Pertanika J. Sci. & Technol. 33 (4): 1849 - 1868 (2025)

PERTANIKA JOURNALS

SCIENCE & TECHNOLOGY

Journal homepage: http://www.pertanika.upm.edu.my/

Morphological and Genetic Relationship of Ancient Shan Tea Tree (*Camellia sinensis* var. *assamica*) from Ecogeographical Regions in Northern Vietnam

Lien Thuy Bui[1,2,3], Phong Xuan Ong[1,2], Thiep Van Nguyen[1], Khang Tan Do[4], Huy Gia Tran[4], Phi Bang Cao[5], Ha Duc Chu[6], Dung Phuong Le[7] and Hong Viet La[1,2]*

[1]*Institute for Scientific Research and Application, Hanoi Pedagogical University 2, 32 Nguyen Van Linh Street, Xuan Hoa Ward, Phuc Yen City, Vinh Phuc Province, 280000, Vietnam*
[2]*Faculty of Biology, Hanoi Pedagogical University 2, 32 Nguyen Van Linh Street, Xuan Hoa Ward, Phuc Yen City, Vinh Phuc Province, 280000, Vietnam*
[3]*Department of Secondary Education, Hoa Lu University, Ninh Binh City, Ninh Binh Province 430000, Vietnam*
[4]*Institute of Food and Biotechnology, Can Tho University, Campus II, 3/2 street, Xuan Khanh, Ninh Kieu, Can Tho City 900000, Vietnam*
[5]*Faculty of Natural Sciences, Hung Vuong University, Nong Trang Ward, Viet Tri City, Phu Tho Province 35000, Vietnam*
[6]*Faculty of Agricultural Technology, University of Engineering and Technology, Vietnam National University Hanoi, 144 Xuan Thuy Street, Cau Giay District, Ha Noi City 122300, Vietnam*
[7]*Department of Science, Technology and Environment, Vietnam Ministry of Education and Training, 35 Dai Co Viet, Le Dai Hanh Ward, Hai Ba Trung Dist, Ha Noi City 122300, Vietnam*

ABSTRACT

The genetic diversity of ancient tea plants is a crucial natural resource that helps understand plant evolution, diversification, and domestication. However, northern Vietnam's genetic diversity of natural ancient tea populations remains unclear. This study investigated the morphological, genetic, and population structure diversity of ancient Shan tea (*Camellia sinensis* var. *assamica*) genotypes across Lao Cai, Yen Bai, and Ha Giang provinces in northern Vietnam. Nineteen tree stems, leaves, and shoots morphological traits were analyzed, revealing significant leaf size, bud characteristics, and trunk circumference variability. Principal Component Analysis identified key morphological traits contributing to diversity, particularly leaf length, bud length, and shoot weight, with distinct groupings among the tea plants. Genetic profiling using ISSR markers amplified 96 bands, with 94 showing polymorphic characteristics, indicating

ARTICLE INFO

Article history:
Received: 05 December 2024
Accepted: 17 March 2025
Published: 11 June 2025

DOI: https://doi.org/10.47836/pjst.33.4.08

E-mail addresses:
btlien@hluv.edu.vn (Lien Thuy Bui)
ongxuanphong@hpu2.edu.vn (Phong Xuan Ong)
thiepvcph@gmail.com (Thiep Van Nguyen)
dtkhang@ctu.edu.vn (Khang Tan Do)
tghuy@ctu.edu.vn (Huy Gia Tran)
phibang.cao@hvu.edu.vn (Phi Bang Cao)
hachu_amser@yahoo.com (Ha Duc Chu)
lephuongdungkstn@gmail.com (Dung Phuong Le)
laviethong@hpu2.edu.vn (Hong Viet La)
* Corresponding author

a high level of genetic diversity. ANOVA revealed that 88% of the genetic variance occurs within populations, further supported by UPGMA clustering and Principal Coordinates Analysis, highlighting the genetic distinctions and similarities among the three tea populations. The study concludes that the morphological and genetic diversity of Shan tea is influenced by eco-geographical factors, underscoring the need for conservation efforts to preserve these valuable germplasm resources for future breeding and research.

Keywords: Ancient Shan tea, eco-geographical regions, ISSR marker, morphological, Northern Vietnam

INTRODUCTION

Camellia sinensis (L.) O. Kuntze, a member of the Theaceae family, is a globally important crop cultivated in over 52 countries, with China and India leading in production (Chen & Chen, 2012; FAOSTAT, 2023). The species includes several varieties, notably *C. sinensis* var. *sinensis* and *C. sinensis* var. *assamica*, both extensively used in tea cultivation (Meegahakumbura et al., 2017). Believed to have been domesticated in southwest China around 3000 BCE (Wambulwa et al., 2017), tea plants have adapted to a range of environmental conditions, flourishing in humid, tropical, and subtropical regions with acidic, well-drained soils (Ahmed & Stepp, 2025; Xia et al., 2020). In Vietnam, the ancient Shan tea, classified as *C. sinensis* var. *assamica*, is primarily found in the high mountainous areas of Son La, Ha Giang, Yen Bai, and Lao Cai provinces. Renowned for its large leaves and distinctive biochemical profile, this variety is highly valued for its tea production (Liu et al., 2017; Xia et al., 2017). Preserving ancient Shan tea is crucial, as it represents a unique genetic resource essential for maintaining biodiversity, supporting sustainable cultivation, and preventing genetic erosion due to environmental and human influences.

The preservation of plant genetic resources has garnered much attention lately. Understanding the genetic diversity within and between populations is crucial for designing effective and economical conservation approaches for plant genetic resources (Guney et al., 2021). Genetic diversity analysis in plants, mainly through DNA markers, is crucial for plant breeding and conservation (Nwosisi et al., 2019). For studying the genetic diversity of tea trees, various molecular markers, such as simple sequence repeat (SSR) (Li et al., 2024) and SSR combined start codon targeted (SCoT) markers (Samarina et al., 2021), have been used. Recently, the genetic diversity of *C. yuhsienensis* was assessed using leaf structure and inter-simple sequence repeat (ISSR) markers; most of the markers have effectively assessed this diversity and can aid in conserving and utilizing these valuable genetic resources (Zou et al., 2024).

Ancient Shan tea has revealed its potential for cross-compatibility with other tea varieties, particularly *C. sinensis* var. *sinensis* (Kumarihami et al., 2016). This cross-compatibility has been attributed to a late-acting self-incompatibility system or post-

zygotic barriers (Kumarihami et al., 2016). Furthermore, the chloroplast and mitochondrial genomes of Shan tea have been deciphered, providing valuable resources for genetic and comparative genomic studies (Zhang et al., 2019). Zhao et al. (2021) investigated the genetic diversity of ancient tea plants. They suggested that the genetic and phenotypic diversity of 145 ancient tea plant germplasm resources from five populations in Sandu County, Guizhou Province, China, was relatively high. The analysis of molecular variance showed that genetic variation within the populations was more significant than among the populations (Zhao et al., 2021).

This study investigated the morphological diversity and genetic relationships of ancient Shan tea trees across three northern provinces of Vietnam, including Lao Cai, Yen Bai, and Ha Giang. The study evaluated 19 morphological traits of leaves and shoots and used ISSR molecular markers to assess the genetic diversity within and between tea populations. Through the analysis of principal components, genetic clustering, and principal coordinates analysis, the study seeks to explore the impact of environmental factors and geographical distances on the morphological and genetic characteristics of ancient tea trees, providing insights into their conservation and breeding. Our findings will provide further insights into the genetic relationships of ancient tea sources in Northern Vietnam based on leaf morphology and ISSR markers, providing a scientific basis for the protection and utilization of this ancient tea plant.

MATERIALS AND METHODS

Sample Collection

Thirty-six individuals of ancient Shan tea were obtained from three locations that belonged to three provinces in northern Vietnam, including Cao Bo commune (Vi Xuyen district, Ha Giang province, denoted as HG.LT 1-14), Suoi Bu commune (Van Chan district, Yen Bai province, denoted as YB.SB 1-7), and Ta Thang commune (Muong Khuong district, Lao Cai province, denoted as LC.TT 1-16) (Figure 1).

