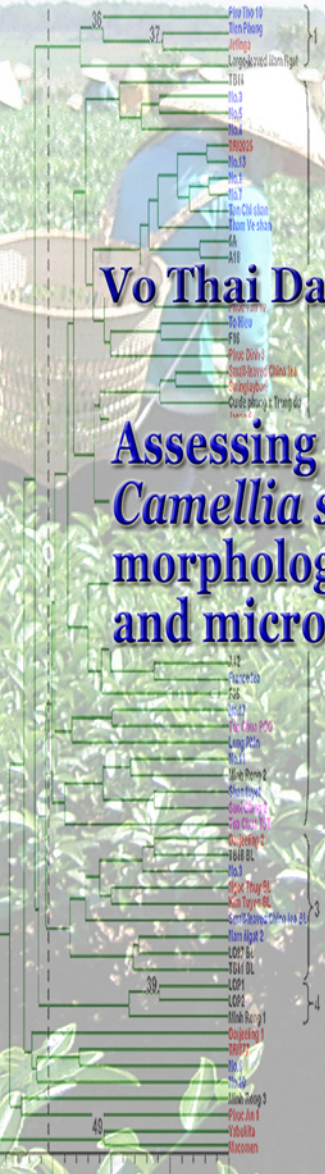
Georg- August University Göttingen
Department of Crop Science



Vo Thai Dan

Assessing genetic diversity in Vietnam tea *Camellia sinensis* (L.) O. Kuntze using morphology, inter-simple sequence repeat (ISSR) and microsatellite (SSR) markers

Göttingen, October 2006

Georg-August University Goettingen

Department of Crop Science

Vo Thai Dan

Assessing genetic diversity in Vietnam tea [*Camellia sinensis* (L.) O. Kuntze] using morphology, inter-simple sequence repeat (ISSR) and microsatellite (SSR) markers

Goettingen, October 2006

Assessing genetic diversity in Vietnam tea [*Camellia sinensis* (L.) O. Kuntze] using morphology, inter-simple sequence repeat (ISSR) and microsatellite (SSR) markers

Dissertation

to obtain the Ph.D. degree

in the Faculty of Agricultural Science,

Georg-August University Göttingen, Germany

Presented by

Vo, Thai Dan

Born in Khanh Hoa province, Vietnam

Supervisor: Prof. Dr. Heiko C. Becker

Co-referee: Prof. Dr. Reiner Finkeldey

Goettingen, December 2006

D7

Name of referee: Prof. Dr. Heiko C. Becker

Name of co-referee: Prof. Dr. Reiner Finkeldey

Date of dissertation: 01/02/2007

Table of content

List of Tables

List of Figures

List of Appendices

Chapter 1

General introduction

1.1 Background and Objectives

The tea plant *Camellia sinensis* (L.) O. Kuntze is used to produce the oldest and most popular non-alcoholic soft beverage across the world and is one of the most important cash crops of many countries, including Vietnam (Table 1.1). Being indigenous to Southeast Asia, in an area stretching from Assam in the West to China in the East and down to Vietnam in the South (Matthews and Stephen, 1998), tea spontaneously grows widely from tropical to temperate regions in Asia. Nowadays tea is commercially cultivated in more than 20 countries, from the Republic of Georgia in the North (42°N) to New Zealand in the South (27°S) (Yamamoto *et al.*, 1997), and becomes a reasonable foreign currency income of many countries. The price for exported tea was low and slightly decreased during the last 25 years (Table 1.1) and the portion of cultivated land for tea growing can not be expanded unlimitedly. In order to increase the foreign currency income from tea production, a feasible method practiced in many countries is the utilization of new highly productive tea cultivars/clones in tea production. Due to its specific characteristics (woody perennial, highly heterogeneous and self-incompatible), tea breeding is costly (money, time and labor), therefore selection based on natural populations play an important role in introducing new potential cultivars/clones. For such purposes, extensive collections of tea have been made in Vietnam and several other countries.

Known as a freely cross-pollinated plant, with many overlapping morphological, biochemical and physiological attributes (Purseglove, 1968; Wickremasinghe, 1979; Banerjee, 1988), tea can not be separated into discrete groups to identify various taxa (Wickramaratne, 1981). It is difficult to say if the original description of tea as *Thea sinensis* by Linnaeus (1752) relates to the species mostly cultivated today (Banerjee, 1992). Tea taxonomy is still a challenge today, but did not receive the attention it deserved possibly because of the complexities involved (Banerjee, 1992). Furthermore, since hybrids are used and clonal propagation is recommended, the widespread cultivation of clonal tea can diminish genetic diversity if care is not taken to use clones of dispersed

origin. Information on taxonomic characteristics, genetic diversity and biogeography of tea in the living collections may help in identifying genotypes with high productive potentials which could be used as progenitors to improve the existing genetic base of commercially grown tea (Banerjee, 1992). Revealing the genetic diversity by using molecular markers has assisted breeders assigning appropriate parents to be used in crosses.

Table 1.1: Statistical data on tea export in 2004 of the whole world, some continents (Asia, Africa, Europe) and the top six tea exporter countries (from FAO, 2006)

Area	**Quantity of tea export**		**Price**	
	Quantity in 2004 (ton)	Average change yearly from 1980 to 2004	Price in 2004 (USD.kg^{-1})	Average change yearly from 1980 to 2004
World	1613633	26243 (76345)	2.028	-0.001 (0.221)
Asia	1026337	14333 (53992)	1.884	-0.009 (0.232)
Africa	405657	9327 (50781)	1.513	-0.004 (0.291)
Europe	90384	1947 (6281)	6.821	0.088 (0.462)
Sri Lanka	298909	4758 (25090)	2.451	0.018 (0.349)
Kenya	284309	8327 (55597)	1.631	-0.017 (0.361)
China	282643	6930 (17234)	1.605	-0.021 (0.125)
India	174728	-2659 (21718)	2.162	-0.012 (0.325)
Vietnam	99400	3768 (10500)	0.961	-0.001 (0.199)
Indonesia	98572	1015 (15548)	1.177	-0.014 (0.350)

(Numbers in the parentheses are the standard deviations)

Objectives

With a longstanding history of cultivation and consumption of tea, Vietnam is believed to be highly rich in genetic diversity of tea in situ. Beside that, the existence of old Shan giant wild teas under the forest canopy at Suoi Giang, Nghia Lo (Ha Giang Province) could also provide important genetic resources for tea breeding. Therefore understanding the genetic diversity within and between wild giant shan teas, local cultivated tea accessions, improved tea cultivars and cultivars imported from other countries will provide the important informative scientific basic for tea breeding programs in Vietnam.

The main objectives of this study are:

- Assessing the morphological diversity of tea grown at Lam Dong province – the main tea producing province of Vietnam;

- Collecting accessions of wild giant shan tea, local tea, selected/improved tea and imported tea to assess the genetic diversity on molecular level by using inter simple sequence repeat (ISSR) and simple sequence repeat (SSR) markers.

This will help to identify parents for hybridization and to reduce the number of accessions needed to maintain a broad range of genetic variability.

1.2 Main properties and types of molecular markers

The molecular markers based on polymorphisms in proteins or DNA have been used in several disciplines such as phylogeny, taxonomy, ecology, genetics, and plant and animal breeding. Properties of molecular markers and their application in various areas of research have been reviewed by many authors (Winter and Kahl, 1995; Yang *et al*., 1996; Smith *et al*., 1997; Gilbert *et al*., 1999; Gupta *et al*., 1999; Gupta and Varshney, 2000; Navajas and Fenton, 2000; Varghese *et al*., 2000; Virk *et al.*, 2000; Charcosset and Moreau, 2004; Varshney *et al*., 2005; Weising *et al*., 2005; Anne, 2006).

Weising *et al*. (2005) mentioned the following properties to be desirable for molecular markers: high polymorphism; co-dominant inheritance (which allows the discrimination of homo- and heterozygous states in diploid organisms); unambiguous assignment of alleles; frequent occurrence in the genome; even distribution throughout the genome; selectively neutral behavior (i.e., no pleiotropic effects); easy access (i.e., by purchasing or fast procedures); easy and fast assay (e.g., by automated procedures); high reproducibility; easy exchange of data between laboratories and low cost for both marker development and assay. Up to now, no single type of molecular markers fulfills all of these criteria. However, different marker systems combine some – or even most – of the above-mentioned characteristics.

Protein electrophoresis has been used over many decades to detect the genetic polymorphism of allozymes, sometimes also called isozymes (Weising *et al*., 2005). The main limitations of this type of markers are the relatively low number of loci available and the limited amount of polymorphism (Navajas and Fenton, 2000). Therefore, today for nearly all applications, markers based on DNA polymorphism are preferred.

Table 1.2 summarizes and compares some technical requirements and main characteristics of different DNA markers commonly used. Marker types included are restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA

(RAPD), amplified fragment length polymorphism (AFLP), inter-simple sequence repeat (ISSR), simple sequence repeat (SSR) and single nucleotide polymorphism (SNP).

Table 1.2: Comparison some main characteristics of different DNA marker techniques (after Gustavo and Gresshoff, 1997; Gillet, 1999; Vignal *et al*., 2002; Anne, 2006; modified)

	RFLP	RAPD	AFLP	ISSR	SSR	SNP
Restriction enzyme used	+	-	+	-	-	-
PCR used	-	+	+	+	+	+
DNA quality[(1)]	HMW, D	MMW, C	C, D	Standard	Standard	Standard
Primer species-specific	-[(2)]	-	-	-	+	+
Inheritance[(3)]	c	d	d	d	c	c
Development effort	High	Very low	Low	Low	High	High
Genotyping effort	High	Very low	Very low	Low	Low	Medium
Reliability	High	Low	High	High	High	High
Accuracy	Very high	Very low	Medium	High	High	Very high
Possibility of automation	-	+	+	+	+	+

[(1)] HMW high molecular weight DNA; MMW medium molecular weight DNA; C constant quality among individuals; D DNA must be digestible

[(2)] but specific probes for hybridization required

[(3)] d dominant; c co-dominant

Polymerase chain reaction (PCR)

All widely used marker techniques today are based on the polymerase chain reaction (PCR), which can reduce the time, effort and expense required. There are many types of PCR-based DNA markers but in this chapter just the most commonly used ones are reviewed.

PCR is a molecular biology technique, based on the enzymatic in vitro amplification of DNA. Figure 1.1 schematically presents the principle of PCR. In a typical PCR assay, three temperature-controlled steps can be discerned, i.e., denature, annealing and extension, which are repeated in a series of 25 to 50 cycles.

- Denature: the double-stranded DNA has to be heated to 94 - 96°C to separate the strands by breaking apart the hydrogen bonds that connect the two DNA strands.

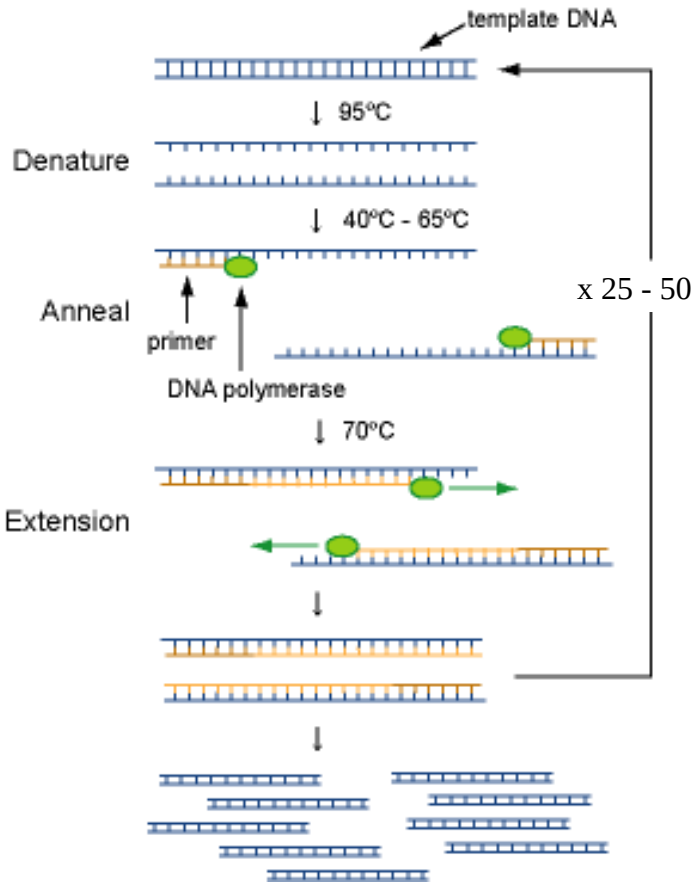


Figure 1.1: PCR scheme (online resource, http://www.246.ne.jp/~takeru/chalk-less/lifesci/images/pcr.gif)

- Annealing: after separating the DNA strands, the temperature is lowered so the primers can attach themselves to the single DNA strands. The temperature of this stage depends on the primers and is usually 5°C below their melting temperature (45 - 60°C).

- Extension (elongation): the DNA polymerase has to copy the DNA strands. It starts at the annealed primer and works its way along the DNA strand. The extension temperature depends on the DNA polymerase. *Taq polymerase* elongates optimally at a temperature of 72°C.

Practically, prior to the first cycle, the DNA is often denaturated for an extended time to ensure that both the template DNA and the primers have completely separated and are now single-strand only. A final extension step is also frequently used after the last cycle to ensure that any remaining single stranded DNA is completely copied.

Restriction fragment length polymorphism (RFLP) markers

Among the various DNA markers, RFLPs were developed first and were initially used for human genetics (Botstein *et al*., 1980). RFLP is based upon hybridization of a probe (a specific DNA sequence designed to hybridize with and thus detect a target sequence) to fragments of genomic DNA following digestion with restriction enzymes (Henry, 1997). These enzymes cleave DNA at specific sites with sequences (four or more base pairs) recognized by the enzyme (Henry, 1997).

RFLPs are co-dominant markers and can be derived from the nuclear, chloroplast, and mitochondrial genome (Weising *et al*., 2005). However RFLP analysis has some limitations since it is time-consuming, labour-intensive, requires a high DNA quality and in most cases also the use of radioactive isotopes. Therefore today RFLP is hardly used any more.

Random amplified polymorphic DNA (RAPD)

The RAPD technique uses the PCR principle for amplification of random DNA sequences (Williams *et al*., 1990). RAPDs involving the use of a single short primer (8 – 19 base pairs) under low annealing temperature conditions enhancing multiple binding at sites scattered throughout the genome to direct amplification of discrete random sequences (Williams *et al*., 1990). RAPD method is much faster and cheaper than RFLP technique and requires only small amounts of DNA (Winter and Kahl, 1995). The main limitation of RAPDs is partly owing to the low level of polymorphism detected and sometimes also partly owing to the lack of reproducibility of results. Another limit of these markers is that the RAPD patterns display dominance, preventing identification of heterozygote (Navajas and Fenton, 2000).

Amplified fragment length polymorphism (AFLP)

AFLP, based on selective PCR amplification of DNA fragments generated by restriction enzymes, is a combination of RFLP analysis and PCR technique. AFLP technology is applicable to all organisms without previous sequence information, and generally results in highly informative fingerprints (Weising *et al*., 2005). The relative efficiency in detecting polymorphism of AFLP is the highest in comparison with RFLP and RAPD. The high frequency of identifiable AFLPs coupled with high reproducibility makes this technology an attractive tool for detecting polymorphism (Gupta *et al*., 1999).

Microsatellites (SSR)

Microsatellites or simple sequence repeats (SSRs) are defined as short DNA fragments (approximate 100 bp) containing patterns with two to six base-pairs repeated in tandem. During evolution, the number of repetition units of microsatellites can be rapidly changed. This makes a large number of alleles per microsatellite locus available for population analysis (Navajas and Fenton, 2000). Microsatellites are co-dominant markers. They show a high level of polymorphism, relative to RFLPs and RAPDs, and are frequent in the genome. Microsatellites are therefore excellent for studies in population genetics (Jarne and Lagoda, 1996).

Figure 1.2 schematically presents the development and application of genic SSR markers. With the establishment of expressed sequence tag (EST) sequencing projects for gene discovery in several plant species and the generation of sequence data of many fully characterized genes and full-length cDNA clones, the wealth of DNA sequence information has been generated. Thus the characterization of genic SSR markers is relatively easy and inexpensive because they are a by-product of the sequence data from genes or EST which are publicly available. Therefore genic SSR markers have been identified, developed and used in a variety of studies on several plant species (Varshney ***et al***., 2005) (Figure 1.2).

Inter-simple sequence repeats (ISSRs)

ISSR technique is a PCR based method, which involves amplification of DNA segment present at an amplifiable distance in between two identical microsatellite repeat regions oriented in opposite direction. The technique uses microsatellites, usually 16 – 25 bp long, as primers in a single primer PCR reaction targeting multiple genomic loci to amplify mainly the inter- SSR sequences of different sizes. The primers can be di-nucleotide, tri-nucleotide, tetra-nucleotide or penta-nucleotide. The primers used can be either unanchored (Gupta ***et al***., 1994; Meyer ***et a***l., 1993; Wu ***et al***., 1994) or more usually anchored at 3' or 5' end with 1 to 4 degenerate bases extended into the flanking sequences (Zietkiewicz ***et al***., 1994) (Figure 1.3).

The technique combines most of the benefits of AFLP and microsatellite analysis with the universality of RAPD. ISSRs have high reproducibility possibly due to the use of longer primers (16- to 25-mers) as compared to RAPD primers (10-mers) which permits the subsequent use of high annealing temperature (45 – 60^{o}C) leading to higher stringency. ISSR has high reproducibility; only the faintest bands are not reproducible. About 92 – 95% of the scored fragments could be repeated across DNA samples of the same cultivar and across separate PCR runs when detected using polyacrylamide (Fang and Roose, 1997; Moreno ***et al***., 1998). 10 ng template DNA yielded the same amplification products as did 25 or 50 ng per 20µl PCR reaction. The annealing temperature depends on the GC content of the primer used and usually ranges from 45 to 65^{o}C.

ISSRs segregate mostly as dominant markers following simple Mendelian inheritance (Gupta ***et al***., 1994; Tsumura ***et al***., 1996; Ratnaparkhe ***et al***., 1998). However, they have also been shown to segregate as co-dominant markers in some cases thus enabling

distinction between homozygote and heterozygote (Wu *et al*., 1994; Akagi *et al*., 1996; Sankar and Moore, 2001).

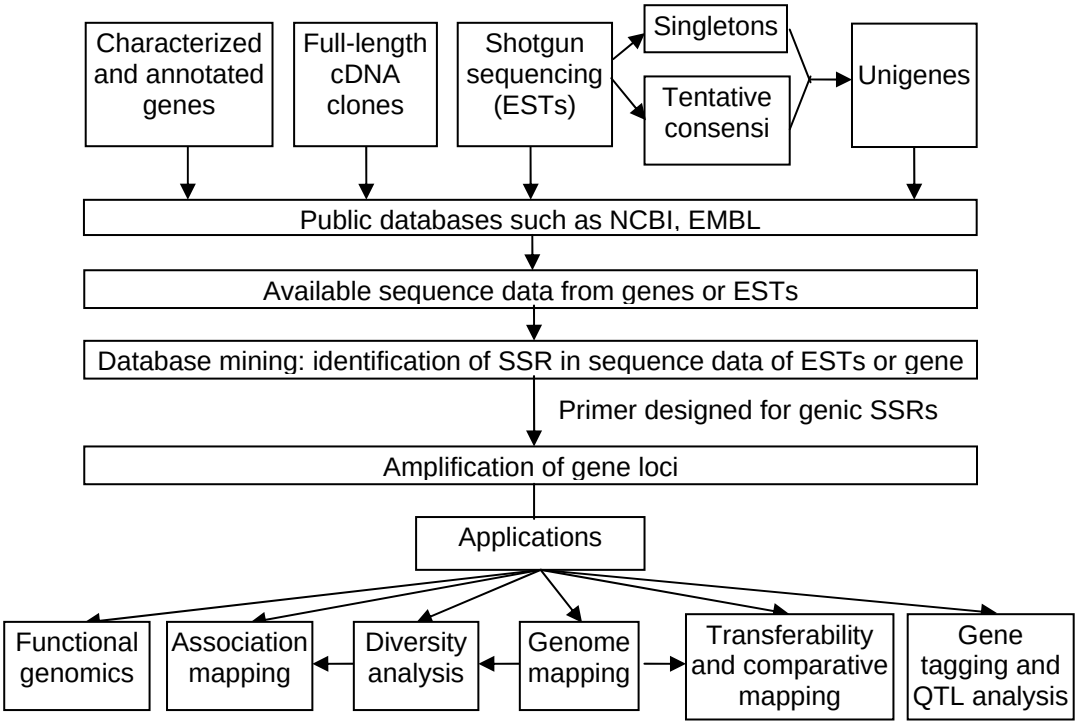


Figure 1.2: A schematic representation of the development and application of genic simple sequence repeat (SSR) markers. ESTs: Expressed sequence tags; NCBI: National Center for Biotechnology Information; EMBL: European Molecular Biology Laboratory; QTL: Quantitative trait loci (Varshney *et al*., 2005)

ISSR markers are highly polymorphic and are useful in studies on genetic diversity, phylogeny, gene tagging, genome mapping and evolution biology (Gupta *et al*., 1994; Becker and Heun, 1995; Wolff *et al*., 1995; Akagi *et al*., 1996; Wolfr *et al*., 1998; Charters and Wilkinson, 2000; Joshi *et al*., 2000; Sankar and Moore, 2001; Reddy *et al*., 2002).

Single nucleotide polymorphism (SNP)

A single nucleotide polymorphism (SNP) marker is a single base change in a DNA sequence, with two possible nucleotides at a given position (Vignal *et al*., 2002). SNPs have been developed and were extensively used since the end of last century in human genetics (Gupta *et al*., 1999). They are suitable for rapid and highly automated genotyping, and have a high abundance (more than 1 SNP per 1000 bp) (Gupta *et al*., 1999). Today SNP are also used more and more in plants (Yanagisawa *et al*., 2003; Domon *et al*., 2004;

Jeong and Saghai Maroof, 2004; Lopez *et al*., 2005; Giancola *et al*., 2006; Soleimani *et al*., 2006).

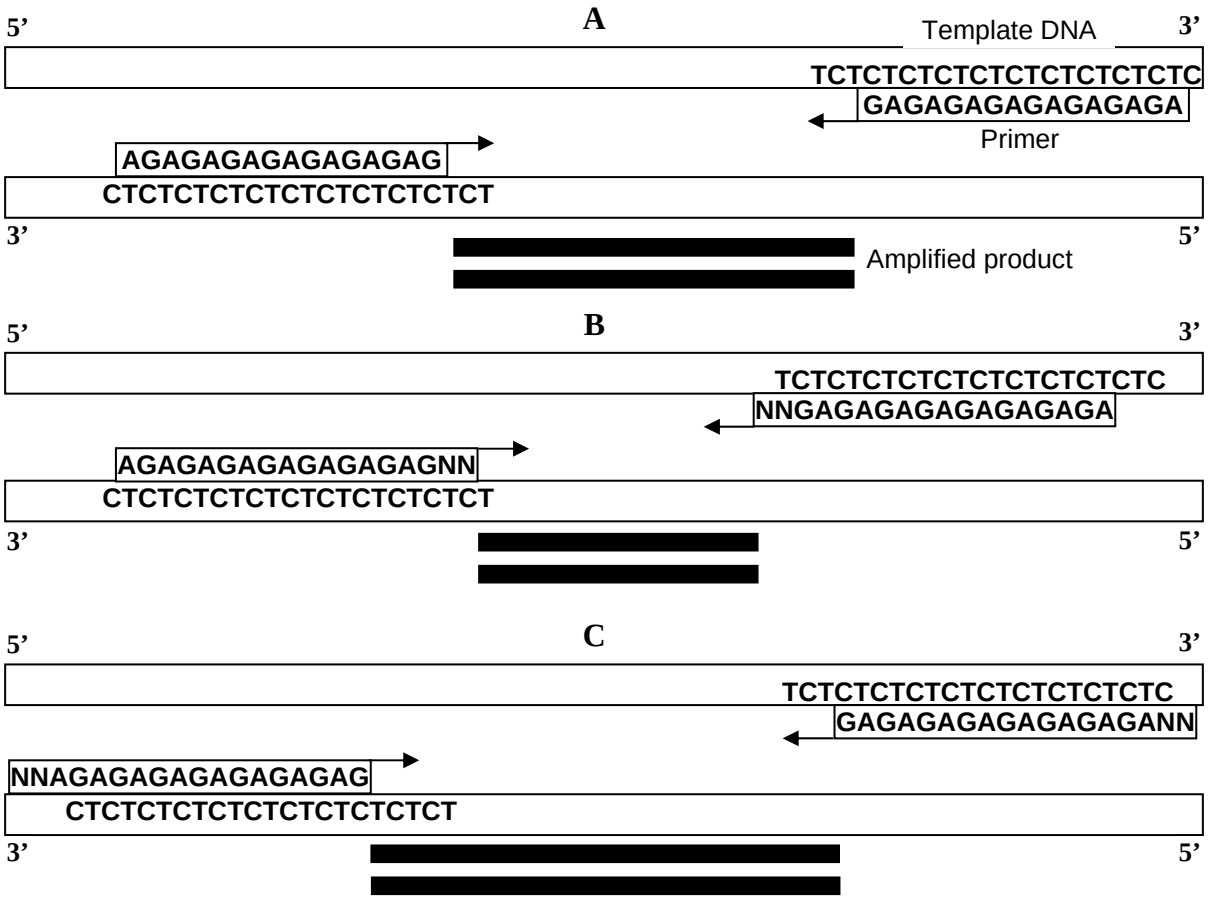


Figure 1.3: ISSR: A schematic representation of a single primer $(AG)_8$, unanchored (A), 3'-anchored (B) and 5'-anchored (C) targeting a $(TC)_n$ repeat used to amplify inter simple sequence repeat region flanked by two inversely oriented $(TC)_n$ sequences. (A) Unanchored $(AG)_n$ primer can anneal anywhere in the $(TC)_n$ repeat region on the template DNA leading to slippage and ultimately smear formation. (B) $(AG)_n$ primer anchored with 2 nucleotides (NN) at the 3' end anneals at specific regions on the template DNA and produces clear bands. (C) $(AG)_n$ primer anchored with 2 nucleotides (NN) at the 5' end anneals at specific regions and amplifies part of the repeat region also leading to larger bands. (Reproduced from Reddy *et al*., 2002)

1.3 Tea *Camellia sinensis* (L.) O. Kuntze: an overview

1.3.1 Morphological characteristics

Tea plant *Camellia sinensis* (L.) O. Kuntze belongs to the family *Theacea* and is economically most important of all *Camellia* species. Tea infusion is the oldest non-alcoholic, caffeine containing beverage in the world. Documentarily the tea plant has been

closely associated with people's life since the dawn of history. Chinese were the first to use tea as medicinal drink, later as beverage (used to be a luxury drink) and have been doing so for the past 3000 years (Eden, 1958). The first tea to reach Europe came by way of the Dutch in the early part of the 17th century (Weatherstone, 1992).

