0.294	0.191
ZDJ	43.70%	0.888	1.226	0.208	0.135
ZHB	52.10%	1.05	1.279	0.256	0.168
TBQ	67.79%	1.346	1.375	0.323	0.211
LDH	60.78%	1.227	1.302	0.285	0.185
DLQ	45.94%	0.927	1.226	0.212	0.138
SHZ	56.58%	1.148	1.301	0.273	0.179
TDT	62.46%	1.266	1.344	0.313	0.206
JPX	45.66%	0.924	1.226	0.214	0.138
TBT	52.66%	1.064	1.266	0.249	0.162
MSJ	45.66%	0.933	1.215	0.208	0.133
Mean	54.34%	1.099	1.276	0.257	0.167

Table 5. genetic variation of different Alkekengi cultivated germplasm based on AMOVA analysis.

Source	df	SS	MS	Est.Var.	%Total	Fst	NM	P-value
Among pops	11	2804.689	254.972	13.134	29%	0.033	7.402	<0.001
Within pops	192	6117.752	31.863	31.863	71%			

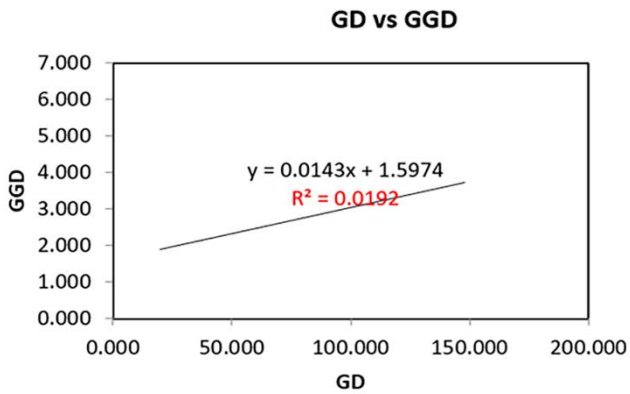

Fig. 3. Mantel test of geographical distance and genetic distance based on SSR markers.

Discussion

Expected to the scarcity of genome or associated sequence data, no study has been doing on *Physalis peruviana* landrace. Given the above, we sequenced some regions; ITS2, matK, rbcL, of the genome of this essential species and performed relative analysis with associated retrieved kind from the GenBank.

ITS2 sequence is short, usually only 200-300 bp, carrying short genetic information, which is usually used for the identification and differentiation of species within the genus. If the difference between various groups below species is to be analyzed, it needs to be combined with other DNA barcodes (Chen *et al*., 2021; Xian *et al*., 2022). RbcL sequences are conservative, with a length of about 700-750 bp and the variation level is above the interspecific level, with little interspecific and intraspecific variation (Narayani *et al*., 2024). The matK sequence is located in the chloroplast intron, which is the main DNA barcode recommended by CBOL for object classification and identification. (China Plant BOL Group, 2011) The sequence length is about 1500 bp. Compared with ribosomal DNA, it mainly encodes the shear mature enzyme K involved in the RNA transcription type II intron. Longer sequences also allow them to hold richer genetic information. (Nguyen *et al*., 2023) The single bar codes of ITS2, rbcL and MatK in this experiment were not effective in distinguishing the 12 plasmic germplasm resources, which was also consistent with the above conclusions.

China Plant BOL Group (China Plant BOL Group, 2011) inform of that the plastid area rbcL gain the highest position of comprehensiveness in both angiosperms and gymnosperms. The point was also confirmed by genetic locus analysis of the *Physalis peruviana* landrace. MatK had highest number of haplotypes and the highest diversity of haplotypes. ITS2 sequence was the highest of the nucleotide diversity.