Morphological Description

The assessment of nineteen morphological characteristics of the stem, leaf, and bud of the ancient Shan tea tree used the International Plant Genetic Resources Institute's guidelines (IPGRI, 1997), with a few minor modifications (Vo, 2007). The young shoots' fifth leaves were chosen for description. The tea bud with one tip and three leaves, located at position 2/3 of the internode between the third and fourth leaves, is where the buds selected for analysis are harvested. Digital calipers with a 0.01 cm sensitivity were used to measure the shoots' weight using precision scales that were sensitive to ± 0.01 g. Meanwhile, shoot length, leaf, and stem measurements were collected using the same equipment. Each morphological trait was measured in 30 replicates per plant, and 36 individuals were

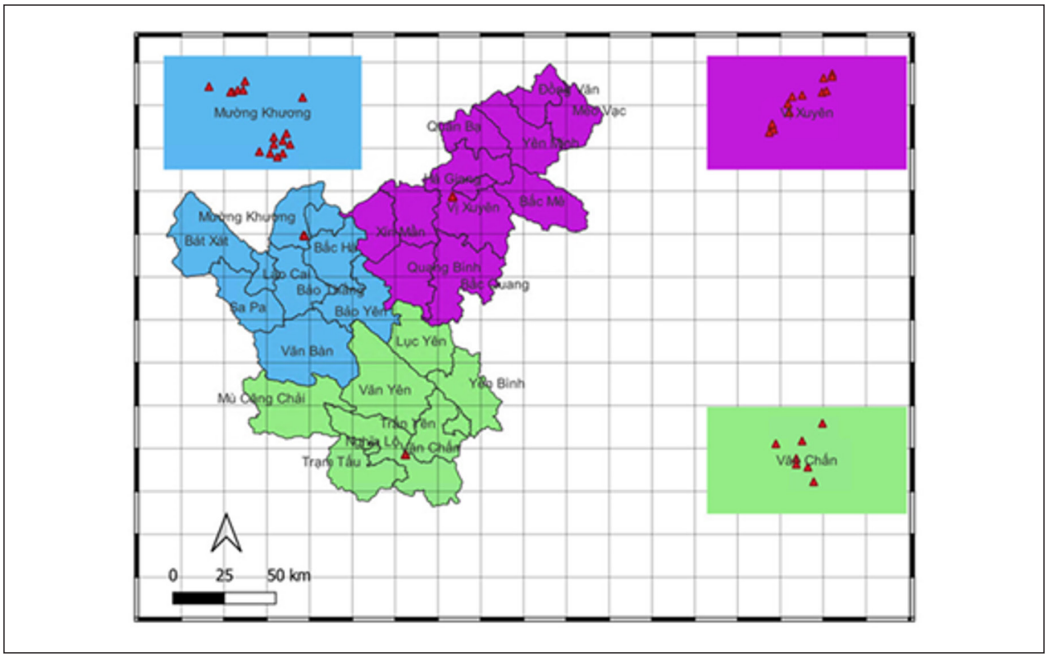

Figure 1. A map of three ecogeographical locations, Yen Bai, Lao Cai, and Ha Giang provinces, was expressed in green, purple, and blue, respectively. Tea genotypes were collected and used in morphological and molecular analyses

assessed across the three studied populations. The means of these replicates were used for statistical analysis, ensuring accuracy and minimizing measurement variability.

Genetic Diversity and Relationship of Ancient Tea Trees

DNA Isolation

Healthy leaves were sterilized with 70% (v/v) alcohol to remove dust and microbes on the surface. Liquid nitrogen was utilized to grind the leaf samples into homogenized powder. The DNA extraction was conducted following the cetrimonium bromide (CTAB) based protocol (Rogers & Bendich, 1989) with appropriate modifications. The quantity and quality of DNA were measured by the Nanodrop One spectrophotometer (Thermo Scientific, USA). DNA integrity was performed by 1% (w/v) agarose electrophoresis at 100V in Tris-acetate-EDTA 1X.

Amplification of ISSR Markers

Ten ISSR primers were used to characterize the genetic diversity (Table 1). The 10 µL reaction consisted of 2 µL deionized water, 5 µL master mix 2X (Biohelix, Taiwan), 1 µL primer (20 µM), and 2 µL DNA template (equivalent to 100 ng). Amplifications were performed by a Mastercycler X50s (Eppendorf, Germany) with the thermal cycle program:

an initial denaturation of 95°C in 4 minutes, 40 cycles included three stages; 94°C in 30 seconds, an appropriate annealing temperature (Ta) in 1 minute, 72°C in 2 minutes; a final extension of 72°C in 10 minutes. PCR products were kept stable at 4°C until use. The PCR products were tested by 2% agarose gel electrophoresis (25V, TAE 1X) in 1 hour and 30 minutes. Band patterns were visualized under UV light in the Gel Doc. XR system (Bio-Rad, USA).

Table 1
Ten primers were used for the PCR products

Primer	Nucleotide sequences (5'-3')	Annealing temperature (°C)
ISSR825	$(AC)_8T$	46
ISSR855	$(AC)_8CT$	46
ISSR866	$(CTC)_6$	52
ISSR827	$(AC)_8G$	50
ISSR811	$(GA)_8C$	45
ISSR813	$(AC)_8CA$	51
ISSR818	$(GA)_8CT$	44.7
ISSR823	$GAA(GT)_7$	51
ISSR826	$(AC)_8C$	46
ISSR889	$ATG(AC)_7$	52

Construction of a Dendrogram Tree

Scored bands were transferred into binary data with Microsoft Excel software version 2021; the presence and absence of a band were scored as 1 and 0, respectively. The dendrogram was constructed using the unweighted pair group method with the arithmetic mean (UPGMA) method using NTSYS-pc 2.1 software. Correspondence analysis was conducted using Biodiversity Pro software. Genetic diversity indices of each marker include PB (polymorphic band), PIC (polymorphism information content), EMR (effective multiplex ratio, MI (marker index, D (discriminating power), and R (resolving power) were calculated by the iMEC web-based program (Amiryousefi et al., 2018). Analysis of molecular variation (AMOVA) and principal coordinate analysis (PCoA) were utilized by the GenAlEx software version 6.51b2 (Smouse et al., 2017).

Data Analysis

All data were analyzed and presented as mean values. Differences between the mean values were tested using ANOVA and ranked using the least significant difference (LSD) method at $P < 0.05$ with SPSS 20.0 software. Principal component analysis (PCA) was analyzed using Minitab statistical software.

RESULTS

Morphological Traits of Ancient Tea Leaves and Shoots

Morphological Description

Nineteen morphological characteristics of ancient Shan tea tree stems, leaves, and shoots were recorded and evaluated in Lao Cai, Yen Bai, and Ha Giang provinces. Regarding tree shape, most trees have an arbor or semi-arbor shape (Figure 2). All trees have large trunk

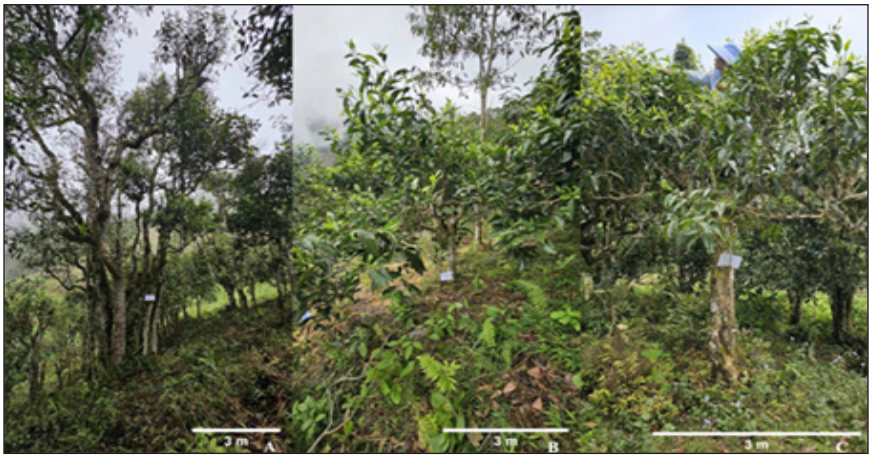

Figure 2. Ancient Shan tea tree shapes from three locations in northern Vietnam (A: LC.TT 15; B: YB.SB 5; C: LC.LT 9)

circumferences, the LC.TT15 tree has the largest trunk circumference, reaching 270 cm, and the lowest is the YB.SB 5 tree, reaching 48 cm (Table 2).