Figure 1.4: Tea plants growing naturally can reach the height of more than 15 m.

Tea plant is an evergreen, perennial, cross-pollinated plant and grows naturally as tall as 15 m (Figure 1.4). However, under cultivation, the bush height of 60 – 100 cm is maintained for harvesting the tender leaves (Figure 1.5), which can continue even more than 100 years. The main vegetative characteristics of tea were summarized in Table 1.3. The flowers are white in color and born singly or pairs at the axils. The fruits are green to dark brown with 1 - 4 seeds and start bearing within 1 – 6 years after planting. Flush shoot (P + 2,3), comprising a bud (furled leaf) and 2 to 3 true young leaves (Figure 1.6), is the normally harvested part of tea plant every 7 to 60 days depending on the varieties, climatic conditions and agro-techniques applied. Due to the differentiation of fermentation during processing, tea products are classified into three main classes: green tea (non-fermented tea), black tea (fermented tea) and oo-long tea (semi- fermented tea).

Figure 1.5: Cultivated tea bushes are kept at the height of 0.6 – 1.0 m

According to Cohen-Stuart (1919) (cf. Yamamoto *et al*., 1997), tea plant is subdivided into four varieties: small-leaved China tea (*C. sinensis* var. *microphylla* or var. *bohea*) (Figure 1.7), large-leaved China tea (*C. sinensis* var.

macrophylla), Shan tea (*C. sinensis* var. *Shan* or var. *burmaensis*) with densely pubescent shoots (Figure 1.8) and Assam tea (*C. sinensis* var. *assamica*) (Figure 1.9). However, all taxa freely interbreed and therefore tea plants are highly diverse and heterogeneous (Willson and Clifford, 1992). Natural hybridization has been so extreme that it is today difficult to find true archetypal China (small- and large-leaved), Shan and Assam varieties.

Figure 1.6: Harvested tea shoots consist of a bud and 2 – 3 leaves.

Figure 1.7: Small-leaved China tea shoots

Figure 1.8: Flush shoots of Shan tea.

Figure 1.9: PH_1 clone, Assam tea.

In its natural habitat, China tea is a dwarf tree with small, dark green, narrow, largely serrated erect leaves (5.5 – 6.1 cm in length and 2.2 – 2.4 cm in width). The plants only produce single flowers. China teas are also known as cold resistant and suitable for making green and oolong teas. The Assam type is a taller tree with larger, less serrated leaves which form a greater angle to the stem and tend to droop at their outer point (leaf size: 16.0 – 19.0 cm length and 7.0 – 9.0 cm width). Leaf color varies, but the green is usually lighter than that of China type and is sometimes even very light, almost yellow. The flowers are born in clusters of two to four. Assam teas are less resistant to cold and are

suitable for making black tea. In general, the vegetative characteristics of tea plant vary continuously over wide ranges (Table 1.3) with high degree of plasticity (Willson and Clifford, 1992; Yamamoto *et al*., 1997).

Table 1.3: Variability in vegetative characteristics of tea

Characteristics	Range of variability
Mean of leaf angle (degrees)	50 – 120
Laminar angle (degrees)	110 – 125
Internodal length (mm)	15 – 70
Individual leaf area (mm2)	120 – 200
Leaf area index (LAI)	3.5 – 8.5
Leaf length/breadth ratio	2.0 – 2.8
Height (cm)	184 – 539
Girth at collar (cm)	25 – 42
Branching habit	Acutely orthotropic to plagiotropic
Thickness of branches at 60 cm from ground level (cm)	1.4 – 4.4
Length of internode between the second and the third leaves from the apical bud of flush shoot (cm)	0.9 – 3.2
Length of the third leaf from the apical bud of growing shoot (cm)	2.0 – 6.0
Breadth of the third leaf from the apical bud of flush shoot (cm)	1.5 – 3.8
Angle between the third leaf of flush shoot and the internode above (degrees)	35 – 65
Color of mature leaf	Light green to dark green
Pubescence on the bud and abaxial side of the first leaf	Glabrous to densely pubescent
Anthocyanin pigmentation in young leaves and petioles	Nil to dark
Dry weight flush shoot (three leaves and a bud) (mg)	60 – 350

Sources: Satyanarayana and Sharma (1986); Banerjee (1987)

1.3.2 Genome diversity

The number of species, reported by various authors, within genus *Camellia* had changed from time to time; of genus 82 species were reported in 1958 (Sealy, 1958), later over 200 species were described (Zhijian *et al*., 1988), and in 2000 more than 325 species were distinguished (Mondal, 2002a). Such changes indicate a taxonomic problem mainly due to hybridization among species. Presently, world-wide over 600 cultivated tea varieties are

available, of which many have unique traits such as high caffeine content, blister blight disease tolerance, drought resistance, frost tolerance etc (Mondal, 2002a).

Owing to extensive hybridization between different *Camellia* taxa, several intergrades, introgressants and putative hybrids have been formed. These can be arranged in a gradient based on morphological characters that extend from China types through intermediates to those of Assam types. Because of the extreme hybridization, existence of the pure archetypes of tea is doubtful (Visser, 1969). Till date, numerous hybrids currently available are still referred to as China, Assam or Shan tea depending on morphological proximity to the main taxon (Banerjee, 1992). Naturally tea hybridizes well with wild relatives and thus taxonomists have always been interested to identify such hybrids due to suspected involvement in tea domestication. It is generally agreed that at least three taxa i.e. *C. assamica*; *C. sinensis*; *C. assamica* sub *sp. lasiocalyx* and to some extent *C. irrawadiensis* have mainly contributed to the genetic pool of tea. The term 'tea' should therefore cover progenies of these taxa and the hybrids between them.

1.3.3 Economic importance, health and other benefits

Tea was initially used as a medicine and subsequently as beverage and is potential an important raw material for the pharmaceutical industry. Presently, tea is the most consumed drink in the world after water, well ahead of coffee, beer, wine and carbonated soft drinks. It has little nutritional value, but is refreshing, mildly stimulating, and produces a feeling of well-being. It is mainly consumed in the form of black tea (78%); green tea (20%) and oolong tea (2%) are mainly popular in the area of East Asia (Yang and Landau, 2000).

Drinking tea can yield the positive health effects such as improving the growth of beneficial micro flora in the intestinal, protecting cells and tissues from oxidative damage by scavenging oxygen-free radicals (Rietveld and Wiseman, 2003), reducing tumors and mutations, promoting antioxidant and antimicrobial activity, preventing dental caries and cardiovascular disease, lowering blood cholesterol, inhibiting the increase of blood pressure and blood sugar, killing bacteria and influenza virus, normalizing diabetes, increasing thermo-genesis and bone density (Chen, 1999; Fujiki, 1999; Hour *et al*., 1999; Kang *et al*., 1999; Sasazuki *et al*., 2000; Yang and Landau, 2000; Suganuma *et al*., 2001; Yanagawa *et al*., 2003). The medical values can be explained mainly by the high amount of flavonoids in tea.

On the economic aspect, at the household level, tea plant is so-called the crop of the poor, especially in the tropical mountainous areas, because even with the minimal investment required, tea can be planted and harvested weekly or each ten-day period on hard and sloping soils where the other food crops or cash crops (such as coffee, cocoa) could not grow effectively; at the nation level, tea export industry contributes a reasonable amount of foreign currency to the income of tea producing countries. Developing tea production at the remote areas also provides many jobs to local farmers and certainly contributes to the development of local infrastructures.

Tea is served as daily drink for two third of the world population. Drinking tea became a special culture ceremony in many countries (such as Japan, China, and Vietnam). Tea also can not be absent in many cultural events such as traditional New Year and wedding ceremony.

Besides being used as beverage, green leaves are also used as vegetables in many areas of Burma and Thailand. Other parts of tea plant are also used: the tea seed oil is used as lubricant, yet extraction from seed is not economical; tea seed cakes contain saponins but has got poor value as fertilizer and animal feed due to low nitrogen, phosphorus and potassium content but can be used successfully as nematocide (Wealth of India, 1950; cf. Mondal *et al*., 2004). Planting tea plant on the remote mountainous areas is considered as an effective method to cover the spare sloping lands.

1.3.4 World tea production

Tea is now commercially cultivated in more than 20 countries in Asia, Africa and South America, from Republic of Georgia in the north to New Zealand in the south; in which Asian countries are the main producers contributing up to 88 % of world harvested tea area in 2005 (FAO, 2006) (Table 1.4). From 1980 to 2005, while the harvested area of the world and many main tea producer countries increased or decreased slowly, the dry yield is always increasing yearly.

Most of tea production is traded around the world. The world-wide demand for tea is predicted increasing at the rate of 4 – 5% in the next few years. Tea produced in the Middle East, Iran, Turkey and the former USSR is almost entirely consumed in the producing countries, as is the small Australian output (Yamamoto *et al*., 1997).

1.3.5 Tea breeding

The two main processes of most plant breeding, including tea breeding, are hybridization and selection. Hybridization can be done either naturally or by hand. With natural pollination, it is difficult to know the pedigree of the cultivars (as pollens may come from any male) hence the chance of reproducibility is low and least preferred presently. Alternatively, controlled crosses, despite being an important approach, has had limited success in tea breeding. However, recently, few clones have been released using this technique.

Table 1.4: The harvested area and yield of cultivated tea of the world, Asia and some main tea producer countries in 2005 and the average change yearly during 1980 and 2005 (FAO, 2006)

Area	**Harvested area**		**Dry yield**	
	Area (ha)	Average change yearly from 1980 to 2004	Yield ($ton.ha^{-1}$)	Average change yearly from 1980 to 2004
World	2561001	7661 (62190)	1.335	0.021 (0.045)
Asia	2259920	7745 (64529)	1.259	0.021 (0.043)
Africa	245080	3013 (5182)	1.976	0.032 (0.124)
China	952500	-4625 (57195)	0.987	0.027 (0.037)
India	500000	4724 (14017)	1.661	0.007 (0.093)
Sri Lanka	210620	-1364 (10111)	1.463	0.027 (0.123)
Kenya	140000	2538 (2617)	2.107	0.037 (0.216)
Indonesia	116200	1201 (10145)	1.475	0.010 (0.260)
Vietnam	104000	2772 (5529)	1.058	0.018 (0.074)
Turkey	100000	1848 (6576)	2.020	0.010 (0.358)
Japan	49000	-480 (1532)	2.041	0.015 (0.090)

(Numbers in the parentheses are the standard deviations)

Tea is essentially a cross pollinated plant and self-incompatible. From the very early days of tea growing, it was recognized that breeding of tea creates problems that are somewhat unique to the plant. This is so because, firstly, unlike other woody perennials, in tea only a part of the total biomass constitutes the harvest, and secondly the plant is highly heterogeneous and self-incompatible (Banerjee, 1992). It is propagated either through seeds or cuttings.

Selection is the most popular, longstanding practice in tea breeding. Since commercial tea plantations earlier were established with seedlings, hence lots of variability exists among them. Often elite plants have been identified among existing bushes and released as clones. The majority of the tea clones have been developed through selection. However, pedigrees of such clones remain unknown. Tea plant selection is mainly based on the morphological characteristics for yield, quality, biotic and abiotic stress resistance.

Until now tea plantation is developed largely from the selected genotypes based on the performance of yield, quality, biotic and abiotic stress resistance amongst the previously existing planting materials. As a consequence, widespread cultivation of clonal tea can diminish the genetic diversity if care is not taken to use clones of diverse origin.

Conventional tea breeding is well established and contributed much to tea improvement over the past several decades, but the process is slow due to some bottlenecks: tea is perennial nature, long gestation periods, high inbreeding depression, self-incompatibility, unavailability of mutants with tolerance to different biotic and abiotic stress, lack of clear selection criteria (Kulasegaram, 1980), low success rate of hand pollination, short flowering time (2 – 3 months), long duration for seed maturation (12 – 18 months), clonal differences of flowering time and fruit bearing capability of some clones (Mondal *et al*., 2004). A new technology for varietal improvement of tea is genetic transformation. However, central to any successful transgenic technology is an efficient *in vitro* regeneration protocol. While an efficient regeneration protocol is essential for introduction of the foreign gene into plant tissues, micropropagation is important for the transfer of large number of genetically modified plants to the field within a short span of time (Mondal *et al*., 2004).

Though several genetic transformation techniques are available (Klee *et al*., 1987; Kuhlemeiere *et al*., 1987; Hooykaas and Schilperoort, 1992; Smith and Hood, 1995), yet few have been employed to produce transgenic tea and the production of transgenic tea remains difficult mainly due to low transformation efficiency as well as its difficult regeneration system (Mondal *et al*., 2004).

Seed-grown trees show a high degree of variability, therefore, the alternative choice is through vegetative propagation from the cuttings. Recently, grafting as an alternative propagation technique has gained considerable popularity. In such case, both root-stock (commonly a drought tolerant cultivar) and scion (often either good quality or high yielding cultivar) are generally fresh single leaf internode cuttings. Upon grafting, the scion and

stock influence each other for the characteristics and thus composite plants combine both high yield and good quality characteristics. For further improvement, the tender shoots were grafted on young seedlings; hence an additional advantage of grafted tea seedling is the presence of tap root system. Vegetative propagation is an effective method of tea propagation, yet it is limited by several factors such as: slower rates of propagation, unavailability of suitable planting material due to winter dormancy, drought in some tea growing area, poor survival rate at nursery due to poor root formation of some clones and seasonal dependent rooting ability of the cuttings (Mondal *et al*., 2004).

Micropropagation technique appears to be an ideal choice for circumvention of the problems related to conventional propagation. Forrest (1969) was pioneer for initiating the work on the tissue culture of tea; then Kato (1985) did a systematic study on micropropagation of tea, but studies on field performance of micro-propagated tea and commercial exploitation only started at the beginning of the 21st century (Mondal *et al*, 2004). The largest difficulty in micro-propagating tea is to regenerate the adventitious shoots from explants; like other woody perennials, major problems encountered in tea micropropagation are phenolic exudation from explants and microbial contamination in tissue culture medium (Mondal *et al*., 2004).

1.4 Recent advances of assessing tea diversity

Genetic diversity is defined as the genetic variation within a population or species but measuring genetic diversity is not restricted to species. Depending on the system and the questions of interest, other divisions including genus or family may be appropriate. The crop diversity of most areas is greater than species diversity implies because more than one variety of each crop is grown. However, crop varieties, because of there selection for a limited number of traits, have considerably less genetic variability than their wild progenitors (Olson and Francis, 1995). Human activities like urbanization, the replacement of traditional agriculture systems by modern industrial methods or the introduction of modern high-yielding varieties may pose a danger to the biological diversity (Khlestkina *et al*., 2004).

Numerous studies to evaluate tea diversity have been conducted by using morphological characteristics (Guohua *et al.*, 1995; Chen *et al.*, 2005), biochemistry components (Magoma *et al.*, 2000; Chen *et al.*, 2005), allozyme (Yee *et al.*, 1996; Chen *et al.*, 2005) and genetic markers, e.g., CAPs (Kaundun and Matsumoto, 2003), cpDNA (Katoh *et al.*, 2003), RFLPs (Matsumoto *et al.*, 1994; Devarumath *et al.*, 2002; Matsumoto *et al.*, 2002),

RAPDs (Wachira *et al.*, 1995, 1997; Chen *et al.*, 1998; Kaundun *et al.*, 2000; Kaundun and Park, 2002; Park *et al.*, 2002), AFLPs (Paul *et al.*, 1997; Balasaravanan *et al.*, 2003) and ISSRs/microsatellites (Ueno *et al.*, 1999; Lai *et al.*, 2001; Mondal, 2002b). However, most of the materials used in these studies were non-indigenous from countries such as Kenya, Japan, Taiwan and the UK.

Though a number of morpho-biochemical markers have been suggested (Wachira, 1990; Singh, 1999; Ghosh Hazra, 2001), yet they have only marginally improved the efficacy of selection for desired agronomic traits. This is mainly due to the fact that most of the morphological markers defined so far, are influenced greatly by the environmental factors and hence show a continuous variation with a high degree of plasticity. Therefore, base on these markers tea cannot be separated into discrete groups for identification (Wickremaratne, 1981).

1.4.1 Morphological diversity

Tea has been classified into different taxa using morphological characteristics. Mondal *et* al. (2004) cited that Barua (1963) provided morpho-anatomical descriptions, which later was elaborated by Bezbaruah (1971). Morphological parameters such as leaf architecture, growth habits and floral biology are important criteria used by tea taxonomists (Banerjee, 1992). While bush vigor, pruning weight, period of recovery from pruning time, plant height, root mass, root - shoot ratio, plucking point density, dry matter production and partitioning are considered as yield indicator of tea (Banerjee, 1992), green leaf pigmentation (Banerjee, 1992), leaf pubescence (Wight and Barua, 1954) have been used as potential determinants for tea quality. Despite the several disadvantages, these are the most adopted markers used by tea breeders globally.

To described and evaluated tea germplasm, the morphological characteristics of tree shape, branchlets, leaf, shoot, corolla, stamen, pistil, capsules, seed and leaf anatomy were measured and reported (Yu and Xu, 1999; Chen and Yu, 2001). Great variation of morphological characteristics was revealed among 87 accessions in genus *Camellia* in Yunnan province of China (Chen *et al*., 2005). All leaf and most flower quantitative characteristics showed significant differences while all fruit quantitative and most of qualitative characteristics measured did not differ significantly (Chen *et al*., 2005).

1.4.2 Cytological diversity

Cytology of the genus *Camellia* was studied since the early 1970s with many interesting features. Chromosome number has been established for the most available taxa of *Camellia* including tea (Bezbaruah, 1971; Kondo, 1977). Tea is diploid (2n = 2X = 30; basic chromosome number X=15) (Bezbaruah, 1971) and the *r* value (ratio of long arm to short arm) for all the 15 pairs of chromosomes range from 1.00 to 1.91. This consistency in diploid chromosome number suggests a monophyletic origin of all *Camellia* species. However few higher ploidy levels such as triploids, tetraploids, pentaploids and aneuploids have also been identified (Singh, 1980).

In karyotype analysis, grouping by chromosome size was difficult in the *Camellia* taxa since the chromosome vary continuously from the largest to the smallest. Furthermore, even in the best preparation, homologous chromosomes did not appear identical (Kondo, 1978). Relatively little intraspecific karyotypic variation had been observed in the cultivated species of *Camellia* studied (Kondo 1979). Sat-chromosomes in karyotypes within many accessions of certain *Camellia* species are morphologically and quantitatively variable. Thus karyotypes including characteristics of sat-chromosomes are not of taxonomic significance for *Camellia*.

It was shown by Kondo and Parks (1979) that the C-banding method can be applied to the somatic mid-metaphase chromosomes in *Camellia* taxa. This differentially stains bands in somatic mid-metaphase chromosomes and permits the identification of individual chromosomes. Thus it is possible to identify the homologous pairs of chromosomes more precisely and perhaps even to measure chromosome divergence between different clones within the same species with same or similar karyotypes. Karyotypic variability and divergence among seven accessions of *C. japonica* L. *sensu lato* with same acetoorcein-stained karyotype were revealed by C-banding (Kondo and Parks, 1981). However, due to the development of more sensitive biochemical techniques, attention was shifted towards biochemical markers.

1.4.3 Biochemical diversity

Biochemical composition was widely used for characterization of different plant germplasm (Das *et al*., 2002). The presence of calcium oxalate crystals and it’s quantity in paranchymatous tissue of leaf petioles (phloem index), have been suggested to be a suitable criterion for classifying tea hybrids (Wight, 1958). The variation in quantity and

morphology of the sclereids in the leaf lamina were also utilized for differentiating tea taxa (Barua, 1958; Barua and Dutta, 1959).

Takeo (1983) suggested a chemo-taxonomic method of classifying tea clones based on the Terpene Index (T.I), which expresses the ratio between linalool and linalool plus geraniols. With the advancement of high performance liquid chromatography, considerable success has been achieved in the identification of tea quality indicators (Takeo, 1981; Owuor *et al*., 1986). These indicators have also found wider use in distinguishing between Assam and China tea (Owuor, 1989).

Although not fully exploited, the polyphenol oxidase activity, individual polyphenols, amino acids and chlorophyll content are considered to be potential parameters in tea taxonomy (Sanderson, 1964). The presence or absence of certain phenolic substances in tea shoots has also been used in establishing relationships among various taxa (Roberts *et al*., 1958). Quantitative changes in chlorophyll-*a*, chlorophyll-*b* and four carotenoids (*β*-carotene, lutein, violaxanthine and neoxanthine) were used for characterization of Assam, China, and Cambod tea (Hazarika and Mahanta, 1984). Total catechin concentration and the ratio of dihydroxylated to trihydroxylated catechin of green leaf were used to establish genetic relationship among 102 Kenyan tea accession (Magoma *et al*., 2000). Though detection accuracy is higher, yet accumulation of such chemicals is subjected to post-transcriptional modification, which restricts the utility of chemical components (Staub *et al*., 1982).

1.4.4 Isozymes diversity

Genetic analysis of isozyme variation was used for cultivar identification in tea (Hairong *et al*., 1987; Xu *et al*., 1987; Chen *et al*., 2005). Among the isozymes, peroxidase and esterase are extensively studied in different tea cultivars (Ikeda *et al*., 1991; Chengyin *et al*., 1992; Singh and Ravindranath, 1994; Yang and Sun, 1994; Borthakur *et al*., 1995; Chen, 1996). However, other isozymes such as tetrazolium oxidase, aspartate aminotransferase and alpha-amylase were also studied among 7 different tea cultivars along with 3 different species (Sen *et al*., 2000). The electrophoretic analysis revealed both the qualitative and quantitative variation in the isozyme banding pattern among different species of tea and their clones. The tetrazolium oxidase enzyme system showed the highest variability among all the enzymes. Cluster analysis using isozyme banding pattern produced a dendrogram which clearly differentiated characteristics of both the clones and species studied. However, isozyme studies in tea were generally limited to few enzymes with inadequate polymorphism (Wachira *et al*., 1995). Therefore, with the

advancement of molecular biology, efforts were shifted towards various DNA based markers.

1.4.5 Molecular diversity

Recently, development of molecular biology has resulted in alternative DNA-based markers for improvement of tea. These markers can assist the process of traditional breeding. The greatest advantages of molecular markers are that they are free from the environmental influence and detection of polymorphisms is possible at an early stage.

Molecular methods were employed because recent morphological and anatomical studies of tea (*C. sinensis*) and related plant species provide conflicting support for tribal and generic relationships within the family (Prince and Parks, 2001).

High quality DNA (high molecular weight) is necessary for every genomic study. To extract high quality DNA from tea, many studies have been done due to its high polyphenolic contents. Principally tea genomic DNA can be isolated using the basic CTAB procedure (Takeuchi *et al*., 1994; Matsumoto *et al*., 1994). Wachira *et al*., (1995) described the method to isolate tea genomic DNA from silica gel dried tea leaves. Genomic DNA of tea products from the market can be extracted using the procedure introduced by Mahipal *et al*., (1999). Mondal *et al*. (2000) describe the protocol to isolate genomic DNA from tea and other phenol rich plant. Good quality chloroplast DNA from tea could be isolated using the procedure reported by Borthakur *et al*. (1998).

1.4.6 RFLP markers

Restriction fragment length polymorphisms (RFLPs) have been used to investigate genetic diversity in cultivated plants and wild relatives (Tanksley *et al*., 1989). In tea, from RFLP analysis using tea phenylalanine ammonia-lyase (PAL) cDNA as probe, Matsumoto *et al*., (1994) succeeded in distinguishing Assam hybrids and Japanese green tea cultivars with high and low catechin content, respectively, and in grouping Japanese green tea at the cultivar level. Polymorphism in PAL/RFLP pattern revealed close relationship of Chinese and Korean sinensis teas apart from Japanese sinensis tea. Assamica teas greatly differ in PAL-variation from Korean and Japanese sinensis teas, but resemble Chinese sinensis tea. The present green tea cultivars in Japan were originated by crossing and selection from a narrow genetic background. Later Japanese green tea cultivars and 463 local tea trees including mountainous tea were analyzed to determine the process of differentiation of Japanese tea trees (Matsumoto *et al*., 2002). Since the allelic frequencies in

mountainous and local tea trees were the same, it is thought that all these teas have the same origin. The results indicate a process of differentiation from the ancestral material presumably introduced from China to the local tea trees and, finally, cultivars which were produced by selecting local tea trees and crossing.

In a separate study, the genetic diversity of tea was investigated also based on RFLP analysis of PAL, CHS2 and DFR, three key genes involved in catechin and tannin synthesis and directly responsible for tea taste and quality (Kaundun and Matsumoto, 2003). A factorial correspondence analysis carried out on all genotypes and markers separates tea samples into two distinct groups according to their varietals status. The large difference between var. *sinensis* and var. *assamica* in their polyphenolic profile was revealed. Matsumoto *et al*. (2004) used RFLP with PAL cDNA as a probe to evaluate the genetic diversity of Korean tea. A total amount of 297 tea trees collected from the ground of 6 old temples and a tea plantation was analyzed. In Japanese teas the PAL locus is composed of 3 multi-fragment alleles, but at least 10 fragment alleles were apparent in Korean teas. Korean teas showed greater genetic diversity than Japanese teas. Korean teas were divided into two different genetic groups: one group was found around old temples and probably derived from China; the other originated from Japanese teas that were introduced in the 19th and 20th centuries.

Devarumath *et al*., (2002) reported that RFLP fingerprints are useful markers to evaluate genetic integrity of micropropagated tea trees.

1.4.7 RAPD markers

Wachira *et al*. (1995) estimated genetic diversity and taxonomic relationships in 38 different cultivars of Kenyan tea by using RAPD markers. Extensive genetic variability was detected between species, consistent with both the present taxonomy of tea and with the known pedigrees of some clones. RAPD analysis also discriminated all of the 38 commercial clones, even those which can not be distinguished on the basic of morphology and phenotypic traits. Yamaguchi *et al*., (1999) using RAPD markers, reported a narrow variation in Korean and Japanese teas, compared to Chinese, Assamese and Vietnamese teas. After being introduced from China, Korean tea underwent little genetic diversification. On the contrary, Japanese tea showed a closer relationship with their Chinese and Indian counter part, which reveal the fact that tea in Japan might have brought from China as well as India.

Later genetic structure of six Korean populations was investigated by RAPD markers (Kaundun and Park, 2002). The genetic diversity within populations was relatively low due to the narrow genetic base of tea samples introduced from China and considerable reduction in population size following mass destruction of tea population in the 14th century. No geographical trends were observed among tea populations. The genetic variability of 20 abandoned Korean tea plantations was investigated using the RAPD methodology and the bulked DNA procedure (Park *et al*., 2002). *Camellia sinensis* being a highly out crossing species maintains high intra-population variability as compared to its inter-population variability.