In addition, ITS2+rbcL double gene DNA barcode has a higher discrimination precision in genetic distance than rbcL+matk double gene DNA barcode. Finally, ITS2+rbcL is recommended to be used as a DNA molecular barcode to identify germplasm resources of acid pulp culture. SSR molecular marker analysis showed that the genetic diversity of the 12 germplasm resources was high, the genetic differentiation among germplasm resources was very weak, and the gene flow was 7.402, indicating that there may be strong gene exchange among these germplasm resources to resist genetic variation caused by environmental selection (Weppler & Stöcklin *et al*., 2005). The genetic distance and geographical distance between germplasm resources were analyzed by Mantel test. The results showed that the correlation between geographical distance and genetic distance was weak ($p < 0.01$, R^2=0.0192). This indicates that the introduction of species may be frequent between regions.

Conclusion

In this study, three DNA barcodes and their mixture loci were pick to assess the genetic diversity of 12 *Physalis peruviana* L., landraces. The phylogenetic trees of ITS2+rbcL and rbcL+matK landraces of *Physalis peruviana* L., in a separate clade demonstrating that the combinations loci ITS2+rbcL and rbcL+matK have

sufficient efficiency for landraces discrimination of *Physalis peruviana*. We found that 12 landraces had low genetic diversity. The genetic similarity among the local varieties was high, and there was strong gene exchange among the local varieties. The consequence of Mantel check means that the correspondence between geographical space and genetic distance was feeble.

References

Bhat, K.V. 2006. DNA fingerprinting and cultivar identification. National Centre on DNA Fingerprinting. New Delhi, N.B.P.G.

Chen, W.Q., X.F. Wang, X.Y. Chen, C. Peng, J.F. Xu and J. Cai. 2021. Evaluation of the ability of different plant DNA barcodes to identify Dendrobium officinale. *Food Sci.*, 42(22): 131-139.

China Plant BOL Group. 2011. Comparative analysis of a large dataset indicates that internal transcribed spacer (ITS) should be incorporated into the core barcode for seed plants. *PNAS*, 108(49): 19641-19646.

Čurn, V. and J. Žaludová. 2007. Fingerprinting of oilseed rape cultivars. *Adv. Bot. Res*., 45: 155-179.

Egea, L.A., R. Mérida-García, A. Kilian, P. Hernandez and G. Dorado. 2017. Assessment of genetic diversity and structure of large garlic (*Allium sativum* L.) germplasm bank, by diversity arrays technology "genotyping-by-sequencing" platform (dart seq). *Front. Genet*., 8: 98-107.

Feng, S.G., M.Y. Jiang, Y.J. Shi, K.L. Jiao, C.J. Shen, J.J. Lu, Q.C. Ying and H.Z. Wang. 2016. Application of the ribosomal DNA ITS2 region of physalis (Solanaceae): DNA barcoding and phylogenetic study. *Front. Plant Sci.*, 7: 1047.

Govindaraj, M., M. Vetriventhan and M. Srinivasan. 2015. Importance of genetic diversity assessment in crop plants and its recent advances: an overview of its analytical perspectives. *Genet. Res. Int.*, 43148.

Jiang, L., M. Zou, S. Zhao, Y.J. Sun and B.L. Xu. 2019. Investigated the Germplasm Resources of *Physalis alkekengi* L. *Res. Pract. Chin. Med.*, 33(3): 16-19.

Korir, N.K., J. Han, L.F. Shangguan, C. Wang, E. Kayesh, Y.Y. Zhang and J.G. Fang. 2013. Plant variety and cultivar identification: advances and prospects. *Crit. Rev. Biotechnol.*, 33(2): 111-125.

Kwon, Y.S., J.M. Lee, G.B. Yi, S.I. Yi, K.M. Kim, E.H. Soh, K.M. Bae, E.K. Park, I.H. Song and B.D. Kim. 2005. Use of SSR markers to complement tests of distinctiveness, uniformity, and stability (DUS) of pepper (*Capsicum annuum* L.) varieties. *Mol. Cells*, 19: 428-435.

Li, X.X., L.J. Qiao, B.R. Chen, Y.J. Zheng, C.C. Zhi, S.Y. Zhang, Y.P. Pan and Z.H. Cheng. 2021. SSR markers development and their application in genetic diversity evaluation of garlic (*Allium sativum* L.) germplasm. *Plant Div.*, 44(5): 481-491.