The criteria of leaf angle, leaf shape, leaf angle shape, leaf serration, and lower leaf surface are all diverse. The colors of leaves and buds vary; most are green and yellow-green. The leaf size ranges from 9.12 × 3.5 cm (LC.TT12) to 21.06 × 6.78 cm (HG.LT13). Some individuals with large leaf sizes are grouped like trees, HG to the right of Figure 3. LT2, 9, 13, and YB.SB4, 6. HG.LT13 has the highest number of pairs of main veins in the leaf (13.0) and the lowest number of pairs of main veins in the leaf of LC.TT10 (8.4). The color of the buds is mostly yellow-green; a few buds are green or light green; on all buds, there are thick snow hairs. Bud length and bud weight are very different (Table 2). Bud length ranges from 10.4 cm (HG.LT 11) to 17.78 cm (YB.SB6). Our results revealed that a number of individuals in Yen Bai (YB.SB1, 2, 4, 5, and 6) have bud length and weight much higher than the samples collected in Ha Giang and Lao Cai.

Principal Component Analysis (PCA)

PCA analysis and phylogenetic tree showed that eco-geographical regions strongly impacted the clustering of both wild tea (He et al., 2023) and cultivated tea plants (Zhao et al., 2022). In order to find which morphological parameters are essential to the growth of ancient tea trees, the statistical PCA was applied. The original set of variables is changed into a new set known as principal components (PCs), uncorrelated variables. The results of principal component analysis and correlation analysis based on the morphological data of ancient tea from three locations in northern Vietnam are expressed in Table 3. Based on the morphological data of the ancient Shan tea genotype, PCA, correlation analysis, and contribution ratio, it is revealed that the seven components explained 100% of the overall variation. The first main

Table 2
Morphological characteristics analyzed in tea genotypes

Genotype	Tree type**	The stem circumference (cm)	The height of the first branching position (cm)	Leaf pose**	Leaf shape**	leaf apex shape**	Leaf base shape**	Leaf serrula form**	Leaf upper surface**	Color of the leaf**	Leaf length (cm)	Leaf breadth (cm)	Leaf length/ breadth ratio	Number of pairs of main veins on the leaf surface	Length of the leaf petiole (cm)	Color of the shoot**	Pubescence density on bud**	Length of the shoot (cm)	Mean of fresh shoot weight (g/shoot)
LC.TT1	2	72	28	2	4	2	1	1	1	2	15.54^{cde*}	5.92^{bc}	2.63	9.8^{f-j}	0.64^{f-k}	2	3	14.76^{b-f}	1.92
LC.TT2	1	88	150	3	4	2	1	1	1	3	11.08^{nop}	4.58^{h-k}	2.42	9.6^{f-k}	0.54^{k-n}	5	3	12.14^{i-m}	1.53
LC.TT3	2	70	33	2	4	2	1	1	1	2	13.98^{fgh}	6.28^{b}	2.23	8.8^{h-k}	0.64^{f-k}	2	3	12.02^{j-o}	1.52
LC.TT4	2	70	22	4	4	2	1	2	1	2	12.16^{k-n}	4.52^{ijk}	2.69	8.8^{h-k}	0.52^{lmn}	2	3	14.62^{c-g}	1.80
LC.TT5	1	115	43	3	1	2	2	4	1	2	13.62^{f-j}	5.10^{fg}	2.67	9.8^{f-j}	0.76^{b-e}	5	3	17.62^{a}	2.38
LC.TT6	1	150	150	2	4	1	1	1	1	2	14.28^{fg}	5.86^{bc}	2.44	9.8^{f-j}	0.76^{b-e}	2	3	14.32^{c-g}	1.70
LC.TT7	1	200	50	2	1	2	2	1	1	3	15.54^{cde}	6.86^{a}	2.27	10.4^{d-g}	0.78^{bcd}	2	3	13.60^{e-j}	1.45
LC.TT8	1	186	46	2	1	2	1	1	1	2	12.86^{h-l}	3.68^{mn}	3.49	8.6^{ijk}	0.54^{k-n}	2	3	13.86^{d-h}	1.71
LC.TT9	1	112	160	2	4	2	1	2	1	3	14.86^{def}	6.74^{a}	2.20	9.8^{f-j}	0.66^{e-j}	5	3	13.24^{f-k}	1.60
LC.TT10	1	74	112	2	4	2	1	2	1	5	10.76^{op}	4.24^{jkl}	2.54	8.4^{jk}	0.72^{c-g}	5	3	14.4^{c-g}	1.31
LC.TT11	3	117	0	2	4	2	1	2	1	2	12.78^{h-m}	5.20^{d-g}	2.46	9.8^{f-j}	0.68^{d-i}	5	3	15.02^{b-e}	2.01
LC.TT12	3	85	0	3	4	1	1	1	1	2	9.12^{q}	3.50^{n}	2.61	9.8^{f-j}	0.50^{mn}	5	3	15.52^{bcd}	2.10
LC.TT13	1	202	20	2	4	2	1	1	1	2	13.08^{g-k}	5.62^{cde}	2.33	9.2^{g-k}	0.66^{e-j}	5	3	12.56^{h-l}	1.48
LC.TT14	1	160	50	2	2	2	2	1	1	3	13.06^{g-k}	5.74^{c}	2.28	10.4^{d-g}	0.54^{k-n}	5	3	13.04^{g-k}	1.50
LC.TT15	1	270	0	2	4	1	1	1	1	3	12.98^{h-k}	5.54^{c-f}	2.34	9.6^{f-k}	0.56^{j-n}	5	3	12.08^{i-n}	1.29
LC.TT16	1	164	17	2	1	1	2	3	1	2	14.82^{def}	5.98^{bc}	2.48	9.8^{f-j}	0.72^{c-g}	5	3	13.74^{e-i}	1.65
YB.SB1	2	136	19	2	4	1	1	2	1	2	16.06^{cd}	5.68^{cd}	2.83	10.4^{d-g}	0.76^{b-e}	5	3	16.34^{ab}	2.02
YB.SB2	2	114	28	2	4	1	1	3	1	2	14.80^{def}	5.06^{fgh}	2.92	9.6^{f-k}	0.86^{b}	5	3	15.72^{bc}	2.20
YB.SB3	3	94	18	2	4	2	1	1	1	1	11.76^{l-o}	3.96^{lmn}	2.97	8.2^{k}	0.52^{lmn}	5	3	10.72^{mno}	1.45
YB.SB4	2	64	88	2	4	1	1	1	1	3	16.20^{c}	5.14^{efg}	3.15	10.2^{d-h}	0.7^{c-h}	5	3	17.24^{a}	2.91
YB.SB5	2	48	106	2	1	2	2	3	1	2	14.02^{fgh}	5.66^{cd}	2.48	11.0^{b-f}	0.6^{h-m}	1	3	17.54^{a}	2.82