Twenty-five Indian tea cultivars and 2 ornamental species were characterized using RAPD markers (Mondal *et al*., 2004). A dendrogram was constructed on the basis of band sharing which separated the population in to 3 clusters i.e. China, Assam and Ornamental type. The principal coordinate analysis revealed that the Chinese clones are more dispersed than Assam clones. Chen and Yamaguchi (2002) investigated the polymorphism, discrimination and molecular phylogeny of tea plant and its 23 related species and varieties by RAPD analysis. RAPD markers confirmed morphological classification and evolution of section *Thea* in genus *Camellia*. Chen and Yamaguchi (2002), also using RAPD marker, discriminated tea germplasm at the inter-specific level in China.

The diversity of 27 accessions comprising Korean, Japanese and Taiwanese tea was examined with RAPD markers (Kaundun *et al*., 2000). Out of the 50 primers screened, 17 primers generated 58 polymorphic and reproducible bands. A minimum of 3 primers was sufficient to distinguish the accessions. Diversity was greatest within the Korean group followed by Taiwanese and Japanese tea. The relatively high diversity of Korean tea might reflect the larger genetic base of its plantations while the low diversity of Japanese tea could be explained by the long and intensive tea selection programme. A dendrogram clustered the tea accessions into two main groups i.e., Taiwan cultivars on the one side and Korean along with Japanese accessions on the other side. This suggests that the Taiwanese tea studied here might have a different origin from that of Korean and Japanese tea (Kaundun *et al*., 2000).

Lai *et al*., (2001) assessed the genetic relationships in cultivated tea clones and native wild tea in Taiwan using RAPD markers. Three major groups could be recognized, i.e., cultivars of China tea and the cultivars developed in Taiwan from hybridization and

selection; Assam tea; and native Taiwanese wild tea. The native Taiwanese wild teas were most distant in the clustering tree. Previously genetic diversity of 15 tea cultivars grown in the National Germplasm Hangzhou Tea Garden was detected using also RAPD markers (Chen *et al*., 1998). It has been proved that Chinese tea cultivars possess high genetic diversity on DNA level.

Tanaka and Yamaguchi (1996) identified the parents of two Japanese tea cultivars, Yutakamidori and Meiryoku, for registration documents using RAPD marker. Wright *et al*. (1996) used the same technique to characterize 5 different South African tea cultivars. Liang *et al*. (2000) investigated the possibility of classification and identification of tea as well as closely related species using RAPD markers. The results showed that the RAPD markers could specifically discriminate between species and varieties. While both Assam and China tea had a specific band, Japanese tea was closer to Chinese tea than others. Some of the tea varieties from Vietnam were hybrids of Assam and China tea. Tanaka *et al*. (2001) used RAPD technique to identify the pollen parent of the popular Japanese green tea cultivar ‘Sayamakaori’. They have screened the female parent ‘Yabukita’ along with 78 putative male tea plants, most of which were introduced from China and concluded that pollen parent of ‘Sayamakaori’ was not present amongst the tested population.

RAPD analysis was applied to investigate genetic variability of *in vitro* raised tea trees which otherwise were morphologically indistinguishable (Mondal and Chand, 2002). Independently Devarumath *et al*., (2002) also reported that RAPD fingerprints are useful to evaluate genetic integrity of micro-propagated tea trees.

However, due to its dominant inheritance and limited degree of polymorphism, attention was given for alternative more advanced markers.

1.4.8 AFLP markers

Amplified fragment length polymorphism (AFLP) is a reliable and robust DNA marker (Vos *et al*., 1995) that can detect more polymorphisms than RAPDs. Thus AFLP markers offer an opportunity to perform detailed genetic studies in closely related materials (Meksem *et al*., 1995).

Paul *et al*. (1997) were the first to employ AFLP markers in tea to detect diversity and genetic differentiation of 32 clones from India and Kenya. Most of the diversity was detected within populations and Chinese types were more variable than Assam or

Cambod types [(1)]. The dendrogram identified the three known types, i.e. Assam, China and Cambod, which were generally consistent with the existing knowledge on the biosystematics of tea, the known pedigree of some of the genotypes and their geographical origin. Assam clones from India and Kenya clustered closely, indicating a common ancestry.

In the same year, Rajashekaran (1997) reported the AFLP analysis of 42 tea clones including 23 UPASI (United Planters' Association of Southern India) clones, 17 popular South Indian estate clones and 2 Kenyan tea clones. He concluded that 90% of the UPASI clones are inbred and thus unsuitable for commercial cultivation. AFLP could be utilized successfully for developing markers related to Blister Blight diseases resistance.

Later Balasaravanan *et al*. (2003) assessed the genetic diversity among 49 tea cultivars from South India. All these teas could be clearly distinguished into three distinct groups viz. Assam, China and Cambod as well as an intermediate but commonly grown Indian tea germplasm with narrow genetic diversity. Among the populations characterized, Chinese types showed a higher diversity than that of Assam types. Genetic distance between Assam and Cambod was larger than that between Assam and China types.

1.4.9 Microsatellite markers

Inter-simple sequence repeat (ISSR) has been used for genetic characterization of various plant species (Tsumara *et al*., 1996). Because of the great length of ISSR primers, they show great repeatability and stability of map position in the genome comparing genotypes (Zietkiewicz *et al*., 1994). Twenty-five diverse tea cultivars were analyzed using the simple sequence repeat anchored polymerase chain reaction (SSR-anchored PCR) or Inter SSR-PCR (ISSR). A dendrogram revealed three distinct clusters of Cambod, Assam and China type, which concurs with the known taxonomical classification of tea. These results suggest that the ISSR-PCR method can be used for genetic fingerprinting and taxonomic classification of tea genotypes (Mondal, 2002b).

Previously Lai *et al*. (2001) successfully assessed the genetic relationships in cultivated tea clones and native wild tea in Taiwan using ISSR markers. The results showed that Taiwanese native wild teas clustered closely with Assam teas then with China teas and the Taiwanese hybrid cultivars. Among three populations studied, the genetic diversity of the

[1] The taxonomic classification system used in Paul’s report differs from the one applied in this study.

native wild tea population was found to be the highest. The variance component within populations was larger than that between populations.

Beside that, ISSR markers were also used to investigate the genetic integrity of micro-propagated tea trees by analyzing their nuclear, mitochondrial and chloroplast genomes (Devarumath *et al*., 2002).

Highly polymorphic SSR markers were successfully developed and characterized in tea (Ueno *et al*., 1999; Freeman *et al*., 2004). However, as to my knowledge, there is no report on using SSR markers in tea. Ueno *et al*. (1999) developed microsatellite markers in *C. japonica* by screening RAPD profiles. Freeman *et al*. (2004) developed microsatellite primers by screening a microsatellite-enriched library which was constructed according to the enrichment protocol of Edwards *et al*. (1996). The optimal annealing temperature of SSR primers was tested as described in Haddrill *et al*. (2002).

1.4.10 Organelle DNA analysis

Because of the lower evolutionary changes of organelle DNA than nuclear DNA, chloroplast (cp DNA) and mitochondrial DNA (mt DNA) sequences have been widely used to investigate interspecific relationships (Jorgensen and Cluster, 1989; Waugh *et al*., 1990; Olmstead and Palmer, 1994). Chloroplast DNA encodes for many agronomically important genes (Kung, 1977; Bedrock and Kolodner, 1979; Nelson *et al*., 1980). Non-coding regions display higher rates of evolution than coding regions; hence former is a desirable target for phylogenic studies. The resolutions of many such non-coding regions have been amplified by the universal PCR primers (Taberlet *et al*., 1991; Demesure *et al*., 1995). However, the relatively high frequency of insertion/deletions may even, in some cases, make it possible to use the size of PCR product as a genetic marker. The choices of cp and mt DNA sequences that maximize phylogenic information however, depend upon the evolutionary time scale of the plant system.

Wachira *et al*. (1997) analyzed species introgression into cultivated gene pool of tea using 5 different organelle-specific primers. Out of them, 3 non-coding chloroplast regions as well as one mitochondrial region that amplified with universal primers did not reveal any polymorphism. Remaining one cp DNA specific PCR product revealed a single-strand conformation polymorphism (SSCP). This SSCP in the inter-genic spacer indicated that 4 species namely *C. furfuracea, C. assimilis, C. nokoensis* and *C. tsaii* shared a common

haplotype. This may indicate a possible hybridization between species of the sections involved.

Tribal and generic relationships within *Theaceae* were investigated using cladistic analyses of chloroplast-encoded *rbc*L and *mat*K flanking intergenic spacer region data (Prince and Parks, 2001). Parsimony analyses of separate and combined data consistently identified three strongly supported lineages: *Theeae*, *Stewartieae*, and *Gordonieae*. These data supported the broad generic circumscription of *Camellia* and *Stewartia* but did not support the recognition of *Gordonia sensu lato*. *Gordonia lasianthus* and *Gordonia brandegeei* are the basal clade in *Gordonieae*, a position far removed from all other representatives of *Gordonia sensu lato* (*Polyspora* and *Laplacea*) included in this study. The authors recognized all three major lineage at the tribal level, although there was weak statistical support for a sister relationship between *Gordonieae* and *Theeae*. Statistical support for the recognition of the two former subfamilies *Theoideae* and *Ternstroemioideae* as two separate families, *Theaceae* and *Ternstroemiaceae*, was also found.

Later the nucleotide sequences of ribosomal RNA maturase (*mat*K) regions in chloroplast DNA were determined on the native tea varieties of Japan, Korea, China, South East Asia, Sri Lanka and India to assess the genetic diversity (Katoh *et al*., 2003). The results suggested that the cultivated teas of India, Bangladesh, eastern China, Japan, and in the Southeast Asia region (Vietnam, Laos and Thailand) belonged to the group of *C. sinensis*. However, the native cultivars in Myanmar and southern China had a genetic similarity to *C. taliensis* and *C. irrawadiensis*. The native cultivars of Thailand and Vietnam were associated with morphologically close taxa.

1.5 Tea production and research in Vietnam

Vietnamese have a long-standing tradition and customs of drinking, processing and planting tea. Drinking tea is not only a pleasure but also a specifically cultural symbol of Vietnamese. The liking for drinking tea going along with the planting of tea was documented historically. Based on the environmental conditions as well as the historical and scientific documents, tea – a valuable perennial industrial crop in economy, nutrition, pharmaceutical materials, environment protection, and also socio-culture aspects – is growing well in Vietnam, especially at the highland and mountainous areas. There are many centuries old wild giant tea trees found in the high mountainous area such as Suoi Giang and Tua Chua which retains primitive characteristics and original qualities.

Djemukhadze (1981), after investigating the evolution of catechin compounds in wild and cultivated teas of Vietnam, China and India, suggested that tea originated from the jungle region of northwest Vietnam.

Nowadays, tea production has an important position in the economy of Vietnam; Vietnam tea products have been exported to more than 59 counties around the world; about 90% is exported to Iraq, Pakistan, Taiwan, Russia, India, Poland, Japan, Germany, United States and Belgium (Tran, 2003). In 2004, about 90% of the total production was exported, mainly as black tea. Among the main tea producing countries, the price of Vietnam exported tea is still very low, just about 1.0 US dollars per kg (FAO, 2006)

In the national plan for the development of the agricultural sector, tea is treated as the major industrial crop with an area of 110.000 ha and yielding 2.2 ton.ha^{-1} in 2010 propositionally (Table 1.5).

Since 1980, the harvested area (ha), production (ton) and dry yield (ton.ha^{-1}) of Vietnam tea is continuously increasing at a yearly mean rate of 4.37%, 7.28% and 2.48%, respectively (FAO, 2006). However, the average yield is still lower than that of the world (Table 1.4). The main statistical data of Vietnam tea production are presented in Figure 1.10 and Figure 1.11. The main projected figures for the Viet Nam tea industry up to 2010 cited from ‘The strategy for the development of Viet Nam Tea Industry to 2010’ are presented in Table 1.5.

Nowadays, commercial tea estates are mainly located in the mountainous, midland and plateau areas in 32 of the total of 61 provinces and cities of Viet Nam, of which Lam Dong province has the largest area of tea cultivation and is the main tea producing province of the South (Figure 1.12).

However, according to Nguyen (1998), during the process of development, Vietnam tea production system has exposed its limitation, mainly:

- Vietnam tea yield is still too low in comparison with other tea producing countries in the area.

- Vietnam tea quality in general is still low and unstable; type of Vietnam tea products is still not diversified because of the low and unstandardized quality of tea materials.

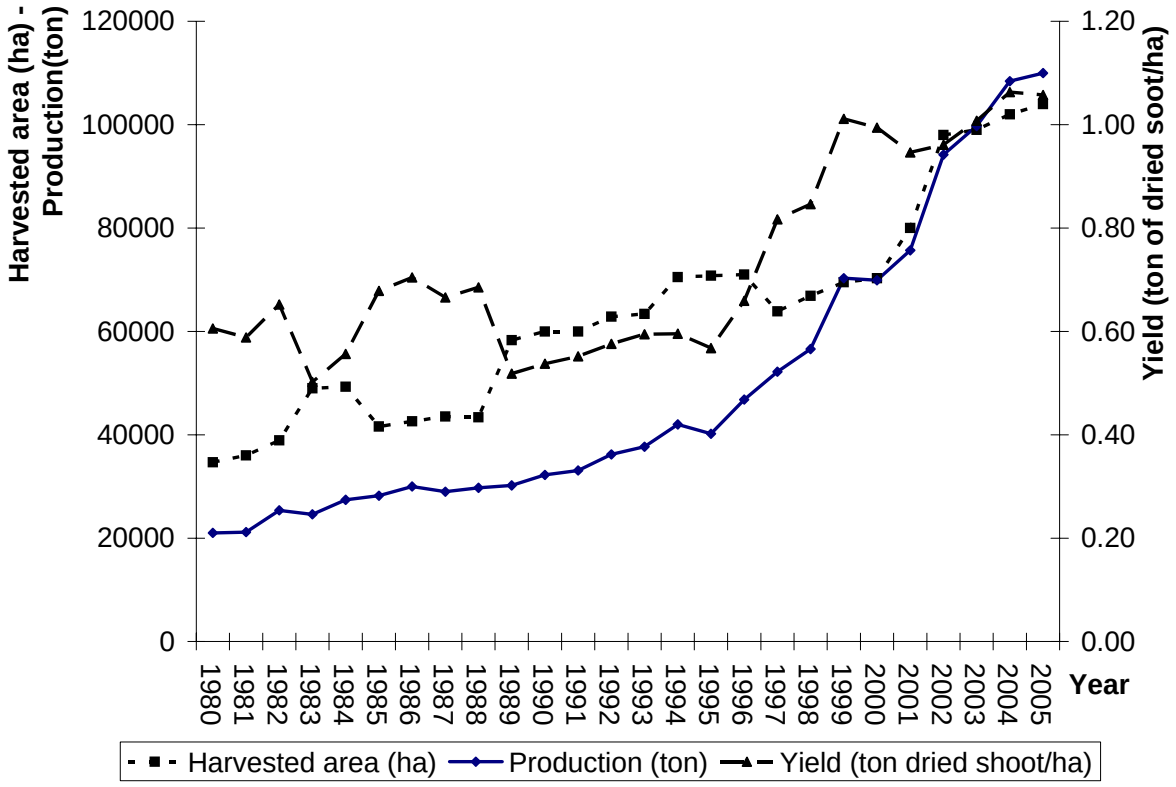


Figure 1.10: The fluctuation of harvested area (ha), production (ton), and dry yield (ton.ha^{-1}) of Vietnam tea production during 1980 and 2005

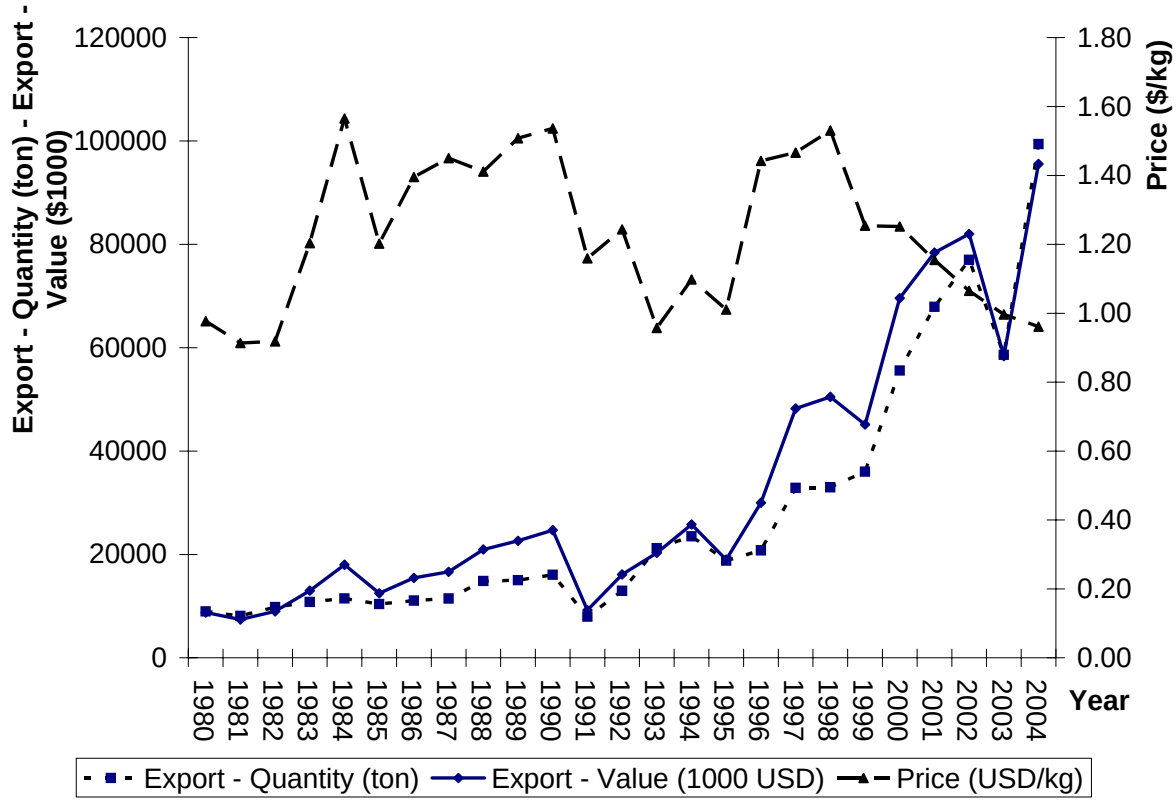


Figure 1.11: The fluctuation of the quantity (ton), value (thousand USD) and price (USD.kg^{-1}) of Vietnam exported tea during 1980 and 2004

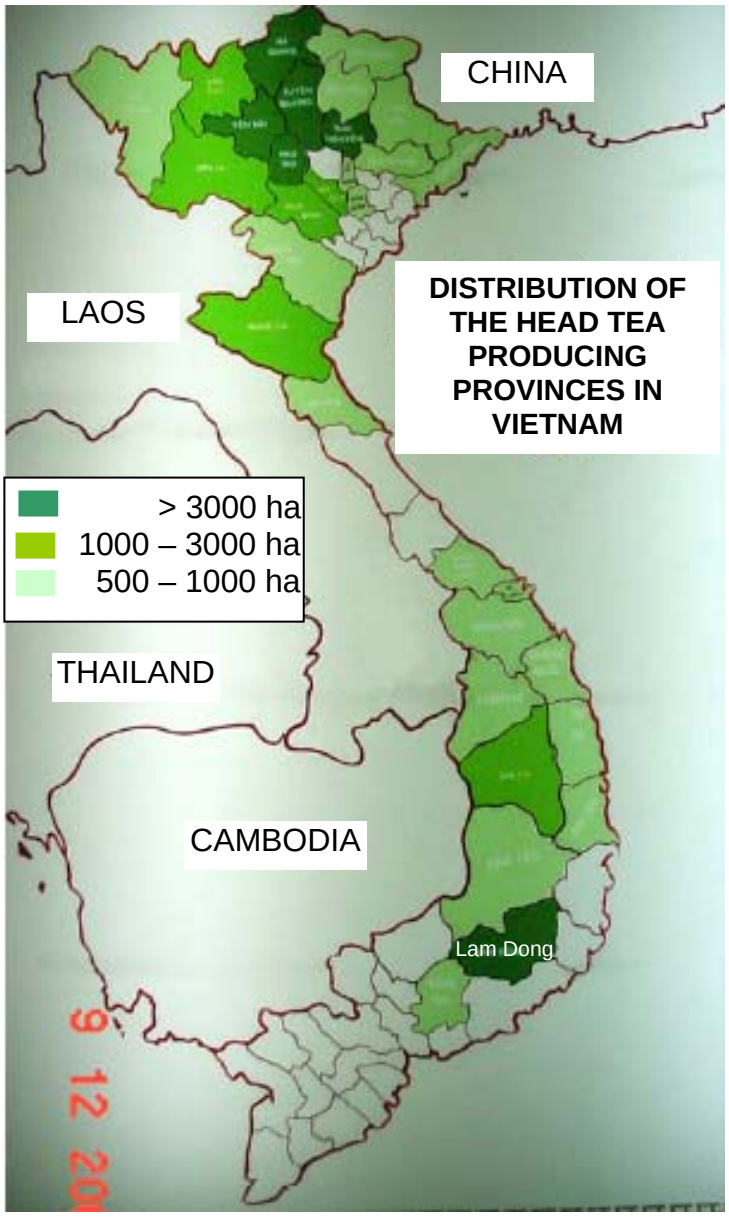


Figure 1.12: Distribution of commercial tea production at Vietnam. Provinces owing more than 3000 ha tea were in dark green; green was used to indicate the provinces having 1000 – 3000 ha tea; and the province cultivating from 500 to 1000 ha tea was in light green (courtesy by Tam Chau Company).

In order to solve these problems, many research has been done focusing on tea breeding, plantation techniques (nursery techniques, pruning techniques, tea protection, nutrition program and fertilization techniques, watering, farming system) and tea processing and biochemistry, which is not within the scope of this review.

According to Tran and Nguyen (1998), during 1988 and 1997, the tea germplasm was collected continuously and reached an amount of 94 ancestors, which was comprised of 37 ancestors of small-leaved Chinese tea (*C. sinensis* var. *microphylla*), 15 ancestors of large-leaved Chinese tea (var. *macrophylla*), 19 ancestors of Shan tea (var. *Shan*) and 23 ancestors of Assam tea (var. *Assamica*). Also during this time, many cultivars and clones imported from India, Sri Lanka, China, Taiwan have been tested for the growth, development, adaptation, disease resistance, yield and quality. PH_1 and 1A were clones selected from the Assamica population; similarly TH_3 was selected from the local midland tea populations. LDP_1 and LDP_2 are the progenies of PH_1 and Dai Bach Tra.

In order to produce seedling for organic tea production, Shan giant wild tea populations at the high mountainous area in the Northern of Vietnam were investigated (Do *et al*., 1998). Shan giant wild teas are distributed on the high remote mountainous areas at the altitude

above 800 m asl with high humidity, low temperature and misty nearly all year round. They can grow scattered or together in the virgin forest. Quality of Shan giant wild teas is good; the contents of soluble substances and tannin in flush shoot are high.

Table 1.5: Projected targets of Viet Nam tea industry up to 2010 (Viet Nam Tea Association, 1999)

Parameters	**2010**
- Total tea area (ha)	110000
- Average yield (Mt of dry shoot.ha-1)	2.20
- Production (dry, Mt)	147000
- Export . Quantity (Mt)	110000
. Value (million US dollars)	200

The hybridizations between Shan giant wild teas and Chinese teas were carried out and investigated (Nguyen and Nguyen, 2004). From 1997 to 2003, the morphological characteristics, growth, resistance, productivity and quality of all tea germplasm were observed. Hand pollination was done and F1 tea trees were assessed. The project is still continued to select new potential tea cultivars. Nguyen *et al*., (1998a, 1998b) review the methodology used in selecting and propagating tea under Vietnam conditions.

The polymorphism of 14 Shan and Trung du tea cultivars/clones at Vietnam Tea Research Institute was analyzed primarily using RADP markers (Nguyen *et a*l., 2004). They were highly diverse based on the morphological characteristics of leaf and shoot. From 82 RAPD primers screened, 9 primers (RA31, RA32, RA36, RA40, RA45; RA46, RA142, RA143 and RTA159) detected polymorphic bands. The results suggested that all investigated teas originated from the same area.

In condition of Lam Dong province, beside the imported Taiwanese tea clones (such as Kim Tuyen, Ngoc Thuy, Tu Quy, Thiet quan am) only specifying for processing oo long teas; TB_{14}, selected clone, well growing, is suitable for local production; its flush shoot can be used to make both green and black teas. LD_{97} grows well and yields high but the size of its flush shoot is rather big so that the appearance of its tea products is coarse. Similarly PH_1 has also high yield and quality but its cultivated area is limited because of the dark color of its tea products. LDP_1, developed from a cross breeding program, is the most promising potential new cultivars for local tea production. (Vo *et al*., 1998; Dong *et al*.,

1998; Chu and Vo, 2001; Pham and Vo, 2001; Vo *et al*., 2001; Nguyen and Vo, 2002; Tran and Vo, 2002; Ha and Vo, 2002; Dang *et al*., 2003; Ha and Vo, 2003; Hoang *et al*., 2005).

For further studies, suggestions for tea breeding research are (Doan and Do, 1998):

- Collecting, reserving and exploiting continuously tea germplasm, particularly focusing on the Vietnam Shan tea germplasm.

- Hybridizing and selecting new potential cultivars fulfilling the hard and strict requirements of the international market, i.e., not only high yielding and adapted to local conditions, but also with potential to produce high quality tea products. Selecting adaptive Shan tea clones from the mountainous giant wild tea populations will contribute effectively to the national plan in re-establishing 5 million hectares of forest to recover the spare hills and mountains. New clones for organic production also are of interest.

Chapter 2

Morphological diversity of tea grown in Lam Dong province (Vietnam)

Abstract

To build up a set of regionally adapted tea populations and clones, a clear understanding of tea genetic diversity is of critical importance. All tea taxa freely interbreed and therefore tea plants with many overlapping morphological, biochemical and physiological attributes are highly diverse and consequently their populations are very heterogeneous. Thirty-one tea accessions belonging to Chinese tea, Indian tea, Shan tea, which are either commercially planted or new promising selections, were morphologically described at Lam Dong province (Viet Nam) and assessed for their genetic diversity. Thirty-four morphological characteristics of stem, the 4th leaf, young shoot and flower were investigated qualitatively and quantitatively following the IPGRI's guidelines. Clusters were constructed using the unweighted pair group method with arithmetic average (UPGMA) based on Euclidean distances of 16 quantitative morphological data. Results showed the high diversity of Lam Dong tea. All tested accessions clustered into 4 groups and all known China, India and Shan teas were clearly separated in sub-groups. The results of this study generally meet with the reputed taxa currently accepted in local tea production.

