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Study on Biological Characteristics and Genetic Relationships of Tea Genetic Resources in Central Vietnam

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ABSTRACT

Background: Tea is an important industrial crop in the world, the drinking is good for human health. The objective of this study is to better understand the biological characteristics and genetic relationships of tea genetic resources collected in the Central Vietnam.

Methods: Ninety-seven tea accessions were studied by analyzing morphological traits and molecular markers. The morphological characters observed according to the Descriptions for tea of International Plant Genetic Resources Institute. Ten primers were used for molecular analysis. The output profile after scoring was used calculate the index reflecting the genetic diversity and relationships among tea accessions such as the number of effective alleles, polymorphic information content and genetic distance.

Result: This study showed that our materials were morphologically diverse in terms of plant type, branches density, leaves and flowers. The RAPD molecular markers (BIO-08-1300 and OPAX-07-750) were effective in assessing genetic diversity with PIC values of 1.990 and 1.953, respectively. The dendrogram tree separated into four clusters. The genetic distance indicated that the tea populations from five provinces of central region (Nghe An, Ha Tinh, Quang Binh, Quang Tri, Thua Thien Hue) were closely related. They were moderately related to the southern central coast tea population but were distantly related to the North group.

Key words: Genetic diversity, Morphology, Population, Tea.

INTRODUCTION

Many scientists agree that the existing cultivated taxa of tea are represented by three types, *C. sinensis* (L.) O. Kuntze (China type), *C. assamica* (Masters) (Assam type) and *C. assamica* sub spp lasiocalyx (Planchon ex Watt.) (Cambod or Southern type). Tea originated in South-East Asia (Wight, 1959) and is cultivated within the latitudinal ranges of 45°N to 34°S, spanning about 52 countries (Deka *et al.,* 2005; Mondal *et al.,* 2004).

Tea is the second most frequently consumed beverage in the world, with two-thirds of the world's population dringking it. Consequently, many countries cultivate tea for commercial purposes such as India, China, Srilanka, Japan, Vietnam (Heneberry, 2006; Bandyopadhyay, 2011).

In Vietnam, tea and its products play an important role in the economy and society, being consumed both domestically and for export. In 2020, the tea growing area in Vietnam reached 123,000 hectares, with a yield of about 180,000 tons, ranking 7th in the world in production and 5th in export (135,000 tons) (Vong, 2021). Drinking green tea is a common habit in many families from rural to urban areas, particularly in Central region. This traditional custom shows solidarity and friendly relationships in meetings. However, each region has a different taste of tea, which may be due to the variety, the harvesting method or the ecological zone. There are still remain unexplored tea genetic resources within this region and their genetic relationships.

Evaluation of genetic resources is crucial for long-term viability, ability to adaptation and for broadening the genetic base of cultivars (Bandyopadhyay, 2011; Makwana *et al.,* 2024). Initially, this evaluation was based on significant morphological characteristics of tea plants, such as leaf, young shoot color and flower traits (Lingaiah *et al.,* 2011; Wickramaratna, 1981; Toyao and Takeda, 1999). However, morphological traits are easily influenced by climate and environmental. Therefore, using DNA molecular markers has provide insights about the variation among genoptypes at the molecular level that become more effective method and reliable approach (Singh *et al.,* 2022; Munsaka *et al.,* 2024). The utilization of Random amplified polymorpic DNA (RAPD) as the first molecular marker to study the genetic diversity and taxonomic relationships of Kenyan tea germplasm (Wachira *et al.,* 1995).