Table 2 *(continue)*

Genotype	Tree type**	The stem circumference (cm)	The height of the first branching position (cm)	Leaf pose**	Leaf shape**	leaf apex shape**	Leaf base shape**	Leaf serrula form**	Leaf upper surface**	Color of the leaf**	Leaf length (cm)	Leaf breadth (cm)	Leaf length/ breadth ratio	Number of pairs of main veins on the leaf surface	Length of the leaf petiole (cm)	Color of the shoot**	Pubescence density on bud**	Length of the shoot (cm)	Mean of fresh shoot weight (g/shoot)
YB.SB6	1	111	40	2	4	2	1	2	1	3	16.68^{c}	6.04^{bc}	2.76	10.6^{c-g}	0.96^{a}	1	3	17.78^{a}	2.12
YB.SB7	2	103	36	2	4	2	1	1	1	2	12.46^{j-m}	3.96^{lmn}	3.15	9.8^{f-j}	0.62^{g-l}	5	3	14.76^{b-f}	2.05
HG.LT1	2	157	40	2	4	2	1	1	1	2	13.74^{f-i}	4.50^{ijk}	3.05	10.2^{d-h}	0.58^{i-m}	5	3	11.20^{l-o}	1.27
HG.LT2	2	96	73	2	4	2	1	1	1	3	16.04^{cd}	5.88^{bc}	2.73	11.6^{bcd}	0.70^{c-h}	5	3	15.68^{bc}	1.72
HG.LT3	2	103	42	2	4	2	1	3	1	2	15.62^{cde}	4.80^{ghi}	3.25	11.8^{abc}	0.78^{bcd}	5	3	12.36^{h-m}	1.28
HG.LT4	2	112	46	2	4	2	1	3	1	2	14.90^{def}	5.06^{fgh}	2.94	10.2^{d-h}	0.74^{c-f}	5	3	13.16^{f-k}	1.70
HG.LT6	2	110	27	2	4	2	1	1	1	2	11.54^{mno}	4.26^{jkl}	2.71	9.4^{g-k}	0.54^{k-n}	5	3	11.10^{l-o}	1.21
HG.LT7	2	91	47	2	4	2	1	1	1	2	14.62^{ef}	5.10^{fg}	2.87	12.0^{ab}	0.64^{f-k}	5	3	10.46^{no}	1.27
HG.LT8	2	116	31	2	4	2	1	1	1	2	12.28^{k-n}	4.10^{klm}	3.00	10.2^{d-h}	0.50^{mn}	5	3	11.58^{k-o}	1.10
HG.LT9	2	79	126	2	4	1	1	1	1	2	18.48^{b}	5.88^{bc}	3.14	12.2^{ab}	0.80^{bc}	5	3	14.72^{b-f}	1.75
HG.LT10	2	133	22	2	1	1	2	1	1	2	12.30^{k-n}	4.12^{klm}	2.99	8.8^{h-k}	0.46^{n}	5	3	15.16^{b-e}	1.16
HG.LT11	2	108	63	2	4	2	1	1	1	5	12.02^{k-n}	3.78^{lmn}	3.18	10.0^{e-i}	0.56^{j-n}	5	3	10.40^{o}	0.79
HG.LT12	2	162	52	2	4	2	1	1	1	1	12.58^{i-m}	4.74^{g-j}	2.65	11.4^{b-e}	0.58^{i-m}	5	3	13.16^{f-k}	1.07
HG.LT13	2	170	31	2	4	2	1	1	1	2	21.06^{a}	6.78^{a}	3.11	13.0^{a}	0.72^{c-g}	5	3	15.20^{b-e}	1.53
HG.LT14	2	142	26	2	1	2	2	1	1	3	10.28^{p}	3.92^{lmn}	2.62	10.2^{d-h}	0.54^{k-n}	5	3	11.86^{k-o}	0.97
CV %											6.29	7.16		9.57	11.92			8.01	
LSD 0.05											1.09	0.46		1.21	0.97			1.41	

Note. * Different lowercase letters show statistically significant differences between genotypes in column ($p< 0,05$); ** The quality traits were coded as follows: Tree habit/type: Arbour (1), semi-arbour (2), shrub (3); eaf shape: Ovate (1), oblong (2), elliptic (3), lanceolate (4), others (99); Leaf apex shape: Acute (1), blunt (obtuse) (2), attenuate (3), others (99); Leaf base shape: Attenuate (acute) (1), rounded (2), blunt (obtuse) (3); Leaf upper surface: Smooth (1), rugose (2), others (99). Leaf pose (angle): 1–Erect (acute) (<35°); 2–Semi-erect (obtuse) (35°–75°); 3–Horizontal (right) (76°–90°); 4–Drooping (>90°) (90°); The serrula form: Regularly acute (1), regularly blunt (2), irregularly acute (3), and irregularly blunt (4); The leaf and the shoot color: Light green (1), green (2), grayed-green (3), grayed-yellow (4), yellow-green (5), and others (6); Pubescence density on the bud: Glabrous to rare (1), light pubescent (2), dense pubescent layer (3)

Figure 3. Morphological characters of the typical leaves of the ancient tea collection from various northern provinces of Vietnam

Table 3
Principal component analysis, correlation analysis, and contribution ratio-based morphological data of ancient Shan tea genotypes

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Shoot weight	0.39	-0.14	-0.58	-0.13	-0.17	-0.67	0.02
Shoot length	0.43	-0.16	-0.51	0.19	-0.05	0.70	-0.05
Leaf length	0.54	0.18	0.24	-0.04	0.46	-0.03	0.63
Leaf breadth	0.47	-0.28	0.38	-0.30	0.22	-0.02	-0.65
Leaf length/breadth ratio	0.01	0.64	-0.24	0.37	0.45	-0.13	-0.42
Main vein	0.39	0.28	0.37	0.45	-0.65	-0.08	-0.05
Leaf petiole length	0.06	0.59	-0.09	-0.72	-0.28	0.20	-0.03
Eigenvalue	2.63	1.85	1.44	0.58	0.35	0.15	0.01
Proportion (%)	37.57	26.37	20.61	8.30	4.96	2.13	0.07
Cumulative (%)	37.57	63.94	84.54	92.84	97.80	99.93	100

component presented 37.57% of the variation. The values of leaf length (0.54), leaf breadth (0.47), bud length (0.43), bud weight (0.39), and number of primary leaf veins (0.39) are the most significant contributors to the first essential component. All five factors positively correlate with the first principal component (PC1), meaning an increase in any of these factors would raise PC1's value. The second principal component explained 26.37% of the variation. The traits of the ratio of the leaf's length to breadth (0.64) and petiole length (0.59) make up the most significant contribution to the second fundamental component. The shoot weight (-0.58), shoot length (-0.51), and the weight of the leaf breadth (0.38) of principal component 3 (PC3) have high weights, suggesting that PC3 captures the variations

in shoot weight and length. The shoot weight and length values are negative, indicating a negative correlation of the third principal component (PC3) with the shoot properties. Thus, the first three components explained 84.54% of the overall variation (Table 3).

Plotting the PCA scores makes it feasible to visually evaluate sample similarity and establish whether samples may be categorized. In this study, the most significant variables of the main components are those with high weights, such as the leaf length, leaf width, and leaf length/breadth ratio in PC1 and PC2. In a main element, variables with weights of the same sign are positively correlated, whereas variables with weights of the opposite sign are negatively correlated. The primary components that may be used to examine the differences between tea plants are leaf size, bud length and weight (PC1), and leaf length/width ratio (PC2).

HG.LT trees tend to be distributed in the chart's upper left and right parts. This suggests wide dispersion within this group and possibly clearly distinct characteristics between HG.LT trees. HG.LT13 is located furthest toward the top of PC2, showing its unique characteristics that are different from other plants in the HG.LT group. The LC.TT trees are concentrated mainly in the lower left part of the chart; this group has relatively homogeneous characteristics and is different from other groups. YB.SB trees are distributed mainly in the center and right parts of the chart; this group has similarities in characteristics. In particular, YB.SB3 and YB.SB7, located on the left side of PC1, may have more distinct attributes in the YB.SB group. Through the PCA chart, we found the dispersion and grouping of tea plants into three different groups (HG.LT, LC.TT, and YB.SB). LC.TT plants tend to be more concentrated and homogeneous, while HG.LT and YB.SB plants have a wider dispersion and greater diversity of characteristics (Figure 4).