Narayani, M., M. Johnson, S.T. Hajare, S.B. Bhosale, V. Upadhye, S.G. Sadashiv and B. Surwase. 2024. A study on molecular taxonomy of South Indian species Selaginella sweared by DNA barcoding using rbcL. *Ecol. Genet. Genom.*, 30: 100224.

Nguyen, P.A.T, T.T. Tran and T.K. Do. 2023. Genetic diversity in the matK gene of *Dimocarpus longan* varieties in the mekong. *Delta Asian J. Plant Sci*., 22(3): 444-451.

Tran, D.N., M. Carole, S. Uthayakumaran, I.L. Batey and C.W. Wrigley. 2009. Rapid DNA-based identification of wheat and barley varieties. *J. Cereal Sci.*, 50(3): 388-391.

Wan, R., Y.J. Wang, J.H. Zhao, T.H. Ma and Z.G. Shi. 2020. Identifying 10 *Lycium* L., plants based on *trnL-trnF* sequence as barcodes. *Ningxia J. Agri. Fores*, 61(1): 14-15.

Wang, F.G., H.L. Tian, H.M. Yi, H. Zhao, Y.X. Huo, M. Kuang, L.K. Zhang, Y.D. Lv, M.Q. Ding and J.R. Zhao. 2018. Principle and strategy of DNA fingerprint identification of plant variety. *Mol. Plant Breed.*, 16(14): 4756-4766.

Wang, Y., Q.G. Zhang, R.X. Gao, B. Zhen, Y. Wang, X.J. Gong and F. Zhang. 2020. Research status and development trend of *Physalis pubescens*. *Farm Prod.*, 8: 72-76, 81.

Weppler, T. and J. Stöcklin. 2005. Variation of sexual and clonal reproduction in the alpine *Geum reptans* in contrasting altitudes and successional stages. *Basic Appl. Ecol*., 6(4): 305-316.

Wright, S. 1978. Evolution and the Genetics of Populations, Variability within and Among Natural Population. The University of Chicago Press, Chicago, United States. p4: 270-271.

Wu, Y.N., L. Xu, L. Chen, Z.J. Cai, B. Wang, R. Zhao and T.G. Kang. 2016. DNA molecular identification of physalis medicinal plants in solanaceae by ITS2 barcode sequence. *Chin. J. Exp. Trad. Med. Formulae*, 22(8): 22-27.

Xian, Y.S., Y.C. Chen, L.L. Kang, B. Wang, R.J. Yuan, D. Wang and Z.L. Zhang. 2022. Jinping Ginseng species identification based on ITS2 bar code and HPLC technology. *Allelopath. J*., 56: 237-249.

Xie, Q.L, H.B. Zhang, F. Yan, C.X Yan, S.G. Wei, J.H. Lai, Y.P. Wang and B. Zhang. 2019. Morphology and molecular identification of twelve commercial varieties of kiwifruit. *Molecules*, 24(5): 888.

Yan, L.L., L.L. Jiang, M. Zou, S. Zhao, L. Jiang and B.L. Xu. 2020. Investigated the germplasm resources of *Physalis alkekengi* L.var. *franchetii* (Mast.) Makino. *Res. Pract. Chin. Med*., 34(1): 9-12.

Yang, B.Y., X.M. Li, Y. Liu and H.X. Kuang. 2017. Research progress on *Physalis pubescens*. *Chin. Trad. Herb. Drugs*, 14: 2979-2988.

Zhang, J., J.J. Yang, S.Z. Fu, X.F. Zhang, C.X. Xia, H. Zhao, J. Ren and K. Yang. 2022. Comparison of DUS testing and SNP fingerprinting for variety identification in cucumber. *Hort. Plant J*., 8(5): 575-582.

Zhu. Y.J., K.L. Jiao, X.J. Luo, S.G. Feng and H.Z. Wang. 2018. Genetic relationship of physalis plants revealed by simple sequence repeat markers. *Chin. Bull. Bot.*, 53(3): 305-312.

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