2.1 Introduction

The tea tree (*Camellia sinensis* (L.) O. Kuntze) is an important cash crop widely cultivated in tropical and subtropical regions. Vietnam having a longstanding tradition and customs of growing and drinking tea is one of the main tea producers in the world; in 2005, the harvested area occupied 4.1% of the worldwide acreage, which is the sixth position. However its yield was still rather low, just approximately 79 % of the world average, and 38 %, 52 %, 64 % and 72 % of that of Malawi, Japan, India, and Sri Lanka, respectively (FAO, 2006). Therefore increasing the yield should raise effectively the economic value of Vietnam tea production.

Located in the Southern part of the West plateau, Lam Dong province contributes up to one third of the total cultivated area of Vietnam tea. Since the early time of its tea industry (around 1927) until the end of 1990s, there have been many tea types planted mainly

seed-grown tea plants which were not well selected. Using so-called unselected cultivars for a long time has been believed as the main reason of low productivity and quality of local tea production. At present, due to the limitation of the available cultivated land, Lam Dong tea industry tends to stabilize its area and continually increases productivity as well as quality by creating a set of locally well adapted tea cultivars or clones and improving intensive farming techniques (Lam Dong Center for Research and Transfer technology in Industrial crops and Fruit tree Production, 2004).

Tea is an allogamous plant and all tea taxa freely interbreed; therefore tea plants, with many overlapping morphological, biochemical and physiological attributes, are highly diverse and heterozygous (Banerjee, 1992). Because of the extreme hybridization, existence of the pure archetypes of tea is doubtful (Willson and Clifford, 1992). Nowadays the primary recommended propagation method is asexual propagation which could be the cause of further reduction in genetic diversity due to the spread of a few, vigorous, well-adapted clones with a capacity to produce high yield and good quality tea. Assessing the diversity in tea would provide the basic information for tea breeding in selecting suitable parents for hybridizing; creating an efficient and reliable set of locally adapted cultivars/clones as well as reducing the amount of accessions to be kept in germplasm collections.

Therefore, 31 tea accessions, which were commercially planted widespread or new promising selections in Lam Dong province, were assessed for the genetic diversity based on quantitative and qualitative characteristics.

2.2 Materials and Methods

2.2.1 Materials

Thirty-one tea accessions (Table 2.1), which are either cultivars commonly planted commercially at Lam Dong province or new promising clones, were morphologically described.

Clones No1, No3, No4, No5, No6, No7, No8, No9, No10, No11, No12, No13, No14, No15, No16, No17, No18, No19 and No21 are all promising clones which were selected and imported into Lam Dong from the North of Vietnam by the French since the early of Lam Dong tea industry, at around 1920s – 1950s. The morphological characteristics of most of

them are similar to shan tea. Unfortunately due to the war condition, all of the related documents were lost, so that their origins and classifications are unknown.

Yabukita clone (*C. sinensis* var. microphylla) is also a promising clone. It was selected from native Japanese tea plants for green tea, and is one of the well known clones in Japanese Tea Industry, covers over 60% of the total clonal tea area of Japan (Yamamoto *et al*., 1997). Yabukita was imported into Lam Dong in 1988 solely to produce special exported Japanese green tea. Small shoot, very little pubescence, high quality, and a fresh yield of 4 – 5 Mt ha^{-1} are some main characteristics of this clone (Vo *et al*., 2001).

Ngoc Thuy, Kim Tuyen and Tu Quy clones (*C. sinensis* var. microphylla) were imported from Taiwan into Lam Dong in 1989 and solely planted on joined-venture farms, following the specific horticultural techniques and managements, quite different from local techniques. The harvest was done at 45 – 55 day intervals. Its small shoots with a little pubescence are used to process special oolong tea. The fresh yield was rather low, just about 5 Mt ha^{-1} in highly intensive conditions (Vo *et al*., 2001).

PH_1 clone (*C. sinensis* var. assamica) was selected from the population of Manipur (India tea) since 1965 at Vietnam Tea Institute and was introduced to Lam Dong in 1980. This clone grows vigorously and yields on average over 15 – 25 Mt ha^{-1} of fresh shoot per ha. Its quality is only medium. No pubescence was observed on its shoot. This clone is suited for processing exported black tea (Vo *et al*., 2001).

TB_{11}, TB_{14} and TB_{14}CD clones (*C. sinensis* var. shan) originated from the collection of Shan Tran Ninh and were individually selected from a group of 6 cultivars since the 1960s at Lam Dong. The average fresh yield of TB_{14} has been reported to be over 15 Mt shoot ha^{-1} under intensive cultivation (Vo *et al*., 2001). The shoot appearance is attractive with densely white pubescence and is favored by local and international markets. These clones are particularly adapted to the natural conditions of Lam Dong province; thus, they have been widely cultivated locally.

LD_{97} clone (*C. sinensis* var. shan) was also individually selected from the population of local shan collection from 1993 – 1997 at Lam Dong. Its big shoots with white dense pubescence are high in quality and suitable for green tea manufacturing. On intensive farms, a fresh yield was reported at over 15 Mt shoot ha^{-1} was reported (Vo *et al*., 2001). LD_{97} clone is easily planted on various types of soil, and is especially suited for infilling practices due to its vigorous growth.

Table 2.1: Main information of investigated tea cultivars/clones. The taxonomic classification used was based on Cohen-Stuart (1919) (cf. Yamamoto et al., 1997)

Cultivars	Planting materials	Year of cultivation	Location	Recently reputed taxa	Recent situation
No1	Stump	1958	Buffalo farm	unknown	Promising clone
No 3	Stump	1958	Buffalo farm	unknown	Promising clone
No 4	Stump	1958	Buffalo farm	unknown	Promising clone
No 5	Stump	1958	Buffalo farm	unknown	Promising clone
No 6	Stump	1958	Buffalo farm	unknown	Promising clone
No 7	Stump	1958	Buffalo farm	unknown	Promising clone
No 8	Stump	1958	Buffalo farm	unknown	Promising clone
No 9	Stump	1958	Buffalo farm	unknown	Promising clone
No 10	Stump	1958	Buffalo farm	unknown	Promising clone
No 11	Stump	1968	Bao Loc High school(1)	unknown	Promising clone
No 12	Stump	1968	Bao Loc High school	unknown	Promising clone
No 13	Stump	1968	Bao Loc High school	unknown	Promising clone
No 14	Stump	1968	Bao Loc High school	unknown	Promising clone
No 15	Stump	1968	Bao Loc High school	unknown	Promising clone
No 16	Stump	1968	Bao Loc High school	unknown	Promising clone
No 17	Stump	1968	Bao Loc High school	unknown	Promising clone
No 18	Stump	1968	Bao Loc High school	unknown	Promising clone
No 19	Stump	1968	Bao Loc High school	unknown	Promising clone
No 21	Stump	1968	Bao Loc High school	unknown	Promising clone

Table 2.1: Main information of investigated tea cultivars/clones. The taxonomic classification used was based on Cohen-Stuart (1919) (cf. Yamamoto et al., 1997) (cont.)

Cultivars	Planting materials	Year of cultivation	Location	Recently reputed taxa	Recent situation
Yabukita	Cutting	1997	Lam Dong Center[(2)]	*C. sinensis* var. microphylla	Promising clone
LDP_2	Cutting	1997	Lam Dong Center	Hybrid - *C. sinensis*	Promising cultivars
LDP_1	Cutting	1997	Lam Dong Center	Hybrid - *C. sinensis*	Promising cultivars
LD_{97}	Cutting	1997	Lam Dong Center	*C. sinensis* var. Shan	Commercially cultivated
PH_1	Cutting	1997	Lam Dong Center	*C. sinensis* var. assamica	Promising clone
TB_{14}	Cutting	1997	Lam Dong Center	*C. sinensis* var. Shan	Commercially cultivated
TB_{11}	Cutting	1996	Buffalo farm	*C. sinensis* var. Shan	Promising clone
Ngoc Thuy	Cutting	1996	Dramri – Bao Loc	*C. sinensis* var. microphylla	Commercially cultivated
Kim Tuyen	Cutting	1996	Dramri – Bao Loc	*C. sinensis* var. microphylla	Commercially cultivated
Tu Quy	Cutting	1996	Dramri – Bao Loc	*C. sinensis* var. microphylla	Commercially cultivated
Shan tuyet	Seed	1927	Cau Dat – Da Lat	*C. sinensis* var. Shan	Promising clone
$TB_{14}CD$	Cutting	1998	Cau Dat – Da Lat	*C. sinensis* var. Shan	Commercially cultivated

[(1)] Bao Loc Technical and Vocational High school (so-called Bao Loc High school)

[(2)] Lam Dong Center for Research and Transfer techniques on Industrial crops and Fruit tree Production (so-called Lam Dong Center)

LDP_1 and LDP_2 cultivars (*C. sinensis*) are the crosses between the mother, Dai Bach tra (Chinese tea, well known as very high quality, specific natural aroma, but low yielding cultivar), and the father, PH_1 (India tea, known as high yield, but just intermediate in quality). Yearly, the growth of its shoots reportedly starts early and stops late, thus the amount of harvesting time is increased. The shoot size was rather small. On eight-year old farms, the average fresh yield was 9 – 10 Mt shoot ha^{-1} (Tran and Nguyen, 1998, Vo *et al*., 2003)

2.2.2 Study area

Location

Lam Dong province, located at latitude 11°32N and longitude 107°50E, occupies the south of the Western highland stretching from the Middle of Viet Nam. Its altitude ranges from 350 to 1500 m above sea level.

Climatic conditions

The climate of Lam Dong province, belonging to the highland tropical monsoon, is relative stable (Table A2.1) and is largely influenced by either Lam Dong‘s geography and altitude, as well as the topography of Truong Son range due to its obstruction of wind. There are two contrary seasons: the rainy season (from November to March of the next year) and the dry season (from April to September). Mist often appears in the morning from April to October, about 10 – 15 days per month. Temperature mainly decreases following the altitude. The yearly temperature amplitude at only around 4°C is not noticeable. Winter is reportedly warm. The daily temperature amplitude is very high; its average is 10 – 11°C, even up to 15 – 16°C, especially in the dry season. The averages of the mean, maximal and minimal temperature yearly are 20.5°C, 29.0°C and 14.9°C respectively.

Based on the mean value of the past ten years (from 1993 to 2003), the climatic conditions of Lam Dong province generally meets the needs for tea growth (Figure 2.1).

Geology and soil

Lam Dong soil belongs to basalt category and tea plants are mainly cultivated on 4 types of feralit soils: brown-yellow, brown-red, red- yellow and yellow soil.

Generally, the conditions of soil, rainfall and humidity at Lam Dong province are favorable for high-yielding teas, while the topography, altitude and fog create a suitable environment for good quality teas.

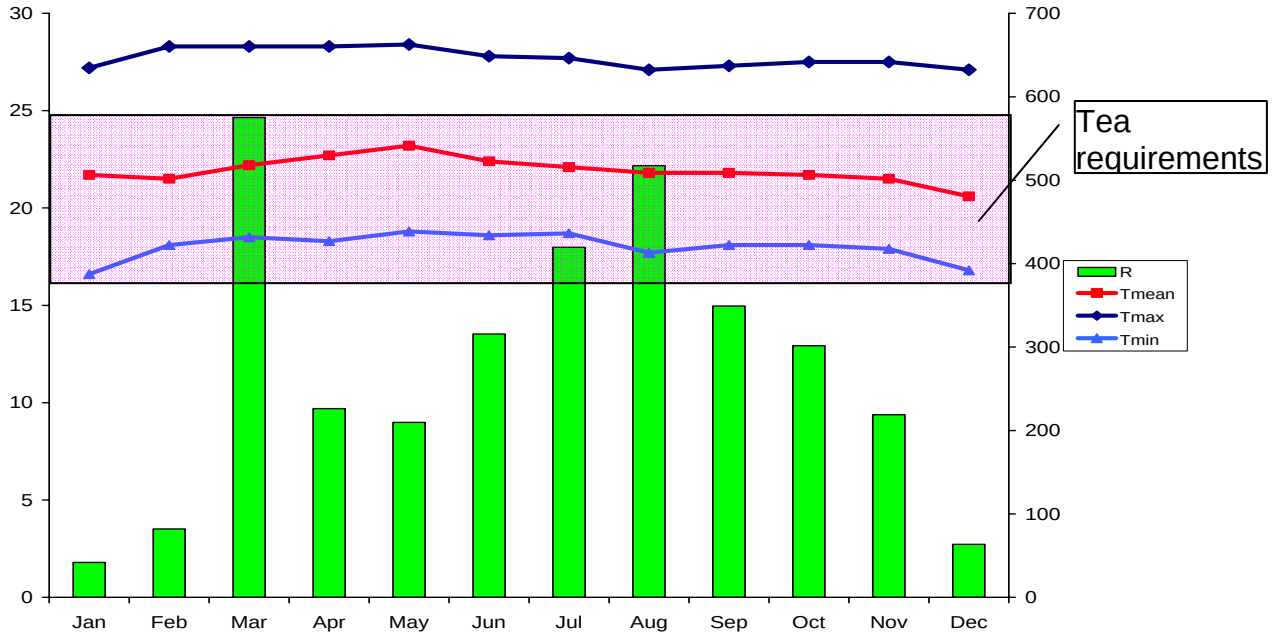


Figure 2.1: Average temperatures and rainfall at Lam Dong province from 1993 to 2003 in comparison with tea requirements (Source: Weather station of Bao Loc Technical and Vocational High school, 2004)

2.2.3 Methods

Thirty three important quantitative and qualitative characteristics of stem, the 4th leaf, flush shoot (P+2) and flower were investigated from the currently harvested tea trees following IPGRI's guidelines (IPGRI, 1997) with some minor adjustments. Tannin content in (P+2) shoots was analyzed at Center for Technology and Management of Resources and Environment, Nong Lam University.

The characteristics of the stem

Stems were described by the following characteristics:

- The stem circumference (cm): measured at 10 cm above ground level (Figure 2.2).

- The height of the first branching position (cm): measured from ground level to the first branching position (Figure 2.3).

Figure 2.2: Measuring the stem circumference at 10 cm above ground level

Figure 2.3: Measuring the branching position from the ground level to the position of the 1st branchlets

- The branch angle (degrees): angle of the first branchlets to the main stem.

- Branching capability: coded as 1- high, 2- mediate and 3- rare.

- The height of plucking surface (cm): measured from ground level to the plucking surface (Figure 2.4).

Figure 2.4: Measuring the height of the plucking surface from the ground level to the surface

- The leaf breadth (cm): measured at the widest position of the lamina.

- The leaf length/breadth ratio: calculated from the data of the leaf length and breadth.

- The length and the breadth of plucking surface (cm).

The characteristics of the 4th leaf

The 4th leaves counted from the bud were exploited by the following characteristics:

- The leaf length (cm): measured from the leaf base to the tip.

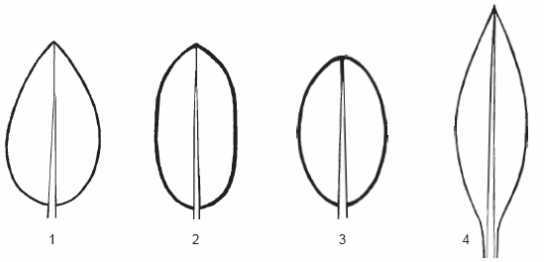


Figure 2.5: Leaf shape (reproduced from IPGRI, 1997)

- The leaf shape: coded as 1- ovate, 2- oblong, 3- elliptic, 4- lancelate and 5- other (Figure 2.5).

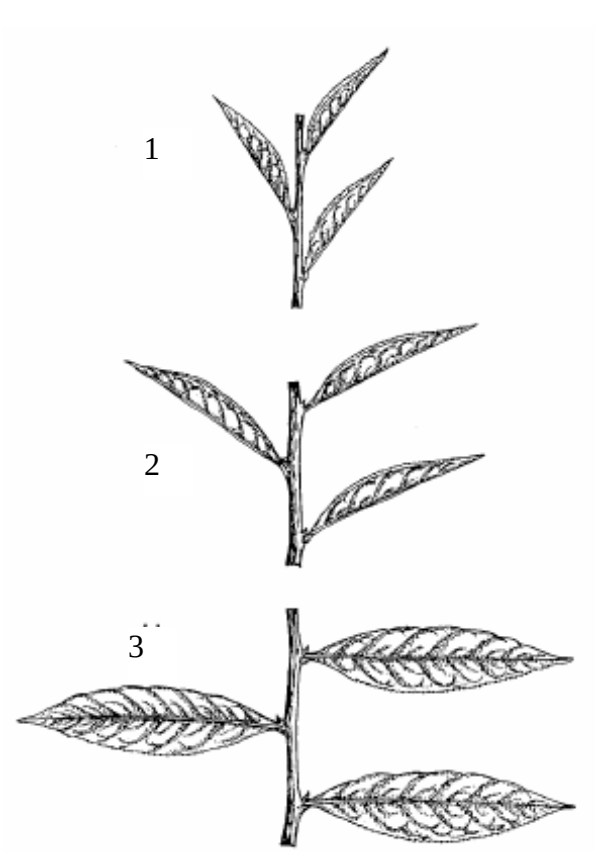


Figure 2.6: Major types of leaf pose of tea plants (reproduced from Banerjee, 1987).

- The leaf angle (degrees): the angle formed between the branchlets and the lamina.

- The leaf pose: coded as 1- erect (<35°), 2- semi-erect (35° - 75°), 3- horizontal (76° - 90°) and 4- drooping (>90°) (Figure 2.6).

- The leaf color: coded as 1- light green, 2- green, 3- grayed-green, 4- grayed-yellow, 5- yellow-green and 6- other.

- The number of pair of main vein on leaf surface: counted the amount of pair of veins uniting into a line near the leaf edge.

- The number of pair of serrulation on leaf margin: counted the number of pair of serrulation on the leaf edge.

- The serrula form: coded as 1- regularly acute, 2- regularly blunt, 3- irregularly acute and 4- irregularly blunt.

- The length of leaf petiole (cm): measured from the branchlets to the leaf base.

- The length from leaf petiole to first serration (cm): measured from the leaf base to the first serration.

- The leaf base shape: coded as 1- attenuate, 2- rounded, 3- blunt and 99- other (Figure 2.7).

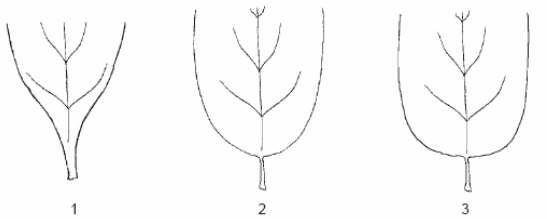


Figure 2.7: Leaf base shape (reproduced from IPGRI, 1997)

The characteristics of flush shoot

The flush shoots were harvested by plucking at the position of 2/3 of the internode between the 2nd and the 3rd leaf from the 2nd leaf and described by the following characteristics:

- The pubescence density on bud: coded as 1- glabrous to rare, 2 – light pubescent indumentums, and 3- dense pubescent layer.

- The pubescence density on lower surface of 1st leaf: coded as 1- glabrous leaves with only hair on the mid-rib, 2- leaves with a few scattered hairs on the lamina near mid-rib, 3- pubescence extends about half-way to the margin, 4- leaves with entire under surface of lamina pubescent and 5- leaves where pubescence forms a dense indumentums.

- The shoot color: coded as 1- light green, 2- green, 3- purplish green and 4- purplish.

- The flush shoot length (cm): measured from the plucked position to the tip of the bud.

- The fresh and dry shoot weight (g): measured the weight of 100 flush shoots after harvesting and after drying, then divided by 100.

- The fresh/dry ratio: calculated from the data of fresh and dry shoot weight.

- Tannin content (% DW): analyzed following the conventional method at the Center for Technology and Management of Resources and Environment, Nong Lam University (Vo *et al*., 2001) (done by the technicians).

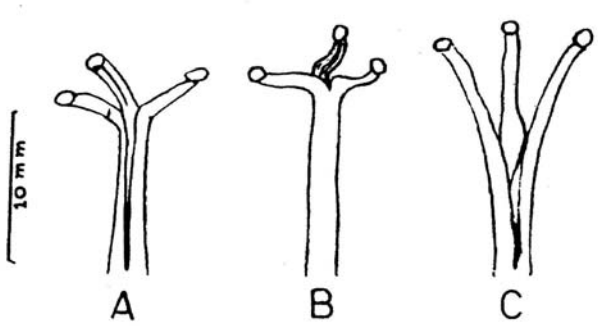


Figure 2.8: Different forms of styles in tea. A- *C. sinensis*; B- *C. assamica* ssp. Assamica; C- *C. assamica* ssp. Lasiocalyx (reproduced from Banerjee, 1992)

The characteristics of flower

Flowers were described as follows:

- The color: coded as 1- white, 2- cream, 3- white with red purple (pinkish) tinge, 4- purple (pink) to purple-violet and 5- other.

- The number of petals.

- The splitting of style: coded as 1- geniculation (free for greater part of their length) (Figure 2.8C), 2- ascending (free for about half their length) (Figure 2.8A) and 3- united for greater part of the length, the free part short, more or less horizontal (terminal) (Figure 2.8B).

- The flowering capability: coded as 3- low, 5- intermediate and 7- high.

- The flowering position: on which branchlets level the flower was generated.

2.2.4 Statistical analysis

Data presented in this report are the mean of observed values with the standard deviations.

To construct a dendrogram representing the relationship among cultivars and clones, the data were first standardized; then the accessions were grouped by hierarchical cluster analysis using the unweighted pair group method with arithmetic average (UPGMA) based on the dissimilarity matrix of Euclidean distances coefficient of 16 investigated quantitative morphological data i.e., the stem circumference (cm), the branching position (cm), the branching angle (degrees), the shoot length (cm), the fresh shoot weight (g), the dry shoot weight (g), the tannin content (% DW), the leaf angle (degrees), the leaf length (cm), the leaf breadth (cm), the number of pair of main vein on the lamina, the number of serrulation on the leaf edge, the petiole length (cm), the length from the petiole to the 1^{st} serration (cm), the number of petal and on which branch level the flower formed.

The principal co-ordinates analysis (PCoA) was also done based on the Euclidean distances coefficient to reveal the relationships among tested accessions.

All the above statistical analyses were performed by using NTSYS 2.1 software (Rohlf, 2000).

The effect of environmental conditions (weather, soil, and even farm techniques applied) onto the morphological characteristics was not considered and interpreted due to the rather homogeneity and stability of the local climatic conditions and the similarity of farming techniques applied. Most of investigated teas were cared and harvested following the official procedure of Lam Dong Center for Research and Transfer Techniques on Industrial Crops and Fruit Tree Production (so-called Lam Dong Center), except for the cultivars of Kim Tuyen, Ngoc Thuy and Tu Quy which were planted according to the specific Taiwanese techniques (for producing oo long teas); besides Shan Tuyet clone at Cau Dat grows freely without pruning.

2.3 Results and discussions

To reveal the genetic diversity of studied tea accessions, all collected data on morphological characteristics were analyzed and presented in Table A2.2, Table A2.3, Table A2.4, Table A2.5 and Table A2.6; and summarized in Table 2.2 below.

2.3.1 Describing the stem

The observed stem characteristics showed a very wide variation between accessions, but rather small variation among measurement within each accession (Table 2.2). Age of studied tea accessions (or the year of cultivation, Table 2.1) and the yearly pruning methods obviously influence the stem circumference as well as stem height. Shan Tuyet (St) clone was planted in 1927 and recently grows freely; hence its stem circumference and height were the highest among the whole investigated tea cultivars and clones.

On the other hand, plant density was strongly influenced by the size of plucking surface: most of investigated teas had the length of plucking surface “smaller than” the breadth except for teas which were planted with low density at Bao Loc High school (No1 to No21) because the development of the canopies was limited by the distances between row and row as well as from tree to tree. Except for St clone, all the other tested teas were under commercially cultivated condition, so that the variation within each accessions was rather low.

Scatter analyze of the branching position separated all tested tea accessions into two groups, i.e., low branching position teas (No1, No3, No7, No10, No15, No16, No18, No19, St and TB_{11}) and high branching position teas (No4, No5, No6, No8, No9, No11, No12, No13, No14, No17, No21, KT, LD_{97}, LDP_1, LDP_2, NT, PH_1, TB_{14}, $TB_{14}CD$, TQ and Yabukita) (Figure 2.9, Figure 2.10 and Figure 2.11).

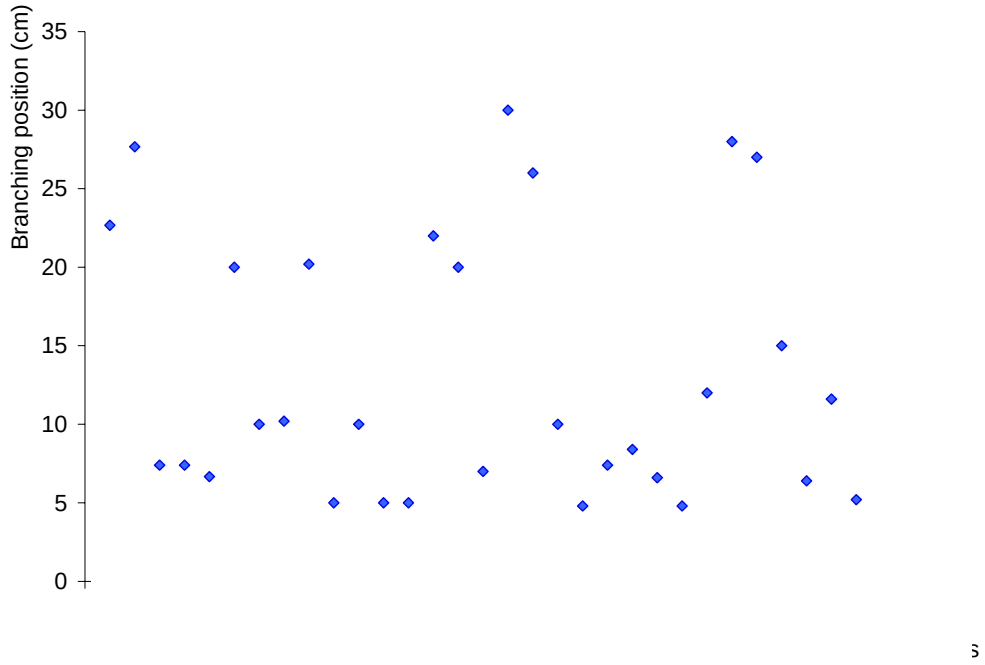


Figure 2.9: Scatter diagram for branching position clearly separates investigated teas into two groups: low and high branching positions.