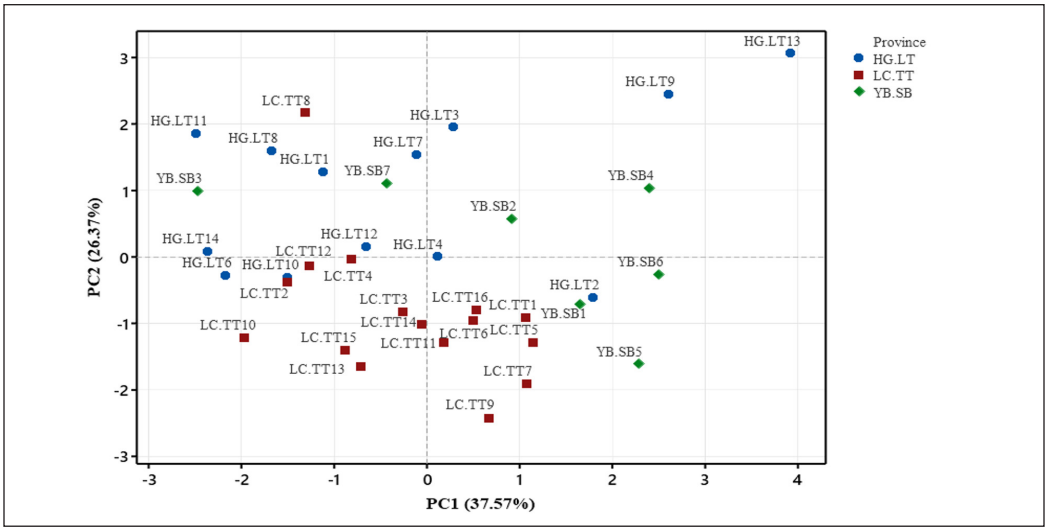

Figure 4. Score plot of morphological data of ancient tea genotypes from the eco-geographical regions of northern Vietnam

Genetic Diversity and Relationship of Ancient Tea Trees

DNA Profile of ISSR Markers

From the electrophoretic spectrum, the data showed that 96 bands were amplified from 36 ancient tea genotypes belonging to three populations, including Yen Bai, Lao Cai, and Ha Giang. In particular, 94 polymorphic bands were recorded, corresponding to a polymorphic rate of up to 97.9%, so for every 1 ISSR primer, there will be an average of 10.44 polymorphic bands. The size of amplified products ranges from 200 to 1800 bp. The UBC816 has the highest band numbers, with 14 bands, and primer UBC888 has the fewest band numbers, with five bands. Two primers, UBC856 and UBC843, amplify the band with the largest size of 1800 bp. In terms of polymorphism, there are seven primers (UBC855, UBC888, UBC825, UBC866, UBC811, UBC816, and UBC843) with a polymorphism rate of 100%. All nine primers are reasonably informative because the PIC index is in the range of 0.25–0.5. There are six primer pairs, including UBC855, UBC840, UBC888, UBC825, UBC866, and UBC816, with the highest PIC index of 0.37 (Table 4).

Table 4
Genetic diversity indices of 9 ISSR markers

Primer	SB	PB	PB%	Size	H	PIC	Rp	Hav.	MI	D	R
UBC855	10	10	100	250–1500	0.50	0.37	5.03	0.00	0.01	0.75	4.72
UBC840	13	12	92	200–1300	0.49	0.37	5.50	0.00	0.01	0.82	5.78
UBC888	5	5	100	250–1500	0.48	0.37	2.06	0.00	0.01	0.83	1.33
UBC825	10	10	100	250–1700	0.50	0.37	4.78	0.00	0.01	0.77	5.11
UBC866	11	11	100	250–1200	0.49	0.37	4.94	0.00	0.01	0.80	4.22
UBC811	9	9	100	200–1000	0.48	0.36	3.50	0.00	0.01	0.85	5.00
UBC856	14	13	93	200–1800	0.43	0.34	4.47	0.00	0.00	0.90	4.94
UBC816	9	9	100	200–1500	0.50	0.37	4.06	0.00	0.01	0.80	3.33
UBC843	15	15	100	200–1800	0.46	0.36	5.47	0.00	0.00	0.87	8.94
Total	96	94				0.37					

Note. SB: Scored band, PB: Polymorphic band, H: Heterozygous, PIC: Polymorphism Information Content, Rp: Resolution power, MI: Multiple index

Genetic Polymorphism

AMOVA analysis showed that the variance between populations was 12%, while within each population, it was up to 88% (Table 5). Our findings are consistent with those of Huang et al. (2022), who demonstrated that genetic variance within populations outweighed genetic differences across tea populations through genotyping analysis using sequencing tea genetic resources (Huang et al., 2022).

Table 5
Results of analysis of molecular variance (AMOVA)

Source	df	SS	MS	Est. Var.	%
Among Pops	2	74.081	37.040	1.990	12%
Within Pops	33	472.503	14.318	14.318	88%
Total	35	546.583		16.309	100%

Dendrogram

The UPGMA clustering dendrogram classified the three tea populations into 2 clusters with genetic similarity ranging from 0.61 to 0.86 (Figure 5). At the threshold of 0.65, cluster I is divided into two sub-clusters: I-A and I-B. The genetic diversity among the three populations is clearly illustrated in subcluster I-A. In particular, at the genetic similarity of 0.79, the data reveal that 11/13 genotypes of the Ha Giang population are in sub-cluster I-A1a. Meanwhile, all genotypes of the tea population in Lao Cai were distributed into sub-clusters I-A1b, I-A2, and I-A3. The Yen Bai population showed the highest genetic diversity due to distribution in all three different sub-clusters, consisting of I-A1a (YBSB1, YBSB3), I-A1b (YBSB5, YBSB6), and I-B (YBSB4, YBSB7). Besides, three genotypes, including HGLT2, YBSB2, and HGLT4, were grouped into cluster II.

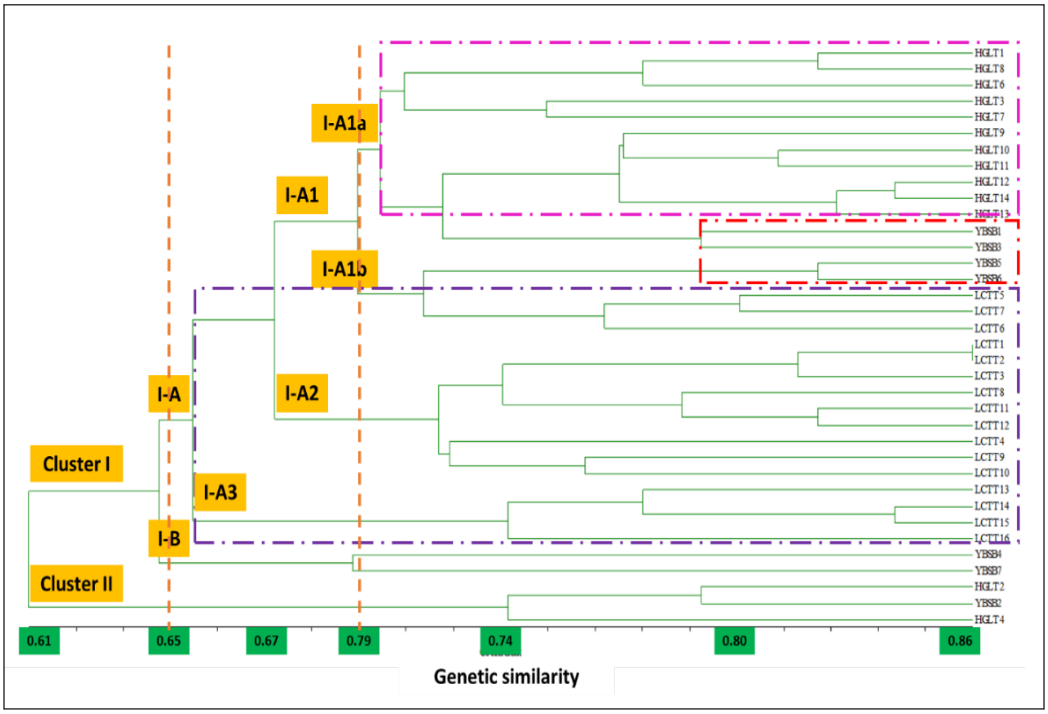

Figure 5. Dendrogram for cluster analysis of 36 *Camellia sinensis* genotypes

Principal Coordinates Analysis

The PCoA diagram visualizes the clustering between the three tea populations of Ha Giang, Yen Bai, and Lao Cai provinces. This information illustrates that the tea genotypes of the Lao Cai population showed more distinctive genetic characteristics than those of the other two populations. In addition, there is a certain overlap in the genetic profile between the two populations of Ha Giang and Yen Bai. Concerning geographical data, the distance between Ha Giang and Yen Bai provinces is closer than that of Lao Cai (Figure 6).