In Vietnam, Yen *et al.* (2012) studied the genetic diversity of tea clones grown in Thai Nguyen City by RAPD and SSR markers, Bich *et al.* (2017) evaluated the golden flower tea plant (*Camellia* spp.) resources by using RADP and ISSR in Quang Ninh province. Huong *et al.* (2010) assessed the genetic diversity of some Shan tea [*Camellia*

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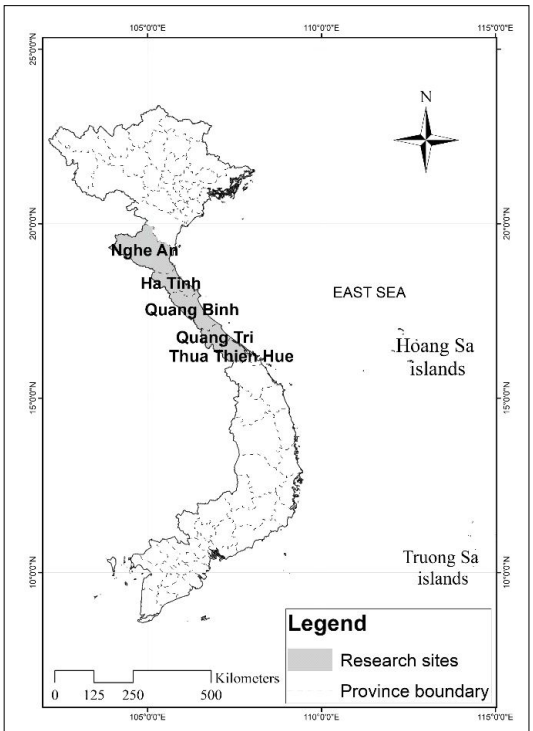
sinensis var. *assamica* (Mast.) Pierre sec. Phamh] lines using RAPD. These studies focused on tea plants in the North region.

Although the stability of RAPD marker is lower than some other markers, RAPD offer a simple, fast and cost effective technique that can analyze very high polymorphisms (Williams *et al.,* 1990). Therefore, RAPD markers provided a practical and effective method to evaluate the genetic diversity and relationships and to identify tea genetic resources (Chen *et al.,* 2005). Until now, there has been no study focusing on the characterization and analyzation of the genetic diversity of tea in the Central region of Vietnam. The preservation of tea germplasm resources and breeding of tea plants have always been vital. Therefore, the objective of this study is to better understand the biological characteristics and genetic relationships of tea genetic resources collected in the Central region, providing more data on Vietnamese tea genetic resources for the conservation and development of this plant species.

MATERIALS AND METHODS

Plant materials

A total of 97 accessions of tea (*Camellia sinensis* (L) O. Kuntze) were used in this study (Table 1). These included 22 accessions from Nghe An province, 15 from Ha Tinh province, 5 from quang binh province, 8 from quang Tri province, 37 from Thua Thien Hue province (Fig 1). Additionally, 6 accessions from southern Coastal central region and 4 from the North of Vietnam region were also collected. The samples collected from Southern Coastal



Tea accessions were collected from Central Vietnam indicated.

Fig 1: Map of Vietnam.

Table 1: List of tea accessions analyzed in this study.