Figure 2.10: Tea tree branches near the ground level (low branching position)

Figure 2.11: Tea tree branches at high position from the ground surface

In general, stem characteristics were strongly controlled by the cultivation techniques applied on the farm; therefore these characteristics were mainly used to describe the studied teas but not involved in the cluster analysis.

2.3.2 Describing the 4th leaf

Figure 2.12: The appearance of tea leaves on tea shoot.

Leaf morphology (Figure 2.12, Figure 2.13, Figure 2.14, Figure 2.15, Figure 2.16, Figure 2.17, Figure 2.18, Figure 2.19 and Figure 2.20) is traditionally an important basis for tea taxonomy. Such characteristics as the ratio of leaf length to leaf width, angle between leaf tip and axis, leaf size, and petiole length are generally used in assigning taxonomic categories (Banerjee, 1992).

The characteristics of leaf and floral morphology, and growth habit are the more important criteria used by Sealy (1958) in assigning taxonomic categories within *Camellia* (Banerjee, 1992).

Leaf characteristics of studied teas also showed a large variation between accessions (Table 2.2). Leaves were categorized into medium, large and extra large leaves (Table A2.3).

Table 2.2: Variability in vegetative and reproductive characteristics of tea accessions at Lam Dong province

Characteristics	Range of variation (with standard deviation within clones)
Stem	
Stem circumference (cm)	18.8 (1.9) – 100.8 (3.7)
Height of the first branching position (cm)	4.8 (0.8) – 30.0
Branch angle (degree)	7.0 (2.0) – 70.6 (3.8)
Height of plucking surface (cm)	68.6 (2.6) – 135.0 (Greatly depending on the cultivation techniques)
Length of plucking surface (cm)	58.4 (2.7) – 320.0
Breadth of plucking surface (cm)	70.0 (7.9) – 200.0
Leaf	
Leaf length (cm)	5.9 (0.6) – 13.3 (0.6)
Leaf breadth (cm)	2.6 (0.2) – 5.7 (0.3)
Leaf length/breadth ratio	1.92 – 3.33
Leaf shape	Ovate – lancelate
Leaf angle (degree)	47.6 (3.2) – 84.3 (2.2)
Leaf pose	Semi-erect – horizontal
Leaf color	Light green – purplish
Number of pair of main vein on leaf surface	5.6 (0.5) – 12.1 (1.0)
Number of pair of serrulation on leaf margin	23.9 (1.5) – 64.7 (2.4)
Serrula form	Regularly acute – irregularly blunt
Length of leaf petiole (cm)	0.18 (0.03) – 0.57 (0.08)
Length from leaf petiole to 1st serrula (cm)	1.4 (0.1) – 2.7 (0.2)
Leaf base shape	Attenuate – rounded
Shoot	
Pubescence density on bud	Nil - dense
Pubescence density on lower surface of 1st leaf	Glabrous leaves with only hair on the mid-rib – leaves where pubescence forms a dense indumentums
Shoot color	Light green – purplish
Shoot length (cm)	5.6 (0.4) – 13.1 (0.6)
Fresh shoot weight (g)	0.33 – 1.10
Dry shoot weight (g)	0.07 – 0.26
Fresh/Dry ratio	3.0 – 5.8
Tannins content (% DW)	**15.23 – 28.56**
Flower	
Flower color	White
Number of petal	4 – 6
Style	3-equal splitting styles – 3-unequal splitting styles
Flowering ability	Few – many

Traditionally small-leaved teas were believed to be low yielding hence they were not planted both on the farm and in the collections which were mostly established in 1950s and 1960s. Similarly, based on the characteristics of leaf shape, leaf color, leaf base, leaf pose and serrula form (Table A2.3 and Table A2.4) all studied teas could be subdivided

into groups. Noticeably none of investigated teas possessed the erect or drooping leaf pose, perhaps because semi-erect and horizontal leaf pose were thought to be advantageous for photosynthetic activity.

Despite marked variation, leaf feature continue to be the basic of classification proposed by Kitamura (1950) and Sealy (1958). According to these authors leaf angle of China tea is less than 50^o, while that of Assam tea is higher than 70^o, Using this classification, only clone No5 and the hybrid LDP_1 belong to China tea; and clone No9, No11, No12, No15, No17, TB_{11} and TB_{14} belong to Assam tea. In fact, currently LDP_1 was known as the hybrid between China and Assam teas, whereas TB_{11} and TB_{14} were selected from Shan collection.

Figure 2.13 : Regularly acute serrulation

Figure 2.14 : Irregularly acute serrulation

Figure 2.15 : Regularly blunt serrulation

Figure 2.16 : Irregularly blunt serrulation

Yamaguchi *et al.*, (1999) summarized that leaf size is gradually shortened from var. *assamica*, China var. *Sinensis* to Japanese and Korean var. *sinensis*.

Related to the quality, leaf color is considered. The light-leaved varieties produce the better quality than the dark-leaves ones, and the optimum ‘greenness’ is essential for

highest quality (Banerjee, 1992). From this point of view, with the light green- and green-leaves, most of studied teas (30 accessions, including all commercially cultivated teas) could have a good quality.

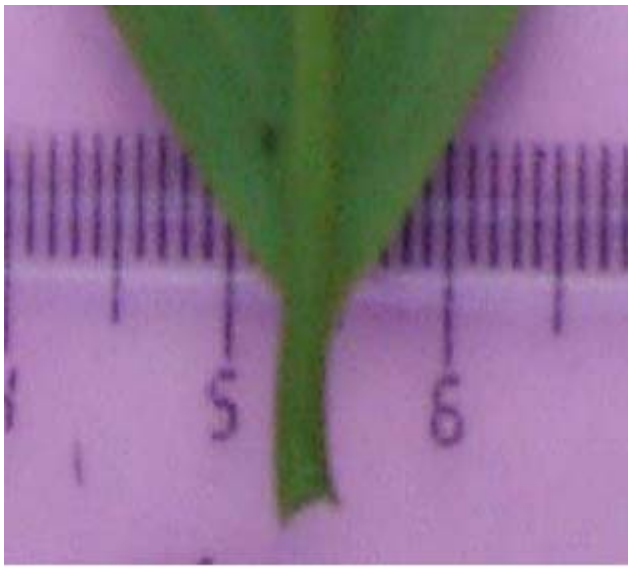


Figure 2.17: Long petiole

Figure 2.18: Short petiole

Figure 2.19: Rounded leaf base

Figure 2.20: Attenuate leaf base

2.3.3 Describing the flush shoot

Flush shoot (Figure 2.21) is the harvested part from tea plant so that the characteristics of flush shoot could imply the productivity and especially the quality of tea. Large variation was observed (Table 2.2). The pubescence density on buds and the lower surface of the 1st leaves were estimated qualitatively (Table A2.5). Among studied teas, only 8 accessions (i.e., No1, No4, No7, No8, LDP_1, LDP_2, PH_1 and Tu Quy) were characterized by glabrous to rare pubescent bud; some of them are commercial cultivated (Tu Quy), or potential cultivars (LDP_1, LDP_2). The rest was identified with light or dense pubescent layer on the bud. Likewise, the lower surface of the 1st leaf of 17 teas was covered by an

indumentums of different density of pubescence (coded as number 3 to 5) (Table A2.6); 14 accessions were characterized by the glabrous 1st leaves or leaves with a few scattered hairs on the lower surface.

Figure 2.21: Tea shoot

According to the literature, the pubescent types produce better teas than glabrous ones irrespective of leaf color (Wight and Barua, 1954). In theory, only Shan teas are characterized by dense pubescence on their buds and lower surface of the 1st leaves (Yamamoto *et al*., 1997). However, due to the free crossing among tea taxa, nowadays many tea clones and cultivars also have pubescence at various levels on buds and lower leaf surface.

As with leaf color, most of flush shoots were in light green and green, only shoot of clone No15 colored purplish; and that of LDP_2, Ngoc Thuy, Tu Quy and Yabukita was in purplish green.

The quantitative traits such as the shoot length (cm) and shoot weight (g) (both fresh and dry) of observed teas were also reported (Table A2.6) and were included in the UPGMA cluster analysis.

Tannin content (% DW) of flush shoot of investigated teas was rather low (15.2 – 28.6%) in comparison with the results in other studies because samples were taken at the end of the year when the tannin content in young shoots is physiologically low.

2.3.4 Describing the flower

Unlike vegetative parts, the reproductive characteristics are more discrete and show relatively less variation; therefore they provide more reliable diagnostic criteria (Banerjee, 1992).

Figure 2.22: Tea flower

All the studied teas were described by white flower formed on 3rd or 4th level of branchlets (Table 2.2, Table A2.6). The flower (Figure 2.22) of most of observed cultivars/clones had five petals, except for that of clone No 13 (six petals), or that of clone No3, No6, No7, No9, No15, No19, LD_{97} and Yabukita (only four petals). Flowering capability of 17 cultivars/clones was scored as low; that of the rest 14 ones was medium. Among the commercially cultivated or promising teas, clone Kim Tuyen, Ngoc Thuy, Tu Quy and Yabukita showed their medium flowering capability; while clone LD_{97}, hybrid LDP_1, LDP_2, clone PH_1, TB_{11}, TB_{14} and TB_{14}CD had low flowering capability. Naturally, tea flowering capability is very high, especially in China tea. However in order to yield high flush shoot (vegetative part) selection was done in the direction of lowering flowering capability.

One important characteristics of tea flower is the splitting of the style (Figure 2.23, Figure 2.24, Figure 2.25 and Figure 2.26). The style consists of three parts which are united for varying length into a column. The style is of taxonomic significance and could be ascending, geniculate or terminal. In this study, none of teas possesses the geniculate style; ascending style was observed in clone No1, No2, No6, No8, No9, No11, No14, No17, No19, No21, Kim Tuyen, LDP_1, LDP_2, Shan tuyet, TB_{11}, TB_{14}, TB_{14}CD and Tu Quy; and the terminal one was recorded in the rest.

Characteristics of style can be used to analyze the genetic similarity of tea collections (Yamaguchi *et al*., 1999) as well as to differentiate between species and sub-species (Wight, 1962).

2.3.5 Assessing the genetic diversity of investigated teas at Lam Dong

The genetic diversity of teas grown at Lam Dong province was revealed using the morphological characteristics via the UPGMA-derived dendrogram (Figure 2.27) and the diagram generated from the first two principal co-ordinates (PCo) (Figure 2.28)

Euclidean distances coefficient values of tested accessions were expressed in dissimilarity matrix table (Table A2.7) and used to construct UPGMA cluster dendrogram (Figure 2.27) and PCoA (Figure 2.28). The accessions exhibited large variation; estimated dissimilarity varied from 2.05, between the most similar accessions Kim Tuyen and Ngoc Thuy, to 8.65, between the most distant accessions Kim Tuyen and No16.

Figure 2.23: Geniculated style of clone Kim Tuyen

Figure 2.24: United style of clone PH_1

Figure 2.25: United style of clone Tu Quy

Figure 2.26: Ascended style of Shan tuyet accession

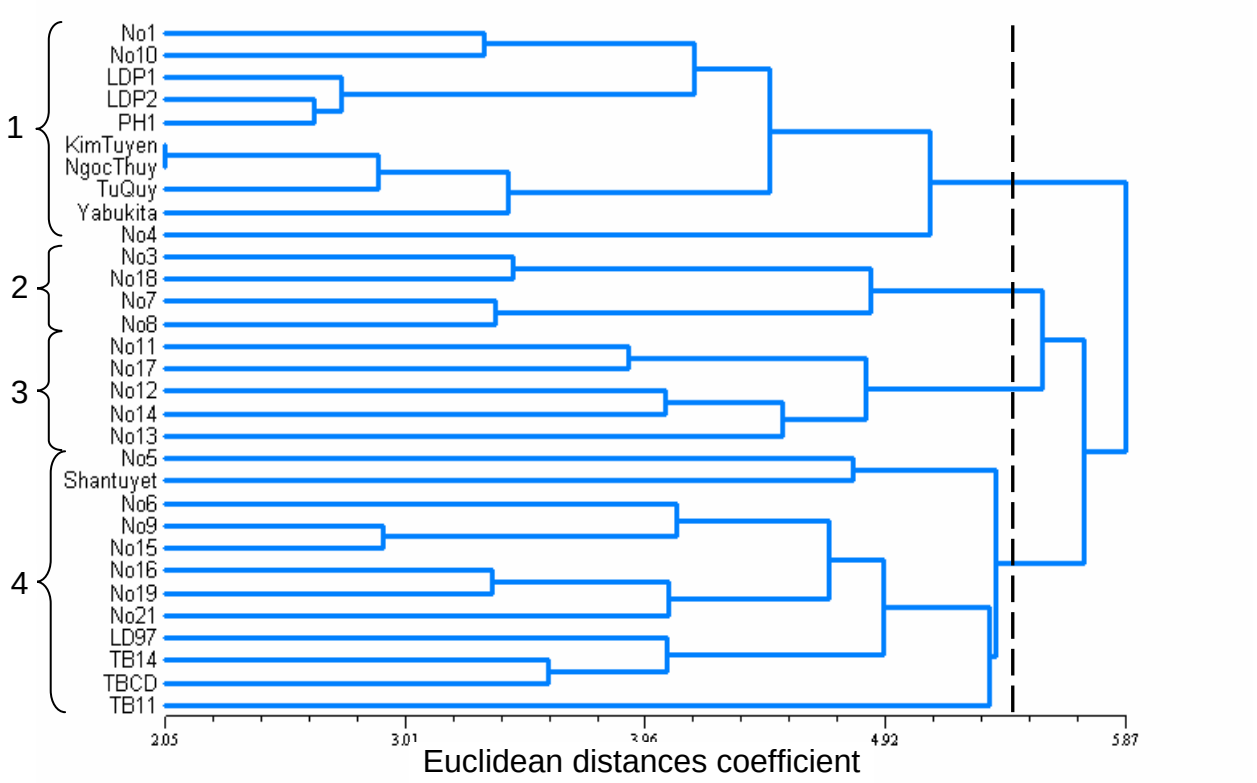


Figure 2.27: UPGMA-derived dendrogram illustrating the relationship among 31 observed teas. All commercially cultivated and promising tea accessions gathered into cluster 1 and 4.

Although the range of Euclidean distances was rather large, the dendrogram failed to show a major grouping. At the value of about 5.40 of Euclidean distance coefficient, all tested accessions were divided into 4 groups (Figure 2.27). The results met the conventional classification system used in Vietnam: within the cluster 1, except for accession No4 (an outlier), two sub-groups can be differentiated. The crosses LDP_1 and LDP_2 gathered to their father PH_1 presenting in one sub-group; in another sub-group, accessions of Kim Tuyen, Ngoc Thuy, Tu Quy and Yabukita, known as small-leaved China teas, came together. While all known shan tea i.e., Shan tuyet, LD_{97}, TB_{14}, $TB_{14}CD$ and TB_{11} appeared under cluster 4; notably commercial shan tea accessions (LD_{97}, TB_{14} and $TB_{14}CD$) formed a sub-group. The promising tea clones (named from No1 to No21) presented in all four clusters, especially cluster 2 and 3 comprised only promising tea clones.

A PCoA was performed based on Euclidean Distances estimator to portray the morphological similarity of 31 tested tea accessions. According to Figure 2.28, the first two principal co-ordinates (PCos) contributed 44.1% of the total variation and the PCoA

depicted relationship that largely agree with the conventional classification system and the clustering patterns presented in the dendrogram (Figure 2.27). All known shan tea accessions are distributed into two groups: in the center (Shan tuyet and TB11) (group 4b) and at the bottom of the figure (TB_{14}, TB_{14}CD and LD_{97}) (group 4a); all known small-leaved China tea accessions (Tu Quy, Ngoc Thuy, Kim Tuyen, Yabukita) are on the middle of the right edge of the figure (group 1b) and LDP_1, LDP_2 and PH_1 are distributed close to each other near the center (group 1a).

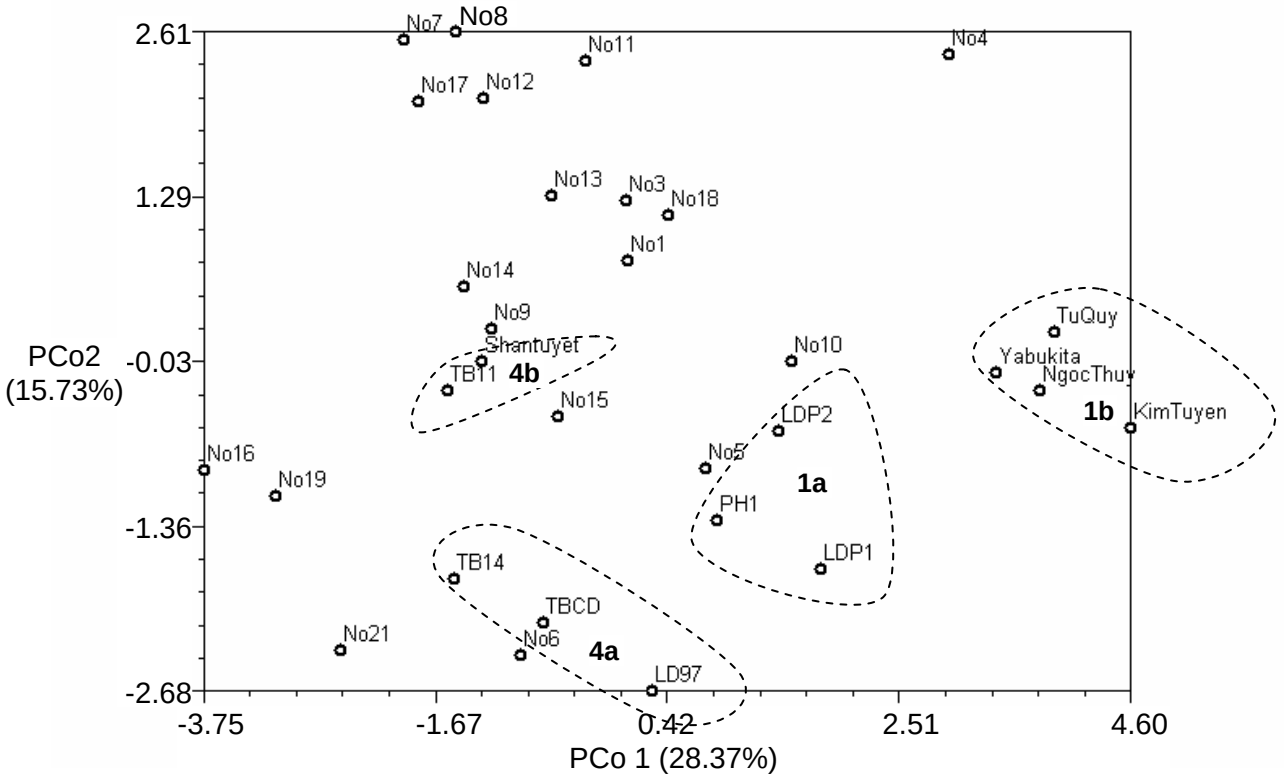


Figure 2.28: Principal co-ordinates analysis of 31 tea accessions grown in Lam Dong province (Vietnam) based on Euclidean distances coefficient calculated from 16 quantitative morphology characteristics

2.4 Conclusions

Investigated tea accessions at Lam Dong showed high diversity based on their morphological characteristics. Although the dendrogram failed in clearly separating the tested accessions, they can be clustered into 4 groups and all known China, India and Shan teas were clearly separated in sub-groups and the results of this study generally meet with the reputed taxa currently accepted in local tea production. A larger diversity was observed in new promising clones which broaden the diversity observed in the established groups.

Appendices

Table A2.1: Average climatic conditions of Bao Loc – Bao Lam zone during 1993 – 2003 (Source: Weather station of Bao Loc Technical and Vocational High school, 2004)

Month	Temperature (^{0}C)			Rainfall (mm)	Humidity (%)		Sunshine duration (h)
	Mean	Maximal	Minimal		Mean	Minimal	
Jan	21,7(0,6)	27,2 (0,2)	16,6 (0,4)	41,8 (22,6)	81,9 (1,8)	54,2 (1,4)	191,7 (24,0)
Feb	21,5 (0,4)	28,3 (0,3)	18,1 (0,3)	82,0 (31,0)	80,0 (2,5)	55,2 (2,0)	243,3 (15,9)
Mar	22,2 (0,4)	28,3 (0,3)	18,5 (0,3)	575,2 (52,5)	81,2 (2,4)	54,7 (1,6)	211,4 (15,6)
Apr	22,7 (0,4)	28,3 (0,2)	18,3 (0,2)	226,3 (68,8)	82,8 (2,1)	54,8 (1,8)	194,8 (10,4)
May	23,2 (0,4)	28,4 (0,2)	18,8 (0,3)	209,7 (45,0)	85,9 (1,1)	54,7 (1,6)	187,8 (10,2)
Jun	22,4 (0,2)	27,8 (0,2)	18,6 (0,4)	315,6 (58,4)	87,4 (1,1)	58,2 (1,8)	160,8 (16,7)
Jul	22,1 (0,3)	27,7 (0,3)	18,7 (0,3)	419,5 (98,2)	88,1 (1,6)	59,9 (1,6)	151,1 (19,7)
Aug	21,8 (0,4)	27,1 (0,4)	17,7 (0,4)	517,4 (93,7)	87,8 (2,0)	68,9 (3,5)	119,7 (9,9)
Sep	21,8 (0,3)	27,3 (0,3)	18,1 (0,4)	349,2 (67,9)	87,0 (1,6)	60,7 (2,1)	132,6 (27,1)
Oct	21,7 (0,3)	27,5 (0,2)	18,1 (0,4)	301,5 (20,5)	86,8 (1,4)	58,7 (1,4)	142,3 (11,3)
Nov	21,5 (0,3)	27,5 (0,3)	17,9 (0,3)	219,0 (47,9)	86,0 (1,5)	55,4 (1,3)	149,8 (17,5)
Dec	20,6 (0,4)	27,1 (0,4)	16,8 (0,4)	63,6 (25,9)	82,5 (2,3)	56,3 (1,8)	180,8 (20,7)
Average/total	21,9	27,7	18,0	3.320,8	84,8	57,4	2.066,1
Tea requirements		17 – 25		1.500 – 2.000 mm.year^{-1}	75 – 80		1.600 – 1.800 h.year^{-1}

(The numbers in the parentheses are standard deviation values)

Table A2.2: Observed characteristics of stem of tea accessions

Var. or clones	Stem circumference (cm)	The 1st branching position (cm)	Branching angle (degrees)	Branching capability(*)	The plucking surface		
					The height (cm)	The length (cm)	The breadth (cm)
1	53.3 (1.5)	22.7 (6.8)	38.3 (7.6)	2	105.0 (10.0)	155.7 (10.1)	135.0 (8.7)
3	34.3 (4.0)	27.7 (2.5)	25.0 (5.0)	1	82.3 (2.5)	125.5 (5.0)	105.3 (4.2)
4	57.8 (2.3)	7.4 (1.8)	24.8 (1.5)	3	83.2 (2.1)	93.2 (2.2)	103.0 (5.7)
5	100.2 (3.5)	7.4 (1.8)	70.6 (3.8)	1	76.8 (2.2)	98.8 (3.4)	119.0 (4.2)
6	55.7 (4.0)	6.7 (1.5)	21.0 (3.6)	1	82.7 (2.5)	68.0 (2.0)	95.0 (1.0)
7	68.0	20.0	8.0 (1.2)	3	105.0	170.0	160.0
8	77.0	10.0	7.0 (2.0)	3	107.0	140.0	150.0
9	84.0 (3.8)	10.2 (4.4)	30.0 (3.8)	2	95.2 (4.0)	121.6 (7.1)	105.0 (3.1)
10	36.2 (4.4)	20.2 (4.2)	60.0 (7.9)	3	85.4 (5.4)	60.0 (7.9)	70.0 (7.9)
11	86.0	5.0	10.0 (3.6)	1	110.0	170.0	135.0
12	85.0	10.0	15.0 (5.0)	3	112.0	215.0	192.0
13	69.0	5.0	37.0 (2.1)	1	110.0	320.0	200.0
14	62.0	5.0	15.0 (4.1)	1	110.0	110.0	85.0
15	47.0	22.0	25.0 (1.9)	1	108.0	180.0	115.0
16	55.0	20.0	23.0 (2.6)	3	122.0	200.0	160.0
17	52.0	7.0	12.0 (2.1)	3	112.0	149.0	120.0
18	42.0	30.0	35.0 (3.0)	3	110.0	194.0	170.0
19	53.0	26.0	11.4 (1.3)	3	135.0	110.0	85.0
21	76.0	10.0	35.6 (2.5)	1	115.0	127.0	120.0
KT	20.8 (2.3)	4.8 (1.5)	60.2 (4.0)	1	73.6 (2.5)	59.6 (2.1)	112.4 (2.5)
LD_{97}	23.2 (2.2)	7.4 (2.1)	64.0 (4.2)	3	114.0 (4.2)	104.0 4.2)	116.0 (4.2)
LDP_1	22.2 (1.5)	8.4 (1.1)	61.0 (4.2)	3	96.8 (2.1)	82.2 (2.3)	117.6 (2.5)
LDP_2	19.2 (1.3)	6.6 (1.1)	47.4 (1.8)	3	68.6 (2.6)	68.0 (2.5)	98.4 (2.5)
NT	19.8 (3.0)	4.8 (0.8)	58.6 (2.6)	1	86.2 (2.4)	58.4 (2.7)	111.8 (2.1)
PH_1	51.0 (2.2)	12.0 (2.1)	70.2 (3.4)	3	104.0 (4.2)	148.4 (5.9)	140.0 (5.4)
St	100.8 (3.7)	28.0 (3.1)	47.0 (2.1)	3	220.0 (7.9)	224.0 (6.5)	190.2 (3.4)
TB_{11}	50.6 (3.4)	27.0 (2.7)	47.8 (3.7)	2	129.0 (2.7)	149.6 (2.1)	112.6 (2.0)
TB_{14}	50.2 (2.9)	15.0 (3.5)	58.0 (2.7)	3	101.6 (3.2)	111.0 (4.2)	128.0 (10.4)
TBCD	26.6 (2.4)	6.4 (1.7)	58.0 (2.1)	3	80.8 (2.3)	64.0 (3.1)	82.0 (2.7)
TQ	50.4 (3.7)	11.6 (3.7)	61.0 (2.7)	2	92.8 (3.1)	73.6 (3.4)	112.2 (2.3)
Ya	18.8 (1.9)	5.2 (0.8)	52.2 (1.5)	3	81.0 (2.7)	83.4 (2.1)	75.0 (2.2)

(*) 1- rare; 2- medium; and 3- high

(The numbers in the parentheses are standard deviation values)

Table A2.3: Observed characteristics of the 4th leaf of tea accessions

Var. or clone	Leaf size		Leaf shape			Color[3]	Leaf base[4]
	The length (cm)	The breadth (cm)	Size[1]	The L/B ratio	Shape[2]		
1	8.7 (0.4)	3.5 (0.3)	L	2.47	3	1	2
3	9.0 (0.7)	4.1 (0.7)	L	2.19	2	1	1
4	5.9 (0.6)	2.8 (0.3)	M	2.14	2	1	2
5	8.5 (0.3)	3.2 (1.1)	M	2.65	3	1	2
6	11.8 (0.5)	3.7 (0.4)	L	3.17	4	1	2
7	11.0 (0.5)	5.3 (0.7)	XL	2.08	2	1	2
8	10.7 (0.4)	4.7 (0.3)	L	2.29	2	1	2
9	10.2 (0.4)	3.8 (0.5)	L	2.67	3	1	2
10	6.9 (0.5)	2.8 (0.4)	M	2.50	2	1	1
11	8.8 (0.5)	2.7 (0.4)	M	3.29	4	1	2
12	7.9 (0.4)	3.8 (0.4)	L	2.08	2	1	1
13	8.8 (0.5)	4.6 (0.3)	L	1.92	1	1	1
14	9.7 (0.4)	3.8 (0.3)	L	2.54	2	1	2
15	11.1 (0.6)	3.8 (0.3)	L	2.92	3	3	1
16	12.4 (0.6)	4.9 (0.4)	XL	2.52	2	1	2
17	11.7 (0.5)	3.8 (0.5)	L	3.08	4	1	1
18	9.5 (0.3)	3.7 (0.3)	L	2.61	3	1	1
19	13.3 (0.6)	4.0 (0.3)	XL	3.33	4	2	2
21	13.1 (0.5)	5.7 (0.3)	XL	2.30	2	2	2
KT	6.8 (0.4)	2.7 (0.4)	M	2.49	2	2	2
LD_{97}	8.7 (0.3)	3.6 (0.5)	L	2.45	2	2	2
LDP_1	9.8 (0.5)	4.7 (0.4)	L	2.06	2	1	2
LDP_2	9.8 (0.5)	3.8 (0.3)	L	2.59	3	2	2
NT	7.6 (0.4)	3.7 (0.3)	M	2.05	2	1	2
PH_1	9.8 (0.4)	4.7 (0.5)	L	2.07	3	2	2
St	9.6 (0.4)	3.9 (0.2)	L	2.48	2	1	1
TB_{11}	10.2 (0.7)	4.8 (0.4)	L	2.13	2	2	2
TB_{14}	9.8 (0.4)	4.7 (0.4)	L	2.07	2	1	2
TBCD	11.7 (0.4)	3.9 (0.3)	L	3.04	4	1	2
TQ	6.7 (0.4)	2.7 (0.3)	M	2.49	2	2	2
Ya	8.7 (0.3)	2.6 (0.1)	M	3.33	4	2	1

[1] M- medium; L- large; and XL- extra large
[2] 1- ovate; 2- oblong; 3- elliptic; and 4- lancelate
[3] 1- light green; 2- green; and 3- grayed-green
[4] 1- attenuate (acute) and 2- rounded

(The numbers in the parentheses are standard deviation values)

Table A2.4: Observed characteristics of the 4th leaf of tea accessions (cont.)