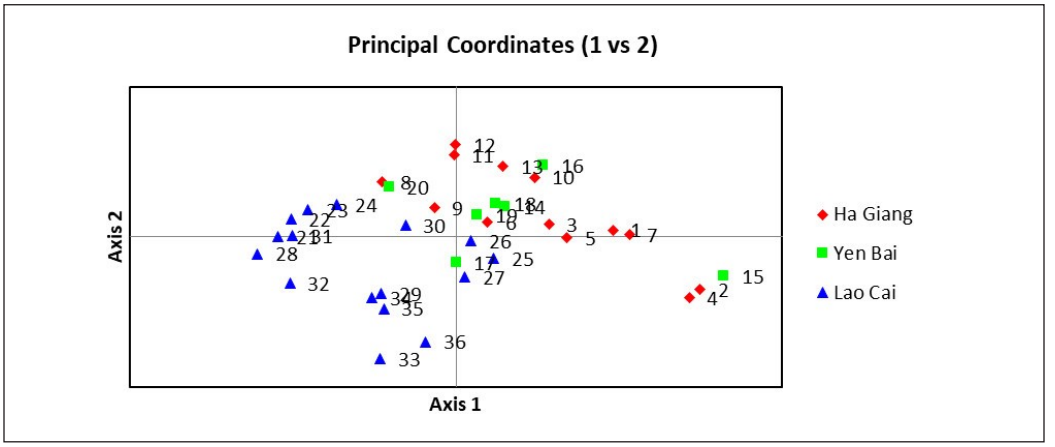

Figure 6. PCoA diagram from three populations of *C. sinensis* based on ISSR marker

DISCUSSION

Leaf morphological traits often adjust in response to different environments to adapt. According to Ran et al. (2023), the growth and development of tea plants and the quality of the tea are also strongly correlated with environmental elements such as temperature, humidity, light, rainfall, and altitude. Amino acid concentration is influenced by altitude; more significant elevations are linked to higher levels (Ran et al., 2023). The morphological diversity observed in the ancient Shan tea trees across Lao Cai, Yen Bai, and Ha Giang provinces suggests significant phenotypic variation, likely influenced by genetic factors and environmental conditions. The recorded variations in tree shape, trunk circumference, leaf size, and bud characteristics highlight the adaptability of *C. sinensis* var. *assamica* to different eco-geographical regions. Notably, larger trunk circumferences and leaf sizes were observed in certain genotypes, such as LC.TT15 and HG.LT13 indicate potential differences in growth rate, age, and environmental adaptation.

Additionally, the observed bud length and weight variations, particularly in the Yen Bai population, suggest regional differences in shoot development, which may impact tea quality and yields. Altitude and the geological environment significantly influenced the distribution, population structure, and evolutionary direction of wild plant germplasm

resources (Bemmels et al., 2016). These findings align with previous studies demonstrating how altitude, climate, and soil conditions contribute to morphological differentiation in tea plants. For example, Clarke et al. (2023) found that regional environmental factors significantly shape the phenotypic characteristics of *C. sinensis*, particularly in relation to leaf structure, shoot development, and adaptability. It highlighted that tea plants grown in warm, humid regions tend to develop broader leaves and greater shoot biomass, a phenomenon similarly observed in the Shan tea populations studied across northern Vietnam. Additionally, soil composition was identified as a key factor influencing leaf morphology, with nutrient-rich, well-drained, acidic soils supporting larger leaves and higher biomass production (Clarke et al., 2023).

These findings correspond with this study's results, where significant differences in leaf size and bud weight were recorded among populations from different provinces, as confirmed by PCA results. Furthermore, altitude was shown to impact morphological adaptation, as higher-elevation tea plants exhibited smaller, more compact leaves, likely in response to cooler temperatures and reduced oxygen availability. This pattern is evident in the distinct leaf and bud characteristics among the studied populations, suggesting that altitude-driven selection pressures contribute to morphological divergence. The UPGMA clustering and PCoA results further reinforce this relationship, as genetic clustering followed geographical patterns, indicating strong eco-geographical influences on genetic variation. These findings emphasize the importance of eco-geographical factors in shaping the morphological and genetic diversity of ancient Shan tea, underscoring their relevance for conservation and breeding strategies.

PCA analysis provided further insights into the key morphological traits contributing to variation among the ancient tea genotypes. The strong contributions of leaf length, bud length, and shoot weight to the first PC1 suggested that these traits play a critical role in distinguishing different tea populations. The clustering of tea plants into distinct groups based on PCA scores confirms that morphological differences correspond closely with geographical origin. The homogeneous characteristics observed in the Lao Cai population (LC.TT) contrast with the greater diversity and dispersion seen in Ha Giang (HG.LT) and Yen Bai (YB.SB), supporting the hypothesis that local environmental conditions and genetic factors drive phenotypic differentiation. Previously, a comprehensive analysis of wild *Camellia* species in the Guizhou Plateau, China, was carried out and provided strong evidence supporting the role of eco-geographical influences in shaping morphological traits (He et al., 2023). Their study revealed significant genetic diversity among *C. tachangensis* and *C. gymnogyna*, which were distributed across different altitude gradients and geological environments.

Specifically, the genetic diversity of *C. gymnogyna* was significantly higher in low-altitude silicate rock areas, whereas *C. tachangensis* exhibited reduced genetic variability

in high-altitude carbonate rock environments, suggesting that altitude and soil composition influence the evolutionary trajectory of tea plant populations. The genetic differentiation coefficient between these two species was 0.075 (He et al., 2023). Additionally, when analyzing populations at different altitudes, the study found that the second altitude gradient (1,100–1,400 m) exhibited the highest genetic diversity. In comparison, the first (>1,400 m) and third (<1,100 m) gradients showed reduced variation (He et al., 2023). These patterns align with the results in this study, where altitude was identified as a key determinant of morphological differentiation in ancient Shan tea populations. The lower genetic diversity at high altitudes may be attributed to restricted gene flow, environmental selection pressures, and adaptation to cooler temperatures. It is consistent with findings in other plant species that exhibit similar genetic constraints in extreme environments.

Molecular marker is a reliable approach to population genetics in natural plants. Regarding data tendency, our study is similar to that of previous publications. Next, the genetic diversity analysis using ISSR markers revealed a high degree of polymorphism (97.9%), indicating substantial genetic variation within the studied tea populations. The high number of polymorphic bands suggests that ancient Shan tea in northern Vietnam possesses a rich genetic pool, which is valuable for conservation and breeding programs. Multiple genetic clusters in the UPGMA dendrogram further support the notion of significant genetic differentiation among populations. Interestingly, the Lao Cai population exhibited more distinct genetic traits, while some overlap was observed between the Ha Giang and Yen Bai populations. In particular, 24 *Camellia* genotypes were collected from the Black Sea region of Turkey and characterized the genetic diversity by 15 ISSR markers (Yoğurtçu & Aygun, 2021). This pattern suggests that geographical proximity may contribute to gene flow and shared genetic characteristics, whereas more isolated populations may have undergone genetic divergence over time.