Sample code	Number of sample	Province	Region
CNA1-1, CNA1-2, CNA2-1, CNA2-2, CNA3-1, CNA3-2, CNA4-1, CNA5-1, CNA5-2, CNA6-1, CNA7-1, CNA7-2, CNA7-3, CNA7-4, CNA8-1, CNA9-1, CNA10-1, CNA10-2, CNA11-1, CNA11-2, CNA11-3, CNA12-1.	22	Nghe An	
CHT1-1, CHT1-2, CHT1-3, CHT2-1, CHT2-2, CHT2-3, CHT2-4, CHT2-5, CHT3-1, CHT3-2, CHT3-3, CHT4-1, CHT4-2, CHT4-3, CHT4-4.	15	Ha Tinh	
CQB1-1, CQB1-2, CQB2-1, CQB3-1, CQB3-2.	5	Quang Binh	Central of Vietnam
CQT1-1, CQT1-2, CQT1-3, CQT1-4, CQT2-1, CQT2-2, CQT3-1, CQT3-2.	8	Quang Tri	
CH1-1, CH2-1, CH2-2, CH3-1, CH4-1, CH4-3, CH5-1, CH5-2, CH6-1, CH7-1, CH9-1, CH12-1, CH12-2, CH13-1, CH14-1, CH15-1, CH16-1, CH17-1, CH20-1, CH21-1, CTr1-1, CTr1-2, CTr1-3, CTr1-4, CTr2-1, CTr3-2, CTr3-3, CTr4-2, CTr5-1, CTr5-2, CTr8-1, CTr8-2, CTr9-1, CTr9-2, CTr10-1, CTr10-2, CTr10-3	37	Thua Thien Hue	
CPY1-1*	1	Phu Yen	
CBĐ1-1*, CBĐ1-2*	2	Binh Dinh	Southern
CQN1-2*	1	Quang Nam	Coastal
CQNg1-1*, CQNg2-1*	2	Quang Ngai	Central
CPH*, CPT*	2	Phu Tho	North of
CTN-1*, CTN-2*	2	Thai Nguyen	Vietnam

(Note: * reference accession).

Central and North of Vietnam region were used as reference accessions. Sampling was conducted from March 2023 to February 2024. The experiment was caried out in Hue University of Agriculture and Forestry, Hue University, Vietnam.

Morphological chacteristics

To observe morphological characteristics, samples collected from five provinces in Central Vietnam (Nghe An, Ha Tinh, Quang Binh, Quang Tri and Thua Thien Hue) were used. The characteristcs observed include plant type, density of branches, anthocyanin coloration at base of petiole, leaf length and width (using the 5th leaf from the tip), pedicel length and flower diameter. Each sample was an individually collected and measured for morphology according to the Descriptions for Tea by the International Plant Genetic Resources Institute (IPGRI, 1997).

DNA extraction and PCR amplification

The tea leaves of each accession were ground individually in liquid nitrogen. Total genomic DNA was extracted using the cetyl-trimethylammonium bromide (CTAB) method (Doyle and Doyle, 1990) with minor modifications. The quality and quantity of each DNA sample were determined with a spectrophotomete before used.

Molecular markers experiment

A total of ten primers were used conducted in this study, including nine RAPD primers (OPAK-15, OPAW-10, OPAD-14, OPE-18, OPAT-08, OPAW-07, OPAX-07, OPW-13) (Valdemar *et al.,* 2004), BIO 08 (Kaundun and Park, 2002) and a single-primer iPBS-2238 developed by Kalendar (2010).

For each PCR mixture was 10 µl volume, containing of 50 ng/µl DNA temple, 2 × PCR master mix (Bioline), 20 pmol primer and add milliQ water up to final volume. Amplification was performed in a thermocycler (Mastercycler, Eppendorf, Germany). The PCR cycle was as follows: An initial denaturing step at 95°C for 3 min, 30-40 cycles at 94°C for 30 sec, 39-54°C for 1 min and 72°C for 2 min. The final extension step was at 72°C for 5 min. The PCR products were electrophoresed on 1.5% agarose gel for 40-50 minutes at a constant voltage of 100V. Agarose gels were stained by 6 × Gel Red TM loading buffer and determined by UV light system. The standard markers were estimated by using the Gene ruler 1kb DNA Ladder (Thermo Scientific) (Fig 2).

Data analysis

The morphological datas were observed and measured to calculate the mean and standard deviation by Excel 2010 software. For molecular marker data, the bands were scored as 1 for present and 0 for absent in each sample. Bands with medium or high intensity were selected for data anlysis. These data were used to compile a binary matrix for cluster analysis using the Numerical taxonomy system (NTSYS-pc) software version 2.1. The Unweighted Pair Group Method with Arithmetic Average (UPGMA) was used to build a dendrogram. The output profile after scoring was used to calculate the number of effective alleles (Ne), polymorphic information content (PIC) (Botstein *et al.,* 1980) and genetic distance (GD) among groups (Nei, 1972). Genetic similarity (GS) among accessions was calculated as described by Apostol *et al.* (1993), along with Shannon's Information Index (I) (Shannon, 1948) and gene diversity (H) (Nei, 1973).