Var. or clones	Leaf angle (degrees)	Leaf pose(1)	Pair of main vein on lamina	No of serrulation on the leaf edge	Serrula form(2)	The length of petiole (cm)	The length from petiole to the 1st serration (cm)
1	63.1 (2.9)	2	7.4 (0.5)	35.2 (1.5)	1	0.20 (0.09)	2.21 (0.17)
3	69.0 (5.2)	2	7.4 (0.5)	36.2 (1.3)	1	0.51 (0.10)	1.73 (0.23)
4	61.0 (5.2)	2	6.0 (0.5)	23.9 (1.5)	1	0.18 (0.08)	1.80 (0.32)
5	47.6 (3.2)	2	6.4 (0.5)	46.5 (1.7)	4	0.42 (0.08)	2.44 (0.18)
6	55.4 (3.2)	2	10.3 (0.5)	64.7 (2.4)	4	0.26 (0.07)	1.90 (0.24)
7	64.0 (3.9)	2	6.6 (0.7)	35.2 (1.2)	4	0.38 (0.08)	2.00 (0.20)
8	67.6 (3.3)	2	8.7 (0.7)	40.1 (1.4)	4	0.21 (0.09)	2.00 (0.21)
9	73.2 (2.6)	2	8.7 (0.5)	45.8 (1.9)	1	0.35 (0.08)	1.64) (0.14)
10	66.4 (4.7)	2	8.4 (0.5)	32.2 (1.8)	1	0.37 (0.08)	1.90 (0.28)
11	77.1 (2.2)	3	7.0 (0.7)	43.9 (1.6)	1	0.45 (0.11)	2.20 (0.27)
12	84.3 (2.2)	3	6.2 (0.4)	31.1 (2.7)	4	0.38 (0.10)	2.60 (0.34)
13	67.6 (2.7)	2	6.8 (0.4)	29.9 (1.4)	3	0.41 (0.07)	1.77 (0.15)
14	65.1 (3.3)	2	8.4 (0.5)	46.3 (2.7)	3	0.18 (0.03)	2.80 (0.27)
15	72.2 (2.5)	2	7.7 (0.8)	49.0 (2.1)	1	0.40 (0.08)	1.79 (0.13)
16	67.5 (5.5)	2	10.6 (0.7)	49.5 (1.6)	3	0.51 (0.14)	2.50 (0.17)
17	83.2 (2.4)	3	6.6 (0.7)	45.0 (1.6)	1	0.44 (0.10)	1.58 (0.13)
18	58.5 (3.2)	2	6.4 (0.5)	29.3 (1.2)	2	0.46 (0.17)	1.80 (0.18)
19	65.5 (5.0)	2	8.8 (0.8)	49.1 (1.7)	1	0.57 (0.08)	2.52 (0.24)
21	57.8 (2.2)	2	9.5 (0.5)	59.2 (2.3)	3	0.54 (0.08)	1.97 (0.18)
KT	50.1 (3.8)	2	5.6 (0.5)	25.4 (1.3)	1	0.28 (0.06)	1.44 (0.10)
LD_{97}	53.5 (3.4)	2	7.4 (0.5)	46.3 (2.7)	1	0.46 (0.07)	1.78 (0.25)
LDP_1	48.8 (3.6)	2	6.4 (0.5)	48.4 (2.3)	4	0.31 (0.07)	1.74 (0.23)
LDP_2	64.9 (4.0)	2	6.6 (0.5)	45.6 (1.1)	3	0.25 (0.07)	1.53 (0.37)
NT	61.3 (3.9)	2	5.7 (0.5)	26.3 (1.2)	4	0.26 (0.07)	1.63 (0.09)
PH_1	68.1 (2.3)	2	6.5 (0.5)	48.0 (2.2)	3	0.29 (0.09)	1.77 (0.23)
St	62.5 (2.1)	2	7.0 (0.0)	44.6 (1.5)	3	0.53 (0.12)	1.50 (0.12)
TB_{11}	76.0 (5.9)	3	6.5 (0.5)	31.0 (0.8)	2	0.48 (0.08)	2.71 (0.19)
TB_{14}	77.0 (5.4)	3	10.1 (1.0)	40.6 (1.3)	1	0.45 (0.05)	1.78 (0.23)
TBCD	57.6 (3.0)	2	9.2 (0.6)	45.5 (1.5)	1	0.50 (0.12)	1.73 (0.24)
TQ	60.1 (2.9)	2	5.8 (0.4)	35.5 (1.8)	2	0.36 (0.10)	1.36 (0.18)
Ya	61.4 (2.5)	2	6.2 (0.4)	24.6 (0.7)	1	0.32 (0.10)	1.54 (0.18)

(1) 2- semi-erect (obtuse) and 3- horizontal (right)
(2) 1- regularly acute; 2- regularly blunt; 3- irregularly acute and 4- irregularly blunt

(The numbers in the parentheses are standard deviation values)

Table A2.5: Observed characteristics of flush shoot of tea accessions

Var. or clones	Pubescence density on		Color[3]	Shoot length (cm)	Weight (g)		The F/D ratio	Tannin content
	Bud[1]	The 1st leaf lower surface [2]			Fresh	Dry		
1	1	1	2	7.4 (0.4)	0.76	0.27	2.81	-
3	3	4	1	4.7 (0.3)	0.62	0.15	4.13	24.16
4	1	1	1	5.7 (0.3)	0.58	0.10	5.80	27.80
5	3	5	2	6.8 (0.6)	0.65	0.16	4.06	26.26
6	2	4	1	7.5 (0.2)	0.55	0.11	5.00	26.12
7	1	1	1	5.0 (0.7)	0.79	0.34	2.32	-
8	1	1	1	4.8 (0.6)	0.72	0.31	2.32	-
9	3	4	1	6.8 (0.6)	0.68	0.27	2.52	-
10	2	2	1	6.6 (0.5)	0.76	0.14	5.43	19.69
11	3	2	1	6.8 (0.4)	0.64	0.16	4.00	16.76
12	2	1	1	8.2 (0.4)	0.93	0.22	4.23	21.37
13	2	1	1	7.2 (0.5)	1.10	0.26	4.23	18.02
14	3	5	1	8.3 (0.6)	0.92	0.22	4.18	16.06
15	3	5	4	6.6 (0.3)	0.57	0.16	3.56	-
16	2	3	1	8.1 (0.5)	1.02	0.22	4.64	18.02
17	3	4	1	7.9 (0.5)	1.03	0.24	4.29	16.62
18	2	1	1	7.5 (0.3)	0.59	0.20	2.95	21.23
19	3	4	1	8.7 (0.3)	0.69	0.22	3.14	-
21	3	4	2	8.3 (0.7)	0.56	0.18	3.11	-
KT	2	3	2	7.7 (0.4)	0.33	0.08	4.13	15.23
LD_{97}	3	5	2	11.3 (0.7)	0.80	0.19	4.21	25.14
LDP_1	1	2	1	5.8 (0.4)	0.53	0.12	4.42	22.77
LDP_2	1	2	3	6.8 (0.4)	0.67	0.17	3.94	20.81
NT	2	2	3	6.7 (0.4)	0.33	0.08	4.13	17.18
PH_1	1	1	1	7.7 (0.6)	0.55	0.12	4.58	22.77
St	3	5	1	7.8 (0.4)	0.91	0.23	3.96	21.09
TB_{11}	3	4	1	8.9 (0.7)	0.83	0.14	5.93	28.56
TB_{14}	3	5	1	10.6 (0.5)	0.83	0.15	5.53	24.16
TBCD	3	5	1	8.7 (0.4)	0.86	0.20	4.30	19.97
TQ	1	2	3	4.7 (0.4)	0.36	0.07	5.14	15.64
Ya	2	3	3	5.4 (0.2)	0.50	0.11	4.55	21.65

[1] 1- glabrous to rare; 2 – light pubescent indumentums; and 3- dense pubescent layer
[2] 1- glabrous leaves; 2- few scattered hairs; 3- pubescence extends about half-way to the margin; 4- entire under surface of lamina pubescent; and 5- dense pubescent indumentums
[3] 1- light green; 2- green; 3- purplish green; and 4- bronze

(The numbers in the parentheses are standard deviation values)

Table A2.6: Observed characteristics of flower of tea accessions

	Color of petal	No of petal	Splitting of style[1]	Flowering position	Flowering capability[2]
1	White	5	2	4	3
3	White	4	2	3	3
4	White	5	3	3	3
5	White	5	3	4	5
6	White	4	2	4	5
7	White	4	3	3	5
8	White	5	2	3	3
9	White	4	2	4	5
10	White	5	3	4	3
11	White	5	2	3	3
12	White	5	3	4	3
13	White	6	3	4	5
14	White	5	2	4	3
15	White	4	3	4	5
16	White	5	3	4	5
17	White	5	2	3	3
18	White	5	3	3	5
19	White	4	2	4	3
21	White	5	2	4	5
KT	White	5	2	4	5
LD_{97}	White	4	3	4	3
LDP_1	White	5	2	4	3
LDP_2	White	5	2	4	3
NT	White	5	3	4	5
PH_1	White	5	3	4	3
St	White	5	2	4	5
TB_{11}	White	5	2	4	3
TB_{14}	White	5	2	4	3
TBCD	White	5	2	4	3
TQ	White	5	2	4	5
Ya	White	4	3	4	5

(*) 1- geniculation; 2- ascending; and 3- terminal
(2) 3- low and 5-medium

Table A2.7: Dissimilarity matrix of Euclidean distance coefficient

	No1	No3	No4	No5	No6	No7	No8	No9	No10	No11	No12	No13
No1	0.00											
No3	5.26	0.00										
No4	4.89	5.45	0.00									
No5	4.64	6.52	5.63	0.00								
No6	5.67	6.21	7.00	5.78	0.00							
No7	5.00	4.21	6.52	6.78	6.72	0.00						
No8	4.35	5.29	5.54	6.32	5.85	3.36	0.00					
No9	4.03	4.89	6.12	5.26	4.27	4.44	4.14	0.00				
No10	3.32	4.81	4.93	4.92	6.18	6.61	6.04	4.82	0.00			
No11	5.09	5.32	5.27	5.65	6.40	5.64	4.64	4.54	5.09	0.00		
No12	4.11	6.21	5.97	5.68	7.19	5.74	5.34	4.67	5.02	4.14	0.00	
No13	4.37	6.83	6.51	5.81	7.69	5.96	5.09	5.30	4.96	5.43	4.19	0.00
No14	3.43	7.02	6.60	5.65	5.52	5.89	4.50	4.55	5.22	4.81	4.04	4.83
No15	4.16	3.48	6.01	5.54	3.90	4.80	5.14	2.92	4.32	4.76	5.23	6.13
No16	5.02	6.22	8.49	6.59	5.71	5.70	5.40	4.84	5.75	5.99	5.40	5.27
No17	5.35	5.58	6.84	7.27	6.92	4.91	4.54	4.54	5.92	3.89	4.77	4.58
No18	4.00	3.43	5.06	5.64	6.77	4.71	5.23	5.37	4.14	4.97	5.67	5.53
No19	5.29	5.22	8.10	6.30	4.96	5.33	6.14	4.52	6.06	5.81	5.76	6.90
No21	6.04	6.42	8.18	5.57	4.45	6.17	5.80	4.82	6.58	6.22	6.64	5.96
KimTuyen	5.56	6.94	5.26	6.12	7.45	8.22	7.73	6.77	4.52	6.59	7.39	6.72
LD_{97}	5.17	6.23	6.87	5.25	5.34	7.01	7.38	5.14	5.01	6.79	6.43	6.48
LDP_1	4.76	5.47	5.64	4.61	5.05	6.35	5.98	5.49	4.65	6.48	6.80	5.73
LDP_2	3.67	5.23	5.08	5.20	4.97	5.96	5.18	4.28	3.65	5.28	5.46	4.68
NgocThuy	5.02	6.06	4.81	5.87	6.88	7.35	6.81	6.07	4.11	6.04	6.46	6.01
PH_1	4.09	5.38	5.62	4.44	5.06	6.21	5.67	4.44	4.13	5.59	5.37	5.10
Shantuyet	4.32	5.34	6.90	4.79	6.46	5.49	5.81	4.16	4.62	5.46	5.09	4.52
TB_{11}	4.31	5.47	6.76	5.62	6.99	5.99	6.42	5.62	5.13	6.37	4.31	5.72
TB_{14}	4.89	6.01	7.18	6.07	5.63	6.85	6.32	4.61	4.58	6.13	5.20	5.16
TBCD	4.71	5.89	7.12	5.29	4.99	6.37	5.96	4.54	4.33	5.83	6.02	4.69
TuQuy	5.39	5.82	5.06	5.51	7.10	7.54	7.03	5.90	3.86	5.62	6.87	6.40
Yabukita	5.19	5.04	4.57	5.79	6.18	6.81	6.82	5.33	3.98	6.01	6.52	6.72

Table A2.7: Dissimilarity matrix of Euclidean distance coefficient (cont.)

	No14	No15	No16	No17	No18	No19	No21	KimTuyen	LD_{97}	LDP_1	LDP_2
No14	0.00										
No15	5.08	0.00									
No16	4.41	4.70	0.00								
No17	5.29	4.83	5.24	0.00							

No18	6.23	4.40	5.90	5.20	0.00						
No19	5.31	3.47	3.35	5.68	5.31	0.00					
No21	5.61	4.74	3.73	6.00	6.14	4.38	0.00				
KimTuyen	7.00	6.20	8.65	7.69	5.67	8.26	7.89	0.00			
LD_{97}	6.11	4.95	6.20	6.52	5.58	5.47	5.75	5.96	0.00		
LDP_1	5.87	4.61	6.39	6.67	5.05	6.49	5.09	4.65	4.95	0.00	
LDP_2	4.78	3.78	5.87	4.92	4.71	6.09	5.53	4.35	4.55	2.72	0.00
NgocThuy	6.34	5.36	7.84	6.92	5.25	7.68	7.13	2.05	5.90	3.73	3.38
PH_1	5.28	3.85	5.74	5.70	4.83	5.92	4.63	5.16	4.66	2.79	2.64
Shantuyet	6.00	4.42	4.93	5.26	4.31	5.06	5.01	7.10	5.49	5.72	5.21
TB_{11}	5.95	4.64	5.09	6.34	4.89	4.57	5.61	7.78	5.70	5.95	5.71
TB_{14}	5.56	4.64	4.23	5.50	5.50	5.12	4.50	7.18	4.43	5.69	4.82
TBCD	5.05	4.45	4.01	5.03	5.06	4.78	4.01	6.05	3.66	4.25	3.63
TuQuy	7.03	5.23	8.16	7.13	5.36	7.83	7.40	2.94	6.66	4.51	4.23
Yabukita	6.68	4.64	7.85	6.78	5.28	7.06	7.60	3.57	5.30	4.44	3.71

Table A2.7: Dissimilarity matrix of Euclidean distance coefficient (cont.)

	NgocThuy	PH_1	Shantuyet	TB_{11}	TB_{14}	TBCD	TuQuy	Yabukita
NgocThuy	0.00							
PH_1	3.91	0.00						
Shantuyet	6.74	4.69	0.00					
TB_{11}	6.68	4.63	5.08	0.00				
TB_{14}	6.33	4.01	4.68	4.22	0.00			
TBCD	5.63	4.15	4.65	5.55	3.57	0.00		
TuQuy	2.86	4.67	6.03	7.45	7.06	6.15	0.00	
Yabukita	3.11	4.81	6.59	6.75	6.59	5.53	3.56	0.00

Chapter 3

Assessment of genetic diversity of tea grown in Vietnam using inter-simple sequence repeat (ISSR) markers

Abstract

Many different markers have been used to reveal the genetic diversity of tea. ISSR-PCR technique that overcomes the main limitations of RAPD, AFLP and SSR markers was used in this study. Seven ISSR primers from a total of 15 primers tested generated polymorphic bands with 71 accessions from a total of 96 template DNAs tested. Cluster analysis based on Dice similarity coefficients was done using the unweighted pair-group method with arithmetic average (UPGMA) to group all studied tea accessions. Analyses of molecular variance (AMOVA) were performed to analyze molecular variance within and between the various groups of material. Except for some accessions remaining distinct and ungrouped, at about 50% similarity level, the dendrogram formed 4 clusters and commonly support the conventional classification. Large and significant variation was found among accessions.

3.1 Introduction

Genetic diversity is defined as the variation among and within species that is attributable to genetic differences. It is caused by selection and various mutational and sexual events and by genome changes ranging from a single base-pair exchange to rearrangements of entire chromosomes. Understanding genetic diversity is important for accurate identification of plant material in a gene bank: without such information breeders have difficulties to select appropriate material for entry into breeding programmes.

To assess the genetic diversity, many different markers have been used i.e., morphological markers, cytological markers, biochemical markers, isozyme markers and molecular markers. In recent years, considerable emphasis has been placed on the development of molecular markers to be used for a variety of objectives, including revealing genetic diversity.

The major limitations of the commonly used polymerase chain reaction (PCR)-based DNA marker systems are the low reproducibility of random amplified polymorphic DNA (RAPD),

high cost of amplified fragment length polymorphism (AFLP) and the need to know the flanking sequences to develop species specific primers for simple sequence repeats (SSRs) polymorphism. ISSR-PCR is a technique that overcomes most of these limitations (Meyer *et al*., 1993; Gupta *et al*., 1994; Wu *et al*., 1994; Zietkiewicz *et al*., 1994; Staub *et al*., 1996; Gupta & Varshney, 2000). Inter simple sequence repeat polymerase chain reaction (ISSR-PCR) analysis is a quick and simple method that combines most of the advantages of microsatellites (SSRs) and amplified fragment length polymorphism (AFLP) to the universality of random amplified polymorphic DNA (RAPD) (Reddy *et al*., 2002) and generates complex band profiles.

ISSR markers have been used to investigate the genetic diversity within and among population of genus *Oryza* (Joshi *et al*., 2000; Qian *et al*., 2001; Saini *et al*., 2004, Bao *et al*., 2006), in tef (Assefa *et al*., 2003), in dent and popcorn maize inbred lines (Kantety *et al*., 1995), among barley cultivars (Fernández *et al*., 2002), in common bean (Métais *et al*., 2000; Galván *et al*., 2003), in blackgram (Souframanien and Gopalakrishna, 2004), in *Lupinus* spp. (Talhinhas *et al*., 2003), in the genus *Cicer* and cultivated chickpea (Iruela *et al*., 2002), in a few members of the *Brassica oleracea* L. (Panda *et al*., 2003), among species of the genus *Diplotaxis* (Martín *et al*., 2000), in genus *Lycopersicon* (Tourn.) Mill. (Kochieva *et al*., 2002), in *Cucurbita pepo* (Paris *et al*., 2003), among pansies (Yockteng *et al*., 2003), of sesame collection (Kim *et al*., 2002), of sweet potato and its wild relatives in *Ipomoea* series *Batatas* (Huang and Sun, 2000), in jute (Roy *et al*., 2006), in hop (Patzak, 2001), of closely related citrus cultivars (Fang and Roose, 1997), of trifoliate orange collection (Fang *et al*., 1997), of *Olea europaea* L. cultivars (Gemas *et al*., 2004; Terzopoulos *et al*., 2005), among coconut accessions (Manimekalai and Nagarajan, 2006), in tea *Camellia sinensis* (Lai *et al*., 2001; Mondal, 2002b; Devarumath *et al*., 2002).

In this study, to support the basic for tea breeding in Vietnam, ISSR technique was used to assess the genetic diversity of 71 tea accessions grown in Vietnam.

3.2 Materials and methods

3.2.1 Tea plant materials

The total of 144 accessions (Table 3.1 and Table A3.1) of wild giant Shan and cultivated teas were collected from six provinces, i.e., Lam Dong, Gia Lai, Phu Tho, Thai Nguyen, Yen Bai and Dien Bien (Figure 3.1), and two tea collections at two Tea research Institute,

i.e., Vietnam Tea Research Institute – VTRI, and Lam Dong Center for Research and Transfer techniques on Industrial crops and Fruit trees Production.

Table 3.1: Classification of studied tea accessions based on their sources.

Source of accession	Number of the accession
- Local cultivated tea[(1)] (so-called local tea)	47
- Improved/selected tea clones[(2)]	34
- Cultivars/clones imported from other countries	51
- Wild giant teas [(3)]	12

[(1)] The term 'local cultivated tea' was used for all the popularly existing teas at certain areas for a long time, of which real origin and acquisition date are still doubted or debatable.

[(2)] The term 'improved/selected tea clone' indicated tea clones which were selected from the local tea populations or tea collections. Tea clones selected from the cross breeding programs were also involved.

[(3)] The term 'wild giant tea' was used for all the giant tea growing wildly for a long time at the high mountainous areas.

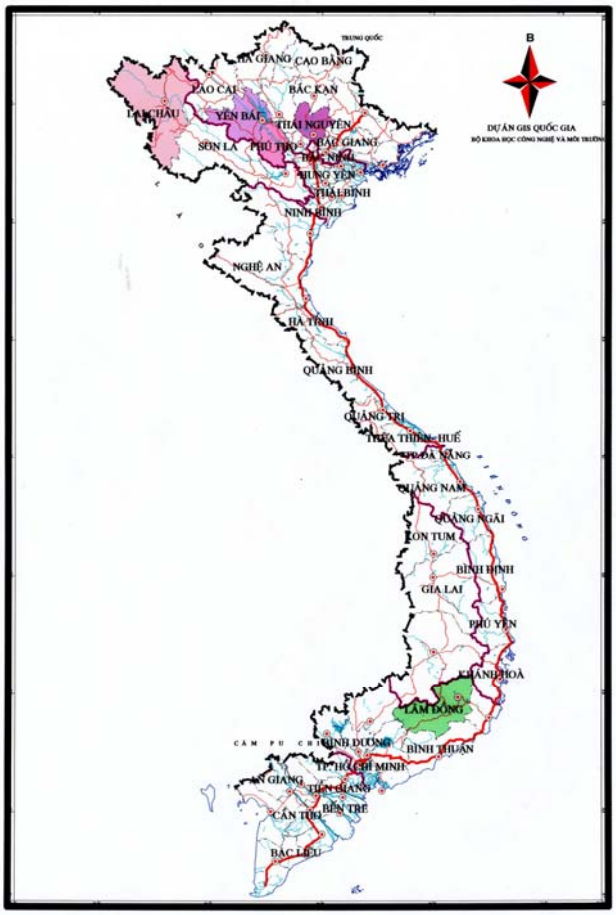


Figure 3.1: Place of the provinces where accessions were sampled (colored areas).

Commercially tea plants have been imported into and planted at Lam Dong province in the early 20th century. Among 23 local cultivated teas sampled at Lam Dong province (Table A3.1), except the 'France' tea and small-leaved China tea BL of unknown origin, the rest was actually acquired also from the North of Vietnam by the French, nevertheless due to the war all the related documents were lost. Contrarily, in the north, tea plants have been grown since a long time at many places and hence there have been many locally specific tea cultivars such as green trung du, mixed trung du, purple-leaved trung du (Figure 3.2), yellow trung du, Tan Cuong, Tan Chi shan, Moc Chau shan.

Figure 3.2: Shoot of purple-leaved trung du tea (local tea).

Some local teas have been believed to be originated from the wild giant shan tea.

Being chosen from the existing local populations, collections or cross breeding programs, most of the improved/selected tea clones involved in the study have been the dominant or promising clones at certain areas. Especially TB_{14} has been the dominant clone at Lam Dong province; 1A and PH_1 have been more popular at the North; LD_{97} and TB_{18} have been identified as the promising ones at Lam Dong.