In order to categorize the variables inside many clusters according to their relationship and normalized value, cluster analysis with a dendrogram is typically utilized. Our AMOVA analysis revealed that most genetic variation (88%) occurs within rather than between populations (12%). The findings showed 96 total bands and 84 polymorphic bands. Likewise, 42 elite UPASI tea clones from India were analyzed by 27 ISSR markers, and the result determined that the total and polymorphic bands were 116 and 70, respectively (Sharma et al., 2022). Furthermore, based on the AMOVA output, there is a high level of intra-genetic variation (90.48%) compared to the low level between populations (9.52%). Liu et al. (2022) also concluded that the genetic variation originated mainly from intrapopulation variation when using single-nucleotide polymorphisms to study the population structure of 137 *C. sinensis* in China. Therefore, the genetic variation within the population of *C. sinensis* is diverse and should be investigated for germplasm conservation of ancient tea (Liu et al., 2022).

This finding aligns with previous studies on tea plant diversity, where intra-population genetic variation has been reported as the predominant source of diversity. The relatively low inter-population variation suggests that tea populations in northern Vietnam maintain a degree of genetic connectivity despite geographical separation, potentially due to natural seed dispersal, human-mediated propagation, or historical gene flow. Meanwhile, the PCoA further corroborates these findings, illustrating a distinct genetic profile for the Lao Cai population while indicating some genetic overlap between the Ha Giang and Yen Bai populations. This result supports the hypothesis that eco-geographical factors, such as altitude, temperature, and soil composition, may significantly shape genetic diversity. Additionally, the genetic clustering observed in the dendrogram and PCoA plot suggests that ancient tea populations in northern Vietnam have retained unique genetic signatures despite sharing a common ancestry. Genetic diversity correlates with geographical distance (Zhang et al., 2022). In tea, He et al. (2023) state that the examined Camellia's genetic differentiation was greatly influenced by the geological environment, soil mineral concentration, soil pH, and altitude (He et al., 2023). Moreover, using AMOVA analysis, ancient Chinese Assam tea (Meegahakumbura et al., 2016) populations were grouped according to the three geographical regions; the genetic variation was partitioned among regions, populations, and within populations (Li et al., 2024).

Overall, these findings emphasize the importance of preserving the genetic resources of ancient Shan tea in northern Vietnam. The observed morphological and genetic diversity highlights the potential for breeding programs to develop new cultivars with desirable traits such as enhanced yield, adaptability to environmental stress, and improved tea quality. Furthermore, conservation efforts should prioritize maintaining this diversity, particularly in populations with unique genetic characteristics. Future research incorporating more comprehensive molecular markers and environmental data could provide deeper insights into these ancient tea populations' evolutionary history and adaptive mechanisms.

CONCLUSION

This study comprehensively analyzes the morphological and genetic diversity of ancient Shan tea across the Lao Cai, Yen Bai, and Ha Giang provinces, demonstrating significant eco-geographical influences on trait variation. The morphological analysis identified slight differences in leaf length, bud length, and shoot weight, with several individuals in Ha Giang genotypes exhibiting larger leaves and thicker buds. At the same time, some Yen Bai samples showed greater shoot biomass. PCA results confirmed that these traits contribute significantly to morphological diversity, with clear population clustering. Genetic profiling using ISSR markers revealed a high polymorphism rate (97.9%), with 94 out of 96 amplified bands being polymorphic. UPGMA clustering grouped Lao Cai genotypes separately from Ha Giang and Yen Bai, while PCoA indicated genetic overlap between Ha

Giang and Yen Bai, suggesting historical gene flow. AMOVA showed that 88% of genetic variation exists within populations, reinforcing strong intra-population diversity. The distinct genetic structure of Lao Cai tea suggests localized adaptation, while shared genetic traits between Ha Giang and Yen Bai indicate potential migration or hybridization. These findings highlight the necessity of in situ and *ex-situ* conservation strategies to safeguard Shan tea genetic resources, ensuring their long-term sustainability and potential for future breeding programs.

ACKNOWLEDGMENT

This research is funded by Hanoi Pedagogical University 2 under grant number HPU2.2023-UT-16 to Hong Viet La.

REFERENCES

Ahmed, S., & Stepp, J. R. (2025). Chapter 3 - Green tea: Plants, artisanal knowledge, processing, manufacturing and production. In V. R. Preedy & V. B. Patel (Eds.), *Tea in Health and Disease Prevention* (2nd ed.) (pp. 21-30). Academic Press. https://doi.org/10.1016/B978-0-443-14158-4.00003-8

Amiryousefi, A., Hyvonen, J., & Poczai, P. (2018). iMEC: Online marker efficiency calculator. *Applications in Plant Sciences, 6*(6), Article e01159. https://doi.org/10.1002/aps3.1159

Bemmels, J. B., Title, P. O., Ortego, J., & Knowles, L. L. (2016). Tests of species-specific models reveal the importance of drought in postglacial range shifts of a Mediterranean-climate tree: Insights from integrative distributional, demographic and coalescent modelling and ABC model selection. *Molecular Ecology, 25*(19), 4889-4906. https://doi.org/10.1111/mec.13804

Chen, Z. M., & Chen, L. (2012). Delicious and healthy tea: An overview. In L. Chen, Z. Apostolides, & Z. M. Chen (Eds.), *Global Tea Breeding: Achievements, Challenges and Perspectives* (pp. 1-11). Springer. https://doi.org/10.1007/978-3-642-31878-8_1

Clarke, C., Richter, B. S., & Rathinasabapathi, B. (2023). Genetic and morphological characterization of United States tea (*Camellia sinensis*): Insights into crop history, breeding strategies, and regional adaptability. *Frontiers in Plant Science*, *14*, Article 1149682. https://doi.org/10.3389/fpls.2023.1149682

FAOSTAT. (2023). *FAO Database*. Food and Agriculture Organization of the United Nations. https://www.fao.org/faostat/en/#data/QCL

Guney, M., Kafkas, S., Keles, H., Zarifikhosroshahi, M., Gundesli, M. A., Ercisli, S., Necas, T., & Bujdoso, G. (2021). Genetic diversity among some walnut (*Juglans regia* L.) genotypes by SSR markers. *Sustainability*, *13*(12), Article 6830. https://doi.org/10.3390/su13126830

He, L., Luo, J., Niu, S., Bai, D., & Chen, Y. (2023). Population structure analysis to explore genetic diversity and geographical distribution characteristics of wild tea plant in Guizhou Plateau. *BMC Plant Biol*, *23*(1), Article 255. https://doi.org/10.1186/s12870-023-04239-2

Huang, F., Duan, J., Lei, Y., Liu, Z., Kang, Y., Luo, Y., Chen, Y., Li, Y., Liu, S., Li, S., & Liu, Z. (2022). Genetic diversity, population structure and core collection analysis of Hunan tea plant germplasm

through genotyping-by-sequencing. *Beverage Plant Research*, *2*(1), 1-7. https://doi.org/10.48130/bpr-2022-0005

IPGRI. (1997). *Descriptors for Tea (Camellia sinensis).* International Plant Genetic Resources Institute.