RESULTS AND DISCUSSION

Morphological chacteristics of collected tea accessions

The collected tea samples from five provinces in the Central region (Nghe An, Ha Tinh, Quang Binh, Quang Tri, Thua Thien Hue) were grouped by province to better understand the differences in their morphology (Table 2). In terms of tree habit, they were divided into three groups: Shrub, semi-arbour and arbour, with semi-arbour and arbour being dominant. Leaf morphology is important, useful and rapid method for species identification (Pi *et al.,* 2009; Lingaiah *et al.,* 2011). The accessions collected in Nghe An province had the largest leaf length and width (12.90 and 5.43 cm, respectively). Table 2 also showed that the standard deviation of leaf length in Nghe An province group was the largest (4.83 cm) compared to other provinces, indicating

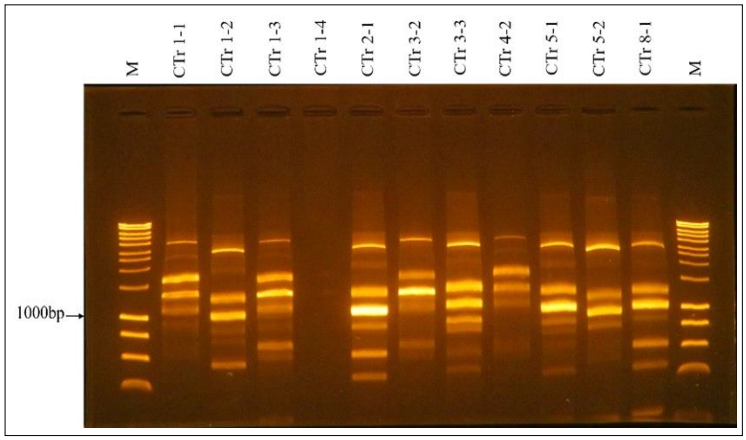


Fig 2: PCR products of BIO-08 primer of some samples; M: DNA molecular size marker 1kb ladder.

Table 2: Some morphological chacteristics of collected tea accessions in province.

Province	Tree habit	Density of branches	Anthocyanin coloration at base of petiole	Length of mature leaf (cm)	Width of mature leaf (cm)	Length/width ratio of mature leaf	Pedicel length (cm)	Flower diameter (cm)
Nghe An	Shrub; Arbour	Sparse; Medium; Dense	Absent; Present	12.90±4.83	5.43±1.57	2.37±0.42	0.96±0.34	4.38±0.47
Ha Tinh	Shrub; Semi-arbour; Arbour	Medium; Dense	Absent; Present	10.73±1.85	4.53±1.01	2.40±0.30	0.83±0.28	4.26±0.75
Quang Binh	Shrub; Semi-Arbour	Medium; Dense	Absent; Present	10.90±1.60	4.20±0.82	2.65±0.51	0.70±0.37	4.06±0.60
Quang Tri	Semi-arbour; Arbour	Medium; Dense	Absent; Present	10.65±1.99	4.38±0.78	2.54±0.35	1.22±0.19	4.58±0.42
Thua Thien Hue	Shrub; Semi-Arbour; Arbour	Sparse; Medium; Dense	Absent; Present	10.46±1.54	4.31±0.78	2.47±0.40	1.24±1.37	3.86±0.52

(Note: Mean±Sd).