Figure 3.3: The plantation of Kim Tuyen tea clone, imported from Taiwan, at Lam Dong.

The group of imported tea consisted of 19 China accessions, 9 Indian, 7 from Sri Lanka, 7 from Taiwan, 4 from Japan, 2 from Laos, 2 from Georgia and 1 Myanmar tea. The clones of Kim Tuyen (Figure 3.3), Ngoc Thuy and Tu Quy were cultivated widely at Lam Dong province to supply specially the materials for processing oo long teas. Yabukita is the dominant tea clone contributing up to 60% of clonal tea area of Japan (Yamamoto *et al*., 1997), but it seems not to be adapted to the Lam Dong condition. Besides Dai Bach Tra was known as the very good quality tea of China; Thiet Bao Tra, Oo long Thanh Tam were also good tea clones of Taiwan to produce oo long teas.

Wild teas at both areas (Suoi Giang – Yen Bai and Tua Chua – Dien Bien) (Figure 3.4) can gather into groups along the slit of the hills or mountains; or scatter in the secondary forest or even in the mountain hamlets. Growing naturally, wild tea plants at Suoi Giang were pruned periodically (some years) while those at Tua Chua were not. Flush shoot of wild teas were also harvested by the local farmers.

For all cultivated teas (local, selected (Figure 3.5) and imported teas), including teas in collections, the samples were taken from the currently harvested tea plants at Vietnam from September to December of 2004. Wild giant teas were sampled during December 2004 and January 2005 from wild giant tea populations at Suoi Giang (Yen Bai province) and Tua Chua (Dien Bien province). Naturally wild tea seeds were thought to be scattered following the stream, therefore the direction of the streamlines or slits and the distribution of wild teas were the main basis for identifying wild giant tea populations.

Figure 3.4: Wild giant shan tea plant at Suoi Giang (Yen Bai).

Figure 3.5: The cross cultivars LDP_1 at Lam Dong province.

3.2.2 Sampling

For each cultivars, clone or population (wild tea), sampling was done on solely one tea plant. The total of 20 normal-grown buds (pekoe, furled-leaf) taken randomly from the plucking surface (or from four direction of the canopy in the case of wild teas) were dried under shading condition (in around 24 hours) and then kept in tubes with silica gel at room temperature during the time of working at Vietnam, and stored at -20^{o}C when working in Germany. The forth leaves were sampled substitutionally in cases where tea plants were in dormant condition.

3.2.3 DNA extraction

Genomic DNA from the dried samples were isolated by using Nucleon PhytoPure, plant and fungal DNA extraction kits (RPN 8511) (Amersham Biosciences Corp.) as commonly used in the Institute (Amersham Bioscience, 2003). Template genomic DNA concentration was estimated using Bio-rad Flourometer.

Three RAPD primers, i.e., OPU10 (5' ACCTCGGCAC 3'), AM16 (5' TGGCGGTTTG 3') and OPC16 (5' CACACTCCAG 3'), were used to check quality of extracted genomic DNA.

3.2.4 The anchored primers

Fifteen ISSR primers, which detected polymorphism in tea samples according to Mondal (2002b), coded as ISSR-801, ISSR-802, ISSR-803, ISSR-805, ISSR-806, ISSR-816, ISSR-821, ISSR-866, ISSR-868, ISSR-869, ISSR-871, ISSR-873, ISSR-877, ISSR-878, and ISSR-879 (Table 3.2), were chosen and ordered from MWG-Biotech AG for revealing the polymorphism of tea accessions.

3.2.5 PCR analysis

PCR mixture (25 µl), adapted to the *Taq* polymerase commonly used in the Institute (FIREPol® DNA polymerase I, Solis Biodyne), contained 10X reaction buffer BD, 25 mM $MgCl_2$, 10 mM dNTPs, 20 µM ISSR primer, 10 ng template DNA, 1 U *Taq*.

ISSR amplification was implemented on the Whatman Biometra© T1 Thermocycle using the protocols of Lai *et al*. (2001) and Mondal (2002b) with a minor modification of the thermal cycles as follows:

- One cycle of pre-denaturation at 94^{o}C for 5 min;

- Forty five cycles of DNA amplification; each cycle consists of three steps, i.e., denaturizing at 94^{o}C for 1 min, then annealing at 50^{o}C for 1 min, and finally extending at 72^{o}C for 2 min;

- One cycle of further extension at 72^{o}C for 7 min; 4^{o}C end.

The amplification products along with a GeneRuler^{TM} 1kb DNA ladder (Fermentas GmbH) were size fractionated on 2.0% agarose gels and electrophoresed in 0.5X TBE buffer [89 mM Tris-HCl (pH 8.3), 89 mM boric acid and 5 mM EDTA] at 90V for 6h, then stained with ethidium bromide, and finally scored for band presence or absence under the UV light.

Table 3.2: Characteristics of ISSR primers used in the present study

Code	Sequence	The length (-mer)	GC percentage (%)	Molecular weight (g/mol)	Melting temperature (T_m)
ISSR-801	5’-ATA TAT ATA TAT ATA TT-3’	17	0.0	5181	31.1
ISSR-802	5’-ATA TAT ATA TAT ATA TG-3’	17	5.9	5206	33.5
ISSR-803	5’-ATA TAT ATA TAT ATA TC-3’	17	5.9	5166	33.5
ISSR-805	5’-TAT ATA TAT ATA TAT AC-3’	17	5.9	5166	33.5
ISSR-806	5’-TAT ATA TAT ATA TAT AG-3’	17	5.9	5206	33.5
ISSR-816	5’-CAC ACA CAC ACA CAC AT-3’	17	47.1	5061	50.4
ISSR-821	5’-GTG TGT GTG TGT GTG TT-3’	17	47.1	5309	50.4
ISSR-866	5’-CTC CTC CTC CTC CTC CTC-3’	18	66.7	5233	60.5
ISSR-868	5’-GAA GAA GAA GAA GAA GAA-3’	18	33.3	5672	46.9
ISSR-869	5’-GTT GTT GTT GTT GTT GTT-3’	18	33.3	5564	46.9
ISSR-871	5’-TAT TAT TAT TAT TAT TAT-3’	18	0.0	5468	33.2
ISSR-873	5’-GAC AGA CAG ACA GAC A-3’	16	50.0	4917	49.2
ISSR-877	5’-TGC ATG CAT GCA TGC A-3’	16	50.0	4881	49.2
ISSR-878	5’-GGA TGG ATG GAT GGA T-3’	16	50.0	5041	49.2
ISSR-879	5’-CTT CAC TTC ACT TCA-3’	15	40.0	4438	42.4

3.2.6 Data analysis

The presence or absence of bands were recorded and used to compute

- The similarity coefficient (Dice, 1945) between two accessions *i* and *j* was estimated as follow:

$$S_{ij} = 2a/ (2a + b + c),$$

where a: number of the common bands; b: number of bands only present in *i* but not *j*; c: number of bands only present in *j* but not *i*.

- Cluster analysis based on Dice similarity coefficient matrix was done using the unweighted pair-group method with arithmetic average (UPGMA) and sequential, agglomerative, hierarchical and nested (SAHN) to group all the studied teas by using NTSYS-pc 2.1 software (Rohlf, 2000). To test the reliability of clustering, bootstrap analysis was done with 4000 replications using PAUP* 4.0 beta version (Swofford, 1998).

- By using Arlerquin 3.1 software (Excoffier *et al*., 2005; Excoffier *et al*., 1992), AMOVA analysis was performed to analyze the molecular variance within and between the various groups of material.

3.3 Results and discussions

3.3.1 DNA extraction

The genomic DNA of samples was extracted and quantified as described above. Due to the highly different quality of the samples (taken from various conditions of tea plants), the concentrations of extracted DNA (ng/µl) were very different and ranged from 2.5 to 785.0 ng/µl, with a mean value of 179 ng/µl and a high standard deviation of 135 ng/µl. The concentration and quality of genomic DNA extracted from the buds was higher than from the 4^{th} leaves. Besides being effected by the content of tannin, the quality of extracted genomic DNA was also strongly influenced by the quality of samples: template genomic DNAs extracted from buds of low tannin content accessions had better quality than those from the forth leaf and/or from high tannin content accessions. Many accessions (such as No19, Manipur Dangri, Lao Chay, Tam Dao, Tan Cuong) could generate high concentration genomic DNAs but theirs DNA did not produce any band with tested RAPD markers. DNA extracted from tannin rich accessions gave brown solutions and did not show any bands.

After testing the DNA quality by RAPD markers and to meet the convention in lab work, only 96 template DNAs (consisting of 39 local cultivated teas, 7 wild giant tea plants, 23 improved/selected tea cultivars and 27 cultivars imported from other countries) were chosen for further study.

3.3.2 ISSR DNA amplification

To estimate the genetic diversity among 96 tea accessions, 15 ISSR primers that gave amplification products in earlier research (Mondal, 2002b) were tested. The preliminary results showed that only 71 accessions produced polymorphic bands with 7 ISSR primers which were characterized by theirs melting temperature between 41.2 to 60.5^{o}C (Table 3.2, Table 3.3 and Table 3.4). The high amount of tannin in some accessions and the long time of storage at room temperature during working time in Vietnam were thought to be the main factors degrading the quality of extracted DNA.

Reddy *et al*. (2002) reported that generally primers with (AG), (GA), (CT), (TC), (AC), (CA) repeats show higher polymorphism in plants than primers with other di-, tri- or tetra-nucleotides. (AT) repeats are the most abundant di-nucleotides in plants but the primers based on (AT) would self-anneal and not amplify. In this study the ISSR primers codes ISSR-801, ISSR-802 and ISSR-803 are (AT) based primers, while ISSR-816 and ISSR-866 are (CA) and (CT) based primers, respectively.

ISSR amplification of these 71 genomic template DNAs with 7 ISSR primers yielded a total of 65 bands of which 64 showed polymorphism (Figure 3.6, Figure 3.7 and Table A3.2). The number of polymorphic bands generated by a primer varied between 2 and 16 (Table 3.4). The size of the polymorphic amplified fragments ranged from around 325 to 2500 bp (ISSR 879). The high ratio of polymorphic bands demonstrates the large variation of the tested accessions. The differences of the total number of bands per primer in this study and Mondal's report (Mondal, 2002b) can be explained by the utilization of different types of *Tag* polymerase and PCR buffer.

ISSR amplification of these 71 genomic template DNAs with 7 ISSR primers yielded a total of 65 bands of which 64 showed polymorphism (Figure 3.7, Figure 3.8 and Table A3.2). The number of polymorphic bands generated by a primer varied between 2 and 16 (Table 3.4). The size of the polymorphic amplified fragments ranged from around 325 to 2500 bp (ISSR 879). The high ratio of polymorphic bands demonstrates the large variation of the tested accessions. The differences of the total number of bands per primer in this study

and Mondal's report (Mondal, 2002b) can be explained by the utilization of different types of *Tag* polymerase and PCR buffer.

Table 3.3: List of accessions showing polymorphic bands with ISSR markers and therefore used for analyzing the genetic diversity

Accession	Type	Currently local taxonomic status(1)	Accession	Type	Currently local taxonomic status
No1	Local	n/a	'France' tea	Local	n/a
No3	Local	n/a	Lung Phin	Local	Shan
No4	Local	n/a	Nam ngat 2	Local	Shan
No5	Local	n/a	Phu Tho 10	Local	n/a
No6	Local	n/a	Shan tuyet	Local	Shan
No7	Local	n/a	Small-leaved China tea BL	Local	Small-leaved China (S-l China)
No8	Local	n/a	Tan Chi Shan	Local	Shan
No9	Local	n/a	Tham Ve Shan	Local	Shan
No10	Local	n/a	Tien Phong	Local	n/a
No11	Local	n/a	To Hieu	Local	n/a
No13	Local	n/a	Suoi Giang 2	Wild giant	Shan
No15	Local	n/a	Suoi Giang purple leaf	Wild giant	Shan
No17	Local	n/a	Suoi Giang 6	Wild giant	Shan
No21	Local	n/a	Tua Chua TST	Wild giant	Shan
Chat tien	Local	Shan	Tua Chua PCO	Wild giant	Shan
Den Sang	Local	n/a	Ho Nam 3	Imported	n/a
1A	Selected	Assam	Kim Tuyen BL	Imported	S-l China
1A BL	Selected	Assam	Phuc An 1	Imported	S-l China
6A	Selected	n/a	Phuc Dinh 1	Imported	S-l China
A18	Selected	n/a	Phuc Dinh 3	Imported	S-l China
A42	Selected	n/a	Phuc Van 10	Imported	S-l China
Cu de phung x Trung du	Selected	Cross	Small-leaved China tea	Imported	S-l China
F16	Selected	n/a	Darjeeling 1	Imported	Assam
F35	Selected	n/a	Darjeeling 2	Imported	Assam
Large-leaved Nam ngat	Selected	Large-leaved China (L-l China)	Manipur Messai	Imported	Assam
$LD_{97}BL$	Selected	Shan	Swinglaybari	Imported	n/a
LDP_1	Selected	Cross	Japan 6	Imported	S-l China
LDP_2	Selected	Cross	Yabukita	Imported	S-l China
Minh Rong 1	Selected	n/a	Macomen	Imported	n/a
Minh Rong 2	Selected	n/a	Jetinga	Imported	Shan
Minh Rong 3	Selected	n/a	Sri Lanka 1	Imported	n/a
PH_1	Selected	Assam	TRI2025	Imported	Shan
PH_1BL	Selected	Assam	TRI777	Imported	Shan
TB_{14}	Selected	Shan	Ngoc Thuy	Imported	S-l China
$TB_{11}BL$	Selected	Shan	Ngoc Thuy BL	Imported	S-l China
$TB_{18}BL$	Selected	Shan			

(1) Taxonomic classification in this study is currently used locally according to Cohen-Stuart (1919); n/a: not available, i.e., the taxonomic classification is not clear or officially accepted by local production.

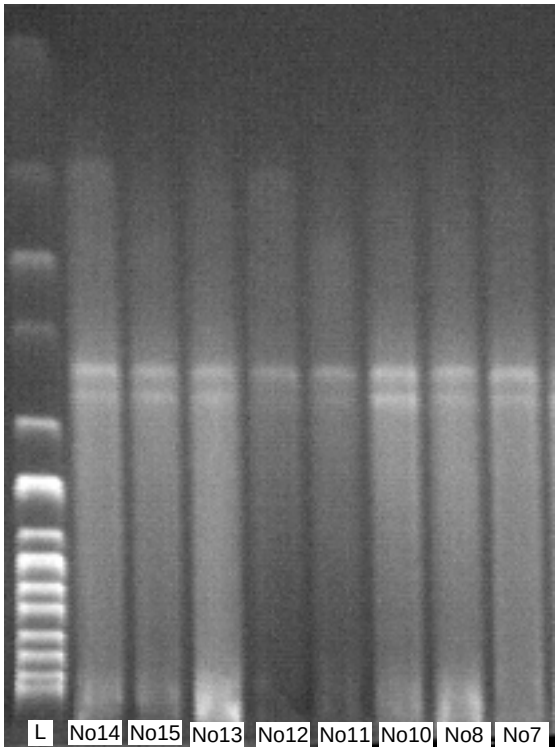


Figure 3.6: Amplification of genomic DNA of some accessions with primer ISSR-879

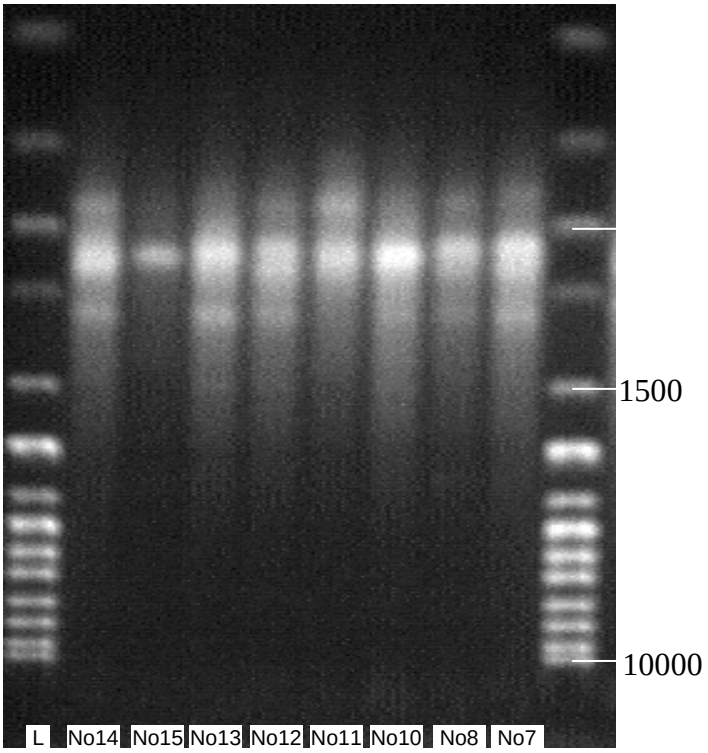


Figure 3.7: Amplification of genomic DNA of some accessions with primer ISSR-866

3.3.3 Cluster analysis

Dice similarity coefficient values are given as similarity matrix table (Table A3.3) and were used for UPGMA cluster analysis (Figure 3.8). The similarity matrix based on the proportion of shared fragments (Dice, 1945) was used to establish the level of relatedness between 71 tested accessions. Estimated similarity ranged from 0.09, between the most distant (dissimilar) accessions $LD_{97}BL$ (selected tea) and Yabukita (imported tea), to 1.00, between the closest (most similar) accessions Chat Tien (local tea) and Suoi Giang 6 (wild giant tea). The tested accessions exhibited more variation than in earlier reports (Wachira et al., 1995; Paul et al., 1997; Modal, 2002b). This great variation may be attributed to the wide variability in origin of the tested accessions. Conventionally, $LD_{97}BL$, Chat Tien and Suoi Giang 6 belong to shan tea, while Yabukita accession is classified as small-leaved China tea.

High frequency of missing data (Table A3.2) may have a certain influence on the result of cluster analysis.

Although the range of Dice similarity coefficient was large (between 0.09 and 1.00), the topology of the dendrogram using UPGMA failed in clearly separating the tested

accessions. However, except for the accessions of Darjeeling 1, TRI777, No6, No10, Minh Rong 3, Phuc An 1, Yabukita and Macomen remaining distinct and ungrouped, at about 50% similarity level, the dendrogram formed 4 clusters of 4, 46, 9 and 3 accessions, respectively (Figure 3.8), i.e., most accessions gathered into one large cluster (cluster 2).

Table 3.4: Number of amplification products generated with 7 ISSR primers

Code	Sequence	Total number of bands	Total number of polymorphic bands	Fragment size (bp)
ISSR-816	5'-CAC ACA CAC ACA CAC AT-3'	8	8	650 – 1500
ISSR-821	5'-GTG TGT GTG TGT GTG TT-3'	11	11	425 - 1200
ISSR-866	5'-CTC CTC CTC CTC CTC CTC-3'	3	2	650 - 1100
ISSR-868	5'-GAA GAA GAA GAA GAA GAA-3'	7	7	675 - 1500
ISSR-873	5'-GAC AGA CAG ACA GAC A-3'	15	15	350 – 1900
ISSR-878	5'-GGA TGG ATG GAT GGA T-3'	5	5	500 – 1300
ISSR-879	5'-CTT CAC TTC ACT TCA-3'	16	16	325 – 2500
	Total	65	64	

The dendrogram showed complicated results in comparison with the conventional classification. The results confirmed the closeness of many accessions, for example in cluster 2, there is the sub-group of India tea (PH_1, Manipur Messai, Sri Lanka 1 and PH_1BL), or the sub-groups of shan tea (sub-group of Chat Tien, Suoi Giang 6, purple-leaved Suoi Giang and Den sang; or sub-group of Shan tuyet, Suoi Giang 2 and Tua Chua TST), or sub-group of China tea (NgocThuy BL, KimTuyen BL, small-leaved China tea BL) in cluster 3.

The study also showed some differences to the conventional classification, i.e., TB_{14} was quite isolated with other known shan tea accessions (i.e., TB_{18}BL, LD_{97}BL, TB_{11}BL); or TRI777 and Yabukita were ungrouped. Previously, Tran and Nguyen (1998) reported that TRI777 originated from Cho Long Shan (Son La province). Beside that, the result also indicated some mistakes in the collection of tea plants (assuming that the sampling in this study was correct). For example, the imported clone Ngoc Thuy collected at Bao Loc genetically differed from the accession with the same name in the tea collection at Vietnam Tea research Institute.

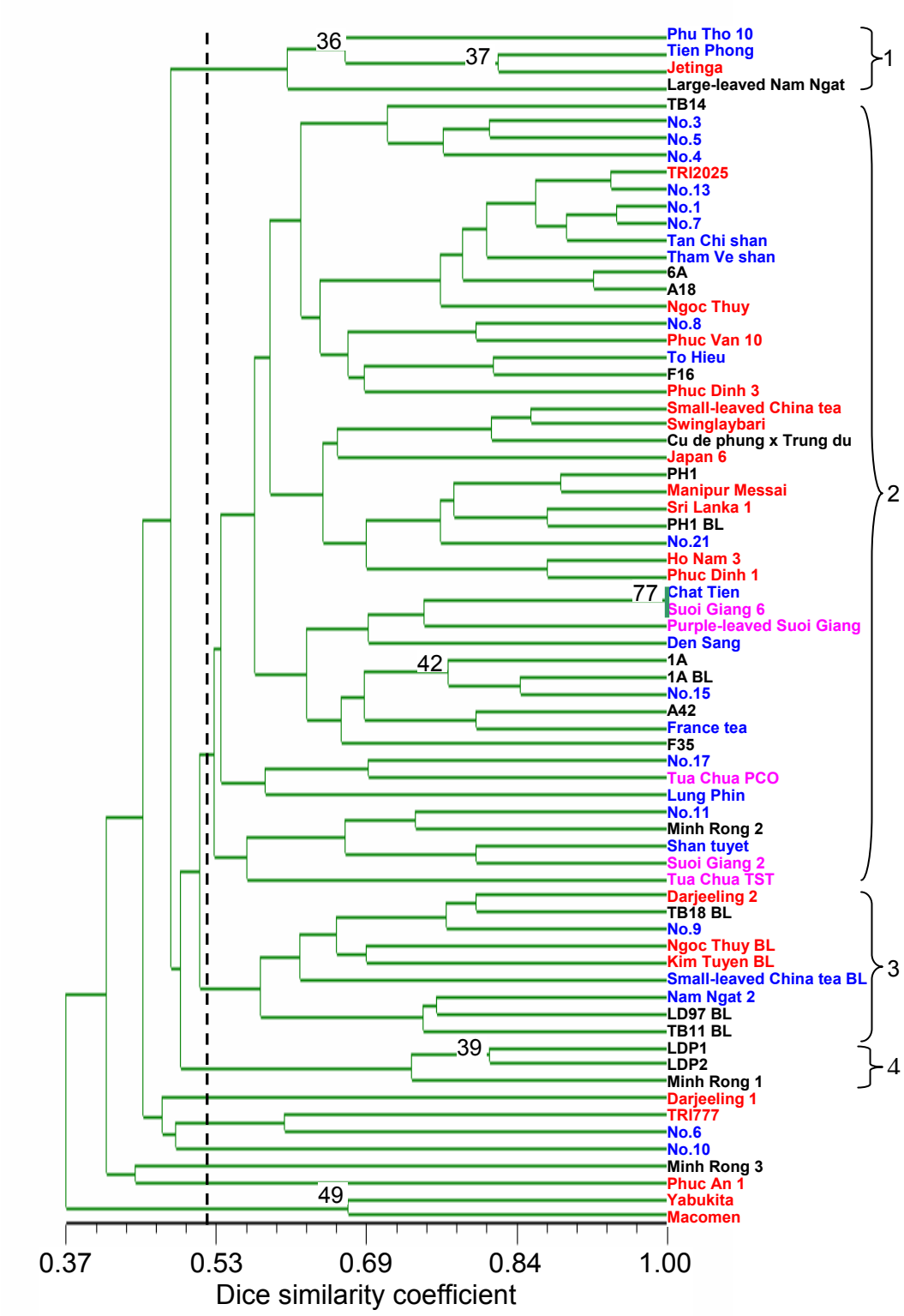


Figure 3.8: Dendrogram illustrating genetic relationships among 71 accessions of local (name in blue), wild (in pink), selected (in black) and imported (in red) teas, generated by UPGMA cluster analysis calculated from 65 ISSR markers produced by 7 primers. The figures attached indicate bootstrap values based on 4000 replicate analyses.

Figure 3.8 shows that the distances between clusters were very low, i.e., no clearly distinct groups could be identified. Also the results of bootstrap values based on 4000 replicate analyses were generally low. The large genetic variance within each group of accessions was the reason. Except for wild giant tea accessions which were expected to be shan tea, the groups of local, selected and imported teas involved accessions of all types of tea.

3.3.4 AMOVA analysis

The AMOVA analysis was used to partition the genetic variation by hierarchical analysis from the Pairwise difference distance matrix. AMOVA showed a significant (P = 0.05) genetic difference between local, wild, selected and imported tea accessions (Table 3.5). The variance within groups was much larger and contributed to 95.26% of the total variation.

Table 3.5: Analysis of molecular variance (AMOVA) for 71 accessions of tea using ISSR markers

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	*P*-value[(1)]
Among groups	3	5.054	0.04589	4.74	0.05
Within groups	67	61.777	0.92204	95.26	
Total	70	66.831	0.96793		
Fixation Index F_{ST}: 0.04741					0.05

[(1)] Significance tests after 4032 permutations

The results of analyzing the comparison of pairs of population samples showed that wild giant tea accessions significantly differ from selected and imported teas (data not shown). If the wild giant tea accessions were believed belonging only to Shan tea; most of accessions belong to local tea were conventionally classified as also shan tea, the accessions of selected and imported teas comprise nearly all four tea taxa, i.e., *C*. *sinensis* var. *microphylla*, var. *macrophylla*, var. *shan* and var. *assamica*. Therefore it was understandable when the variation within the populations was greatly large.

In conclusion, ISSR markers revealed the high variation among tested accessions; however, no clear cluster was identified. Most accessions including widely cultivated teas gathered in one large group. With some exceptions, the results commonly supported the conventional classification.