Kumarihami, H. M. P. C., Oh, E. U., Nesumi, A., & Song, K. J. (2016). Comparative study on cross-compatibility between *Camellia sinensis* var. *sinensis* (China type) and *C. sinensis* var. *assamica* (Assam type) tea. *African Journal of Agricultural Research*, *11*, 1092-1101. https://doi.org/10.5897/AJAR2015.9951

Li, M. M., Meegahakumbura, M. K., Wambulwa, M. C., Burgess, K. S., Moller, M., Shen, Z. F., Li, D. Z., & Gao, L. M. (2024). Genetic analyses of ancient tea trees provide insights into the breeding history and dissemination of Chinese Assam tea (*Camellia sinensis* var. *assamica*). *Plant Divers*, *46*(2), 229-237. https://doi.org/10.1016/j.pld.2023.06.002

Liu, C., Yu, W., Cai, C., Huang, S., Wu, H., Wang, Z., Wang, P., Zheng, Y., Wang, P., & Ye, N. (2022). Genetic diversity of tea plant [*Camellia sinensis* (L.) Kuntze] germplasm resources in Wuyi Mountain of China based on single nucleotide polymorphism (SNP) markers. *Horticulturae*, *8*(10), Article 932. https://doi.org/10.3390/horticulturae8100932

Liu, S., Liu, H., Wu, A., Hou, Y., An, Y., & Wei, C. (2017). Construction of fingerprinting for tea plant (*Camellia sinensis*) accessions using new genomic SSR markers. *Molecular Breeding*, *37*(8), Article 93. https://doi.org/10.1007/s11032-017-0692-y

Meegahakumbura, M. K., Wambulwa, M. C., Li, M. M., Thapa, K. K., Sun, Y. S., Moller, M., Xu, J. C., Yang, J. B., Liu, J., Liu, B. Y., Li, D. Z., & Gao, L. M. (2017). Domestication origin and breeding history of the tea plant (*Camellia sinensis*) in China and India based on nuclear microsatellites and cpDNA sequence data. *Frontiers in Plant Science*, *8*, Article 2270. https://doi.org/10.3389/fpls.2017.02270

Meegahakumbura, M. K., Wambulwa, M. C., Thapa, K. K., Li, M. M., Moller, M., Xu, J. C., Yang, J. B., Liu, B. Y., Ranjitkar, S., Liu, J., Li, D. Z., & Gao, L. M. (2016). Indications for three independent domestication events for the tea plant [*Camellia sinensis* (L.) O. Kuntze] and new insights into the origin of tea germplasm in China and India revealed by nuclear microsatellites. *PLoS One*, *11*(5), Article e0155369. https://doi.org/10.1371/journal.pone.0155369

Nwosisi, S., Dhakal, K., Nandwani, D., Raji, J. I., Krishnan, S., & Beovides-García, Y. (2019). Genetic diversity in vegetable and fruit crops. In D. Nandwani (Ed.), *Genetic Diversity in Horticultural Plants* (pp. 87-125). Springer. https://doi.org/10.1007/978-3-319-96454-6_4

Ran, W., Li, Q., Hu, X., Zhang, D., Yu, Z., Chen, Y., Wang, M., & Ni, D. (2023). Comprehensive analysis of environmental factors on the quality of tea (*Camellia sinensis* var. *sinensis*) fresh leaves. *Scientia Horticulturae*, *319*, Article 112177. https://doi.org/10.1016/j.scienta.2023.112177

Rogers, S. O., & Bendich, A. J. (1989). Extraction of DNA from plant tissues. In S. B. Gelvin, R. A. Schilperoort & D. P. S. Verma (Eds.), *Plant Molecular Biology Manual* (pp. 73-83). Springer. https://doi.org/10.1007/978-94-009-0951-9_6

Samarina, L. S., Matskiv, A. O., Shkhalakhova, R. M., Koninskaya, N. G., Hanke, M. V., Flachowsky, H., Shumeev, A. N., Manakhova, K. A., Malyukova, L. S., Liu, S., Zhu, J., Gvasaliya, M. V., Malyarovskaya, V. I., Ryndin, A. V., Pchikhachev, E. K., & Reim, S. (2021). Genetic diversity and genome size variability

in the Russian Genebank collection of tea plant [*Camellia sinensis* (L). O. Kuntze]. *Frontiers in Plant Science*, *12*, Article 800141. https://doi.org/10.3389/fpls.2021.800141

Sharma, S., Kumar, A., Rajpal, V. R., Singh, A., Babbar, S., & Raina, S. N. (2022). Evaluation of genetic diversity and population structure in elite South Indian tea [*Camellia sinensis* (L.) Kuntze] using RAPD and ISSR markers. *Genetic Resources and Crop Evolution*, *70*(2), 381-398. https://doi.org/10.1007/s10722-022-01433-3

Smouse, P. E., Banks, S. C., & Peakall, R. (2017). Converting quadratic entropy to diversity: Both animals and alleles are diverse, but some are more diverse than others. *PLoS One*, *12*(10), Article e0185499. https://doi.org/10.1371/journal.pone.0185499

Vo, T. D. (2007). *Assessing genetic diversity in Vietnam tea [Camellia sinensis (L.) O. Kuntze] using morphology, inter-simple sequence repeat (ISSR) and microsatellite (SSR) markers* [PhD dissertation, Universität Göttingen]. Semantic Scholar. https://api.semanticscholar.org/CorpusID:247019507

Wambulwa, M. C., Meegahakumbura, M. K., Kamunya, S., Muchugi, A., Moller, M., Liu, J., Xu, J. C., Li, D. Z., & Gao, L. M. (2017). Multiple origins and a narrow genepool characterise the African tea germplasm: Concordant patterns revealed by nuclear and plastid DNA markers. *Scientific Reports*, *7*(1), Article 4053. https://doi.org/10.1038/s41598-017-04228-0

Xia, E. H., Zhang, H. B., Sheng, J., Li, K., Zhang, Q. J., Kim, C., Zhang, Y., Liu, Y., Zhu, T., Li, W., Huang, H., Tong, Y., Nan, H., Shi, C., Shi, C., Jiang, J. J., Mao, S. Y., Jiao, J. Y., Zhang, D., …, & Gao, L. Z. (2017). The tea tree genome provides insights into tea flavor and independent evolution of caffeine biosynthesis. *Molecular Plant*, *10*(6), 866-877. https://doi.org/https://doi.org/10.1016/j.molp.2017.04.002

Xia, E., Tong, W., Hou, Y., An, Y., Chen, L., Wu, Q., Liu, Y., Yu, J., Li, F., Li, R., Li, P., Zhao, H., Ge, R., Huang, J., Mallano, A. I., Zhang, Y., Liu, S., Deng, W., Song, C., …, & Wan, X. (2020). The reference genome of tea plant and resequencing of 81 diverse accessions provide insights into its genome evolution and adaptation. *Molecular Plant*, *13*(7), 1013-1026. https://doi.org/10.1016/j.molp.2020.04.010

Yoğurtçu, B. M., & Aygun, A. (2021). Characterization of tea (*Camellia sinensis* L.) genotypes grown in Turkey by ISSR markers. *Applied Ecology and Environmental Research*, *19*(5), 4103-4114. https://doi.org/10.15666/aeer/1905_41034114

Zhang, C., Jia, C., Liu, X., Zhao, H., Hou, L., Li, M., Cui, B., & Li, Y. (2022). Genetic diversity study on geographical populations of the multipurpose species *Elsholtzia stauntonii* using transferable microsatellite markers. *Frontiers in Plant Science*, *13*, Article 903674. https://doi.org/10.3389/fpls.2022.903674

Zhang, F., Li, W., Gao, C. W., Zhang, D., & Gao, L. Z. (2019). Deciphering tea tree chloroplast and mitochondrial genomes of *Camellia sinensis* var. *assamica. Scientific Data*, *6*(1), Article 209. https://doi.org/10.1038/s41597-019-0201-8

Zhao, Y., Wang, R., Liu, Q., Dong, X., & Zhao, D. G. (2021). Genetic diversity of ancient *Camellia sinensis* (L.) O. Kuntze in Sandu County of Guizhou Province in China. *Diversity*, *13*(6), Article 276. https://doi.org/10.3390/d13060276

Zhao, Z., Song, Q., Bai, D., Niu, S., He, Y., Qiao, D., Chen, Z., Li, C., Luo, J., & Li, F. (2022). Population structure analysis to explore genetic diversity and geographical distribution characteristics of cultivated-

type tea plant in Guizhou Plateau. *BMC Plant Biol*, *22*(1), Article 55. https://doi.org/10.1186/s12870-022-03438-7

Zou, Y., Ge, X., Yan, C., Zhong, Q., Chen, D., Chen, Z., Yuan, Y., Guo, H., Zhou, Y., Wang, J., & Cao, L. (2024). Assessment of genetic diversity of *Camellia yuhsienensis* based on leaf structure and inter simple sequence repeat (ISSR) markers. *Genetic Resources and Crop Evolution*, *71*(8), 4749-4762. https://doi.org/10.1007/s10722-024-01910-x