that the leaf characteristics in Nghe An accesions group were more variable than others. However, length/width ratio of leaf across of all accessions did not show a large difference (2.37-2.65 cm). Compared with the research results of Diep *et al.* (2022) on leaf size of hybrid tea lines, which had leaf length of 7.15-9.5 cm and leaf width of 3.17-4.53 cm, our accessions collected in the Central region were larger. The morphological traits of tea, such as medium and thick branch density, which related to the number of leaves per plant and large leaf size are advantageous and suitable traits for harvesting conditions where fresh leaves are picked to make drinking tea water. Pedicel length and flower diameter also demonstrated the diversity of the collected accessions. The Thua Thien Hue accessions group had the longest average pedicel length (1.24 cm) but the smallest flower diameter (3.86 cm). Meanwhile, the accessions group in Quang Tri had the largest flower diameter (4.58 cm).

Although the morphological and chemical characteristics of plants may vary depending on climate and genetics, morphological characteristics remain an important and fundamental step in plant classification (Ghanbari, 2022).

Genetic diversity of collected tea accessions

Ten primers used for ninety-seven accessions produced fourteen markers. Table 3 showed that all markers exhibited polymorphism. In which, the number of polymorphic bands ranged from fourteen (2238-2000) to forty-two (BIO-08-1300) for accessions from the Central Vietnam region and ranged from fifteen (2238-2000) to forty-five (BIO-08-1300) for all accessions of this study. The mean of effective number of alleles (Ne) and polymorphic information content (PIC) for the Central region were 1.705 and 0.319 respectively, which were equivalent to 1.738 and 0.326 for all accessions. Among the markers, BIO-08-1300 had the highest Ne and PIC of 1.990 and 0.374, respectively. This finding was similar to the result reported by Kaundun and Park (2002), who evaluated the genetic structure of six Kerean tea populations comprising ninety individuals using RAPD and found that primer BIO-08 was higher polymorphic. The PIC of the OPAX-07-750 marker was also higher than others, 0.369. A study on genetic diversity among twenty-five accessions of *Camellia* spp. collected in Quang Ninh (North of Vietnam) using RAPD reported had a similar PIC (0.36) for this marker (Bich *et al.,* 2017). This indicates that these markers are useful for evaluating the diversity of tea accessions in the Central region.

The mean of Ne and PIC values of accessions from in Central Vietnam region were 1.705 and 0.319, respectively, nearly equivalent to those of all accessions, which included reference accessions. The average PIC value of fifteen tea genotypes collected from Northern Mountainous Agricultural and Forestry Science Institute (located in Phu Tho province) in the North Vietnam by Phong *et al.* (2016) using six Inter-primer Binding Sites (iPBS) marker was 0.3, equivalent to our accessions. This indicates that the diversity of tea in the North and Central regions of Vietnam was not significant different.

Genetic relationship

The dendrogram revealing the genetic relationships among ninety-seven tea accessions is shown in Fig 3. All tea accessions were grouped into two major clusters. Cluster I was composed of fifty-eight accessions, most of the accessions collected from the Central region belonged to this group (Table 4). These included Nghe An (19), Thua Thien Hue (15), Ha Tinh (12), Quang Tri (6) and Quang Binh (4) and only two accessions from the Southern central coast.

Table 3: Effective number of allele (Ne) and polymorphic information content (PIC) of collected accessions.

Primer code	Size of polymorphic fragments (bp)	Accessions from in Central vietnam region (n = 87)		All accessions (n = 97)	
		Ne	PIC	Ne	PIC
2238	2000	1.344	0.223	1.354	0.227
BIO-08	1000	1.920	0.364	1.940	0.367
BIO-08	1300	1.981	0.373	1.990	0.374
OPAK-15	750	1.454	0.263	1.540	0.289
OPAW-10	750	1.565	0.296	1.646	0.315
OPAD-14	1400	1.885	0.359	1.926	0.365
OPE-18	800	1.800	0.346	1.856	0.355
OPAT-08	1000	1.344	0.223	1.354	0.227
OPAW-07	500	1.621	0.310	1.697	0.326
OPAW-07	1100	1.776	0.342	1.815	0.348
OPAX-07	400	1.593	0.303	1.593	0.303
OPAX-07	750	1.920	0.364	1.953	0.369
OPW-13	750	1.865	0.356	1.894	0.361
OPW-13	1300	1.800	0.346	1.770	0.340
Mean		1.705	0.319	1.738	0.326

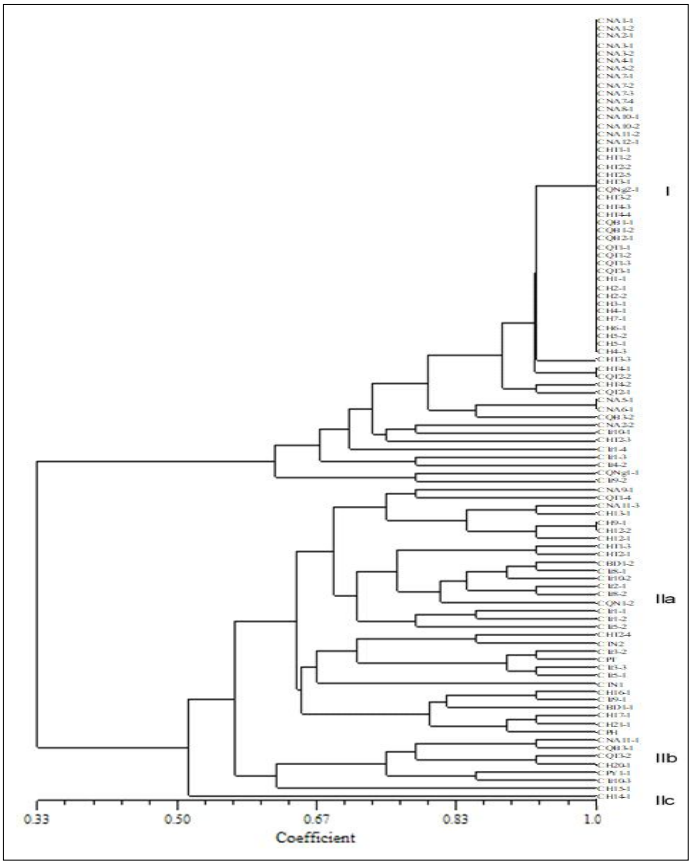


Fig 3: Genetic relationship between 97 accessions.

Cluster IIa had thirty-two accessions, most of which were accessions collected in Thua Thien Hue (18). Of the total six accessions collected in the Southern central coast, three belonged to this group. Notably, all four accessions collected from the North region also belonged to this group. Cluster IIb had seven accessions, including Nghe An (1), Quang Binh (1), Quang Tri (1) and Thua Thien Hue (3) from the Central region and one sample from to the Southern Central Coast group. Cluster IIc had only one accession collected in Thua Thien Hue. These results show that the accessions collected in the Central region had genetic relationships closely with each other and were diverse, as they were presented in all four clusters. However, the accessions collected in Thua Thien Hue had close genetic relationships with all accessions from the Central region, the Southern Central Coast and the North region. Additionally, the accessions collected in Thua Thien Hue province were all present in the four clusters, indicating that the tea accessions in Thua Thien Hue were the most diverse in comparison with the others.

From the result of genetic dissimilarity analysis using the UPGMA method, Chen *et al.* (2005), Shefali *et al.* (2013), Gul *et al.* (2007) and Sahib *et al.* (2011) reported extensive genetic diversity in the tea genotypes through RAPD analysis.

Table 4 also shows the index reflecting the genetic diversity of the populations. The number of effective alleles (Ne) in the Nghe An group was low (1.246), leading to the lowest gene diversity (H) (0.177), equivalent to the group collected in the North region (0.188). The highest gene diversity was in the population collected in Thua Thien Hue (0.447). This result aligns with data shown in Fig 3, as the accessions collected in Thua Thien Hue were present in all four groups, indicating more gene exchange in this group.

The pairwise genetic distance (GD) among the seven groups is shown in Table 5. The greatest distance (0.870) was observed between Nghe An and North tea groups and the lowest (0.009) was between Nghe An and Quang Tri. The GD among Nghe An, Ha Tinh, Quang Binh and Quang Tri did not exceed 0.146, indicating that these four groups are genetically closely related. The GD between Thua Thien Hue and other provinces in Central region (Nghe An, Ha Tinh, Quang Binh, Quang TrË) was moderate, ranging from 0.146 to 0.222.

Similarly, the GD among Southern Central Coast and Nghe An, Ha T) nh, Quang Binh and Quang Tri groups were moderate (0.313-0.397) but closely related to the cultivar group from Thua Thien Hue (0.065). The groups from Nghe An, Ha Tinh, Quang Binh and Quang Tri were distantly related to the North group (0.663-0.870), indicating distinct genetic differentiation among them. In contrast, the accession groups from Thua Thien Hue and Southern Central Coast were related to the North (0.219-0.231). The result of Kaundun and Park (2002) analyzed genetic distances from six populations (including 90 individuals) located in the southwest of Korea, which varied from 0.038 between Pulhoesa and Seryongri and 0.214 between Pulhoesa and Kaktongri populations. This reveals that the

Table 4: Number of tea accessions classified into 4 sub-clusters and their genetic diversity.

Population	No. of acc.	Cluster no.				Genetic diversity		
		I	IIa	IIb	IIc	Ne	I	H
Central region	87	56	24	6	1	1.668	0.576	0.391
Nghe An	22	19	2	1	-	1.246	0.293	0.177
Ha Tinh	15	12	3	-	-	1.507	0.512	0.331
Quang Binh	5	4	-	1	-	1.365	0.300	0.206
Quang Tri	8	6	1	1	-	1.380	0.389	0.250
Thua Thien Hue	37	15	18	3	1	1.832	0.638	0.447
Southern central coast	6	2	3	1	-	1.709	0.586	0.401
North	4	-	4	-	-	1.300	0.281	0.188
Total	97	58	31	7	1	1.477	0.428	0.286

Table 5: Pairwise genetic distance between 7 populations of tea.

Population	Nghe An	Ha Tinh	Quang Binh	Quang Tri	Thua Thien Hue	Southern Central coast	North
Nghe An							
Ha Tinh	0.022						
Quang Binh	0.023	0.039					
Quang Tri	0.009	0.017	0.038				
Thua Thien Hue	0.222	0.146	0.208	0.173			
Southern Central Coast	0.397	0.313	0.366	0.323	0.065		
North	0.870	0.663	0.724	0.795	0.219	0.231	

genetic distances among these populations were closer than those among the tea populations in our study, indicating the diversity of tea populations in central Vietnam.

CONCLUSION

The results of this study showed that our materials were morphologically diverse in terms of plant type, branches density, leaves and flowers. The RAPD molecular markers (BIO-08-1300 and OPAX-07-750) were effective in assessing genetic diversity with PIC values of 1.990 and 1.953, respectively. The dendrogram tree separated the 97 accessions into four clusters with most individuals concentrated in group I and group IIa. The genetic distance indicated that the tea populations from five provinces (Nghe An, Ha Tinh, Quang Binh, Quang Tri, Thua Thien Hue) of Central region were closely related. They were moderately related to the Southern Central Coast tea population but were distantly related to the North group.

Among the five tea populations in the central region, the tea population in Thua Thien Hue province has a close genetic relationship with tea populations in the central, southern central coast and north regions, interestingly. This is the first study on the genetic relationship of tea accessions in the central region of vietnam, contributing to a better understanding of tea genetic resources. It also aids in the conservation and development of genetic resources for this region and provides new research directions.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Conflict of interest

All authors declared that these is no conflict of interest.

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