Appendices

Table A3.1: Basic information on the total of 144 collected accessions consisting of 47 local teas, 12 wild giant shan teas, 34 improved/selected teas and 51 imported tea cultivars/clones

Accessions	Planting materials	Year of plantation	Location(1)	Origin, acquisition place and date
No1	Stump	1958	LD Center	Local, Northern VN
No3	Stump	1958	LD Center	Local, Northern VN
No4	Stump	1958	LD Center	Local, Northern VN
No5	Stump	1958	LD Center	Local, Northern VN
No6	Stump	1958	LD Center	Local, Northern VN
No7	Stump	1958	LD Center	Local, Northern VN
No8	Stump	1958	LD Center	Local, Northern VN
No9	Stump	1958	LD Center	Local, Northern VN
No10	Stump	1958	LD Center	Local, Northern VN
No11	Stump	1968	Bao Loc	Local, Northern VN
No12	Stump	1968	Bao Loc	Local, Northern VN
No13	Stump	1968	Bao Loc	Local, Northern VN
No14	Stump	1968	Bao Loc	Local, Northern VN
No15	Stump	1968	Bao Loc	Local, Northern VN
No16	Stump	1968	Bao Loc	Local, Northern VN
No17	Stump	1968	Bao Loc	Local, Northern VN
No18	Stump	1968	Bao Loc	Local, Northern VN
No19	Stump	1968	Bao Loc	Local, Northern VN
No21	Stump	1968	Bao Loc	Local, Northern VN
Ba Vi	Cutting	1991	VNTI	Local, Ha Tay, 1918
Chat tien	Cutting	1991	VNTI	Local, Ha Giang, 1918
Den Sang	Cutting		VNTI	Local
‘France’ tea	Seed		Da Lat	Local
Gia Vai	Cutting	1991	VNTI	Local, Ha Giang, 1918
Green Cudephung	Cutting	1991	VNTI	Local, Ha Giang, 1935
Green Trung du	Cutting	1991	VNTI	Local, Phu Tho, 1918
Hoc Mon	Cutting	1991	VNTI	Local, Southern VN, 1918
Huong tich son	Cutting		VNTI	Local
Lao Chay	Cutting	1991	VNTI	Local, Ha Giang, 1918
Lung Phin	Cutting		VNTI	Local, Ha Giang, 1996
Mixed Trung du	Cutting	1990	VNTI	Local, Phu Tho, 1918
Moc Chau Shan	Cutting	1990	VNTI	Local, Son La, 1990
Nam ngat 2	Cutting	1991	VNTI	Local, Ha Giang, 1918

Table A3.1 (cont.)

Cultivars	Planting materials	Year of cultivation	Location[(1)]	Origin, acquisition place and date
Nam Ty	Cutting		VNTI	Local
Phu Tho 10	Cutting		LD Center	Local, Phu Tho
Purple-leaved Trung du	Cutting	1991	VNTI	Local, Phu Tho, 1918
Shan tuyet	Seed	1927	Da Lat	Local, Northern VN
Small-leaved China tea BL	Seed		Bao Loc	Local
Su Neo	Cutting		VNTI	Local
Tam Dao	Cutting		VNTI	Local
Tan Chi Shan	Cutting	1991	VNTI	Local, Lang Son, 1963
Tan Cuong	Cutting	1991	VNTI	Local, Thai Nguyen, 1965
Tham Ve Shan	Cutting	1991	VNTI	Local, Ha Giang, 1918
Tien Phong	Cutting		VNTI	Local
To Hieu	Cutting	1990	VNTI	Local, Lang Son, 1963
Trung du Thanh Duc	Cutting	1991	VNTI	Local, Phu Tho, 1918
Yellow Trung du	Cutting	1991	VNTI	Local, Phu Tho, 1918
Suoi Giang	Cutting		VNTI	Wild, Yen Bai, 1997
Suoi Giang 1			Yen Bai	Wild
Suoi Giang 2			Yen Bai	Wild
Purple-leaved Suoi Giang			Yen Bai	Wild
Suoi Giang 4			Yen Bai	Wild
Suoi Giang 5			Yen Bai	Wild
Suoi Giang 6			Yen Bai	Wild
Ta xua	Cutting		VNTI	Wild, Son La, 1996
Tua Chua 1			Dien Bien	Wild
Tua Chua TST			Dien Bien	Wild
Tua Chua PCO			Dien Bien	Wild
Tua Chua 4			Dien Bien	Wild
1A BL	Cutting		LD Center	Selected, Phu Tho
1A	Cutting	1990	VNTI	Selected, Phu Tho, 1970
5A	Cutting	1991	VNTI	Selected, Phu Tho, 1970
6A	Cutting	1990	VNTI	Selected, Phu Tho, 1970
A18	Cutting	1991	VNTI	Selected, Phu Tho, 1970
A42	Cutting	1991	VNTI	Selected, Phu Tho, 1970
Cu de phung x Trung du	Cutting	1991	VNTI	Selected, Phu Tho, 1988
Dai Bach tra x PH1	Cutting	1991	VNTI	Selected, Phu Tho, 1988
F16	Cutting	1991	VNTI	Selected, Phu Tho, 1970
F23	Cutting	1991	VNTI	Selected, Phu Tho, 1970
F35	Cutting	1991	VNTI	Selected, Phu Tho, 1970
Free-crossed PH1	Cutting	1991	VNTI	Selected, Phu Tho, 1988

Table A3.1 (cont.)

Cultivars	Planting materials	Year of cultivation	Location(1)	Origin, acquisition place and date
Jetinga 2	Cutting	1991	VNTI	Selected, Phu Tho, 1989
Large-leaved Nam ngat	Cutting	1991	VNTI	Selected, Phu Tho, 1990
LDP1	Cutting	1997	LD Center	Selected, VNTI
LDP1	Cutting		VNTI	Selected, Phu Tho, 1988
LDP2	Cutting	1997	LD Center	Selected, VNTI
LDP2	Cutting		VNTI	Selected, Phu Tho, 1988
LD97 BL	Cutting	1997	LD Center	Selected, Lam Dong, 1997
Manipur	Cutting	1991	VNTI	Selected, Phu Tho, 1970
Minh Rong 1	Cutting		VNTI	Selected, Lam Dong, 1996
Minh Rong 2	Cutting		VNTI	Selected, Lam Dong, 1996
Minh Rong 3	Cutting		VNTI	Selected, Lam Dong, 1996
PH1 BL	Cutting	1997	LD Center	Selected, VNTI
PH1	Cutting	1990	VNTI	Selected, Phu Tho, 1970
PH1 x Dai Bach tra	Cutting	1991	VNTI	Selected, Phu Tho, 1988
PT 95	Cutting		VNTI	Selected, Phu Tho
S28	Cutting	1991	VNTI	Selected, Phu Tho, 1970
TB11 BL	Cutting	1996	LD Center	Selected, Lam Dong
TB11	Cutting		VNTI	Selected, Lam Dong, 1996
TB14	Cutting		VNTI	Selected, Lam Dong, 1996
TB14 BL	Cutting	1997	LD Center	Selected, lam Dong
TB14*	Cutting	1998	Da Lat	Selected, Lam Dong
TB18 BL	Cutting		Bao Loc	Selected
Brao cu	Cutting		VNTI	Imported, China, 1996
Dai Bach tra	Cutting	1991	VNTI	Imported, China, 1963
Ho Nam 1	Cutting		VNTI	Imported, China, 1995
Ho Nam 2	Cutting		VNTI	Imported, China, 1995
Ho Nam 3	Cutting		VNTI	Imported, China, 1995
Kasarigou	Cutting	1991	VNTI	Imported, China, 1919
Ky Mon	Cutting	1991	VNTI	Imported, China, 1959
Phuc An 1	Cutting		VNTI	Imported, China, 1996
Phuc An 2	Cutting		VNTI	Imported, China, 1996
Phuc Dinh 1	Cutting		VNTI	Imported, China, 1996
Phuc Dinh 2	Cutting		VNTI	Imported, China, 1996
Phuc Dinh 3	Cutting		VNTI	Imported, China, 1996
Phuc Van 10	Cutting		VNTI	Imported, China, 1996
Small-leaved China tea	Cutting	1990	VNTI	Imported, China, 1922
Triet Giang 1	Cutting		VNTI	Imported, China, 1996
Triet Giang 2	Cutting		VNTI	Imported, China, 1996

Table A3.1 (cont.)

Cultivars	Planting materials	Year of cultivation	Location[(1)]	Origin, acquisition place and date
Triet Giang 3	Cutting		VNTI	Imported, China, 1996
Triet Giang 4	Cutting		VNTI	Imported, China, 1996
Triet Giang 5	Cutting		VNTI	Imported, China, 1996
Konkhitda	Cutting	1989	VNTI	Imported, Georgia, 1989
Small-leaved Japan tea	Cutting	1989	VNTI	Imported, Georgia, 1989
ACT 49	Cutting		VNTI	Imported, India, 1997
ACT63	Cutting		VNTI	Imported, India, 1997
Assam	Cutting	1991	VNTI	Imported, India, 1918
Darjeeling 1	Cutting		VNTI	Imported, India
Darjeeling 2	Cutting		VNTI	Imported, India
Manipur Dangri	Cutting	1991	VNTI	Imported, India, 1918
Manipur Messai	Cutting	1991	VNTI	Imported, India, 1918
Monochai	Cutting	1991	VNTI	Imported, India, 1919
Swinglaybari	Cutting	1991	VNTI	Imported, India, 1919
Japan 6	Cutting		VNTI	Imported, Japan
Japan 7	Cutting		VNTI	Imported, Japan
Japan 8	Cutting		VNTI	Imported, Japan
Yabukita	Cutting	1997	LD Center	Imported, Japan, 1991
Macomen	Cutting	1991	VNTI	Imported, Laos, 1923
Pousang	Cutting	1991	VNTI	Imported, Laos, 1923
Jetinga	Cutting	1991	VNTI	Imported, Myanmar, 1919
Sri Lanka 1	Cutting		VNTI	Imported, Sri Lanka
Sri Lanka 2	Cutting		VNTI	Imported, Sri Lanka
TRI2023	Cutting	1991	VNTI	Imported, Sri Lanka, 1970
TRI2024	Cutting		VNTI	Imported, Sri Lanka
TRI2025	Cutting	1991	VNTI	Imported, Sri Lanka, 1977
TRI2043	Cutting	1991	VNTI	Imported, Sri Lanka, 1977
TRI777	Cutting	1990	VNTI	Imported, Sri Lanka, 1977
Kim Tuyen BL	Cutting	1996	Bao Lam	Imported, Taiwan
Ngoc Thuy	Cutting		VNTI	Imported, Taiwan, 1995
Ngoc Thuy BL	Cutting	1996	Bao Lam	Imported, Taiwan
Oolong Thanh Tam	Cutting		VNTI	Imported, Taiwan, 1995
Phuc Van Tien	Cutting		VNTI	Imported, Taiwan
Thiet bao tra	Cutting		VNTI	Imported, Taiwan
Tu Quy BL	Cutting	1996	Bao Lam	Imported, Taiwan

[(1)] VNTI: Vietnam National Tea Institute, Phu Tho province;

LD center: Lam Dong Center for Research and Transfer techniques on Industrial crops and Fruit trees Production; Other names: private name of the places or provinces.

Table A3.2: The presence and absence of polymorphic bands generated from 71 accessions with 7 ISSR primers (1: presence; 0: absence; ?: missing data)

ChatTien	10001000	1100000	000001000001000	0001000000000000	00001000000	???	?????
DenSang	00001000	1110000	000000000001000	0000001000100000	00001000000	011	00011
Francetea	00101000	1110000	000000000000100	0001000000000000	00001000000	011	00010
LungPhin	10001000	1110000	000000000001000	1001000000011000	00000100000	010	00100
No1	00100000	1100000	????????????????	????????????????	00101100000	011	00011
No3	10100000	1010000	001000010000100	0000000000000100	00001100000	010	00011
No4	10100000	1000000	001100100000000	????????????????	00001100000	010	00011
No5	10100000	1100000	001000010100000	0000000000000100	00001100000	110	00011
No6	00000001	1100000	000101000000000	0000000000101000	00101000000	010	10011
No7	00100000	1100000	????????????????	????????????????	00001100000	011	00011
No8	10000000	1000000	????????????????	????????????????	00101100001	010	01011
No9	00101010	1100000	????????????????	0001000010000000	01001010100	011	00011
No10	10000000	0100000	000001000000000	0000000000000100	00101000010	111	00011
No11	00101001	1110000	000000010001010	1101011000000000	00101100000	011	00011
No13	00100000	1100000	000000000001010	????????????????	00101100000	010	00011
No15	00101000	1110000	????????????????	0011000000000000	01001010100	010	00010
No17	10101000	1110000	000001000001000	0001010110010000	??????????	???	00011
No21	10100000	1100000	????????????????	0010000000000000	00001010000	010	00010
PhuTho10	00000001	1110000	111011010000000	0000000000000100	??????????	011	10010
SChinaBL	00101000	1110000	????????????????	1000110000000000	01000010100	010	00010
Shantuyet	00001001	1110000	000000001100000	0000000010000000	00101100000	010	00010
TanChiShan	00100000	1100000	????????????????	0001000000000000	00100100000	011	?????
ThamVeShan	00101000	1100000	000000000001000	0001000001000000	00100100000	011	?????
TienPhong	00100000	1110000	101001000000000	0000000000000100	00000100000	???	10010
ToHieu	10100000	0100000	????????????????	0001000000000000	00101100000	010	10000
PlSuoiGiang	10001000	1110000	????????????????	0000100000000000	00101000000	011	?????
SuoiGiang2	00001001	1010000	????????????????	0000000000000000	00001100000	011	00010
SuoiGiang6	10001000	1100000	????????????????	0001000000000000	00001000000	011	00010
TuaChuaPCO	10101000	1010001	000010000011000	0101010000110010	00001100000	010	00011
TuaChuaTST	00000001	0110001	000000000001001	0001100000010000	00001100000	010	00011
1A	00101000	1110000	000000000001100	0101000000000000	00001000000	010	10000
1ABL	00101000	1110000	000000000001000	0101000000000000	01001000100	010	10010
6A	00101000	1100000	000000000000100	0001000000000000	00001100000	010	?????
A18	00100000	1100000	????????????????	????????????????	00001100000	010	00010
A42	00101000	0100000	????????????????	0001000000000000	00001000011	011	00010
CDPxTD	10100000	1000000	????????????????	????????????????	00001000011	011	00010
F16	10101000	1110000	????????????????	0001000000100000	00101100000	010	10000
F35	00110100	1110000	000000000001100	0101010000000000	00001000000	011	00011
KimTuyenBL	00100000	1101011	000000000001000	1011100000000000	01001000100	011	10010
LD97BL	????????	???????	000001001001011	1011011100000000	01001010000	010	00011
LDP1	00100001	1100010	000000010001000	0010010011100001	??????????	???	00010
LDP2	00000001	1100000	000000010001001	0011010010100001	??????????	???	00010
LlNamNgat	00101000	1010010	????????????????	0000010000000000	00000100010	011	00010
MinhRong1	00100001	1100010	000000010001000	0000010010010000	00001100000	010	00010
MinhRong2	00001001	1110010	000000000001010	0000001110000000	00101100000	010	00011
MinhRong3	00001000	0100000	000001000001010	0000000000010000	00001000000	010	?????

NamNgat2	????????	???????	000001001001000	1001010000001000	00001010000	010 00011
NgocThuyBL	00001000	1110011	000001010001000	0011000000000000	01001001000	011 00010
PH1	10100000	1100000	000000010001000	????????????????	00001100000	010 00011
PH1BL	10100000	1100000	????????????????	0001000000000000	01001000000	010 ?????
TB11BL	????????	???????	000000001001001	1011010010000000	00001010010	011 00011
TB14	10101000	1110000	001001100000000	0000000000000100	00001000000	010 10011
TB18BL	10101000	1110011	000000000001000	1001000010000000	01001010100	010 00011
Darjeeling1	01010011	1100000	000001000000000	????????????????	00001100000	011 00000
Darjeeling2	00100000	1100011	000000010001000	0000000010000000	01001010100	010 ?????
HoNam3	10010100	1000000	000000010011000	1010001000000000	01001000000	010 00010
Japan6	10100000	???????	????????????????	1110111000000000	00001000010	010 00110
Jetinga	00100000	1110000	001000000000000	????????????????	00000100000	110 00010
Macomen	00100000	0100000	001000000000000	????????????????	00001000110	110 01000
ManipurMessai	10101000	1100000	000000010001000	1011100010100000	???????????	010 00010
NgocThuy	00100000	1100000	000000000010100	0000000000000100	00100100000	011 00010
PhucAn1	10010100	0100000	000000010001000	????????????????	10011000100	010 ?????
PhucDinh1	10010000	1100000	????????????????	????????????????	01001000000	010 00010
PhucDinh3	10100000	1100000	000010010001000	1000001010000000	00101000000	010 10000
PhucVan10	10010000	1100000	000010000000000	????????????????	00101100000	010 ?????
Schina	00000000	1000000	000001010000000	????????????????	???????????	011 00010
SriLanka1	10101000	1100000	000000010000100	????????????????	01001000100	010 00010
Swinglaybari	10000000	1100000	000001010000000	????????????????	???????????	011 00010
TRI2025	00100000	1100000	????????????????	????????????????	00101000000	010 00011
TRI777	00001001	1110000	011000000010000	0000000000101110	00100100000	010 00011
Yabukita	00100000	1100100	001000000000000	0000000000000110	00000000100	??? 01010

Table A3.3: Dice similarity coefficient matrix of 71 tested accessions based on the presence or absence of ISSR markers generated with 7 primers

	Phu Tho 10	Tien Phong	TB14	Small-leaved China tea	Darjeeling1	Swinglaybari	Yabukita	Macomen	Jetinga
Phu Tho 10	1.00								
Tien Phong	0.78	1.00							
TB14	0.62	0.72	1.00						
Small-leaved China tea	0.60	0.46	0.42	1.00					
Darjeeling1	0.52	0.42	0.40	0.53	1.00				
Swinglaybari	0.64	0.53	0.57	0.86	0.59	1.00			
Yabukita	0.45	0.57	0.50	0.36	0.24	0.46	1.00		
Macomen	0.30	0.35	0.43	0.17	0.30	0.29	0.67	1.00	
Jetinga	0.55	0.82	0.61	0.43	0.40	0.50	0.67	0.56	1.00
TRI2025	0.57	0.57	0.74	0.60	0.44	0.67	0.57	0.50	0.63
TRI777	0.60	0.52	0.56	0.35	0.42	0.42	0.46	0.27	0.64
Ngoc Thuy	0.50	0.60	0.46	0.57	0.48	0.63	0.53	0.32	0.63
No1	0.67	0.67	0.67	0.73	0.60	0.77	0.53	0.44	0.67
No3	0.54	0.61	0.71	0.50	0.35	0.56	0.45	0.38	0.67
No4	0.35	0.50	0.72	0.40	0.36	0.47	0.44	0.40	0.60
No5	0.52	0.61	0.69	0.47	0.42	0.63	0.55	0.55	0.73
No6	0.54	0.43	0.57	0.53	0.55	0.59	0.27	0.30	0.40
No7	0.67	0.71	0.70	0.73	0.63	0.77	0.57	0.47	0.71
No8	0.43	0.38	0.57	0.60	0.40	0.67	0.38	0.33	0.44
No10	0.50	0.40	0.59	0.57	0.45	0.75	0.32	0.50	0.40
No11	0.48	0.40	0.50	0.53	0.52	0.57	0.28	0.32	0.56
No13	0.36	0.50	0.56	0.43	0.45	0.50	0.44	0.40	0.60
No17	0.36	0.48	0.64	0.43	0.35	0.63	0.33	0.29	0.63

Table A3.3 (cont.)

	Phu Tho 10	Tien Phong	TB14	Small-leaved China tea	Darjeeling1	Swinglaybari	Yabukita	Macomen	Jetinga
Chat Tien	0.33	0.35	0.63	0.44	0.50	0.73	0.25	0.31	0.31
Den Sang	0.46	0.38	0.59	0.53	0.48	0.59	0.30	0.32	0.53
Lung Phin	0.30	0.35	0.43	0.29	0.40	0.50	0.18	0.22	0.56
Minh Rong 3	0.30	0.25	0.50	0.36	0.44	0.46	0.13	0.40	0.27
Shan tuyet	0.48	0.43	0.50	0.40	0.52	0.47	0.27	0.29	0.57
Tan Chi Shan	0.62	0.67	0.47	0.75	0.59	0.80	0.46	0.43	0.71
Tham Ve Shan	0.36	0.44	0.43	0.50	0.50	0.57	0.35	0.35	0.59
To Hieu	0.40	0.50	0.57	0.22	0.44	0.55	0.24	0.50	0.50
Purple-leaved Suoi Giang	0.67	0.43	0.74	0.60	0.53	0.83	0.27	0.38	0.50
Suoi Giang 2	0.75	0.53	0.57	0.73	0.63	0.62	0.25	0.24	0.59
Tua Chua TST	0.37	0.33	0.41	0.27	0.45	0.35	0.17	0.30	0.50
Tua Chua PCO	0.30	0.33	0.51	0.33	0.32	0.40	0.28	0.26	0.52
1A	0.38	0.45	0.59	0.27	0.38	0.35	0.29	0.42	0.53
6A	0.30	0.47	0.57	0.36	0.53	0.46	0.38	0.50	0.63
A18	0.62	0.77	0.67	0.67	0.59	0.73	0.62	0.53	0.80
A42	0.50	0.38	0.55	0.60	0.42	0.67	0.35	0.59	0.47
CDPxTD	0.57	0.43	0.60	0.80	0.42	0.83	0.43	0.47	0.47
F16	0.53	0.60	0.72	0.33	0.48	0.57	0.29	0.42	0.63
F35	0.40	0.40	0.52	0.44	0.50	0.50	0.33	0.36	0.55
Large-leaved Nam Ngat	0.56	0.59	0.52	0.67	0.40	0.57	0.33	0.33	0.67
LDP1	0.37	0.33	0.30	0.50	0.40	0.57	0.35	0.33	0.57
LDP2	0.38	0.26	0.23	0.55	0.43	0.62	0.27	0.18	0.46
Minh Rong 1	0.44	0.42	0.41	0.53	0.55	0.59	0.35	0.40	0.60
Minh Rong 2	0.41	0.37	0.50	0.35	0.48	0.42	0.23	0.26	0.52

Table A3.3 (cont.)

	Phu Tho 10	Tien Phong	TB14	Small-leaved China tea	Darjeeling1	Swinglaybari	Yabukita	Macomen	Jetinga
PH1	0.43	0.50	0.64	0.53	0.45	0.71	0.44	0.40	0.60
Suoi Giang 6	0.59	0.40	0.67	0.73	0.56	0.92	0.38	0.38	0.50
France tea	0.48	0.50	0.62	0.53	0.48	0.59	0.42	0.42	0.63
Ho Nam 3	0.30	0.17	0.34	0.53	0.36	0.59	0.17	0.20	0.30
Phuc An 1	0.32	0.11	0.36	0.33	0.36	0.57	0.25	0.42	0.21
Phuc Dinh 3	0.44	0.33	0.48	0.40	0.36	0.59	0.26	0.40	0.40
Phuc Van 10	0.44	0.38	0.50	0.36	0.60	0.62	0.29	0.35	0.47
Manipur Messai	0.33	0.33	0.48	0.53	0.33	0.71	0.35	0.40	0.59
Sri Lanka 1	0.43	0.38	0.62	0.53	0.35	0.71	0.53	0.48	0.48
Darjeeling 2	0.36	0.29	0.40	0.46	0.35	0.53	0.40	0.50	0.40
TB18BL	0.33	0.34	0.59	0.33	0.30	0.50	0.36	0.40	0.48
Ngoc Thuy BL	0.55	0.38	0.50	0.67	0.46	0.70	0.24	0.25	0.42
PH1BL	0.46	0.46	0.71	0.50	0.47	0.80	0.43	0.57	0.57
1ABL	0.46	0.50	0.62	0.40	0.35	0.47	0.43	0.48	0.57
Small-leaved China tea BL	0.53	0.50	0.56	0.55	0.30	0.62	0.48	0.44	0.67
Kim Tuyen BL	0.40	0.37	0.42	0.47	0.40	0.53	0.38	0.43	0.43
No9	0.50	0.38	0.59	0.62	0.52	0.67	0.45	0.48	0.48
No15	0.56	0.50	0.64	0.55	0.38	0.62	0.48	0.53	0.63
No21	0.50	0.50	0.67	0.60	0.44	0.83	0.47	0.50	0.63
Phuc Dinh 1	0.57	0.43	0.63	0.60	0.56	0.83	0.43	0.38	0.50
Japan 6	0.24	0.24	0.45	0.50	0.27	0.67	0.22	0.57	0.50
Nam Ngat 2	0.29	0.22	0.48	0.55	0.46	0.55	0.12	0.27	0.31
LD97BL	0.24	0.17	0.38	0.46	0.38	0.46	0.09	0.22	0.25
TB11BL	0.26	0.10	0.33	0.50	0.40	0.50	0.11	0.35	0.27

Table A3.3 (cont.)

	TRI2025	TRI777	Ngoc Thuy	No1	No3	No4	No5	No6	No7	No8	No10	No11	No13	No17	Chat Tien	Den Sang
TRI2025	1.00															
TRI777	0.67	1.00														
Ngoc Thuy	0.75	0.57	1.00													
No1	0.89	0.70	0.89	1.00												
No3	0.71	0.53	0.58	0.74	1.00											
No4	0.75	0.50	0.48	0.78	0.78	1.00										
No5	0.78	0.52	0.56	0.80	0.81	0.75	1.00									
No6	0.82	0.60	0.42	0.74	0.38	0.55	0.44	1.00								
No7	0.82	0.63	0.82	0.95	0.78	0.82	0.84	0.67	1.00							
No8	0.67	0.60	0.56	0.70	0.74	0.78	0.70	0.63	0.63	1.00						
No10	0.67	0.41	0.52	0.70	0.48	0.45	0.62	0.56	0.63	0.60	1.00					
No11	0.76	0.53	0.50	0.87	0.53	0.52	0.51	0.47	0.82	0.61	0.42	1.00				
No13	0.94	0.58	0.67	0.95	0.61	0.64	0.67	0.64	0.89	0.74	0.55	0.81	1.00			
No17	0.77	0.41	0.36	0.77	0.48	0.56	0.48	0.40	0.77	0.62	0.48	0.65	0.71	1.00		
Chat Tien	0.60	0.27	0.25	0.55	0.33	0.40	0.44	0.47	0.60	0.55	0.53	0.48	0.53	0.70	1.00	
Den Sang	0.71	0.55	0.43	0.74	0.48	0.48	0.46	0.56	0.78	0.53	0.50	0.67	0.67	0.58	0.63	1.00
Lung Phin	0.38	0.47	0.33	0.44	0.38	0.40	0.37	0.31	0.47	0.44	0.24	0.53	0.50	0.62	0.63	0.48
Minh Rong 3	0.60	0.26	0.22	0.50	0.21	0.25	0.30	0.44	0.55	0.36	0.44	0.44	0.63	0.53	0.67	0.56
Shan tuyet	0.67	0.60	0.50	0.70	0.46	0.43	0.52	0.54	0.63	0.60	0.40	0.59	0.61	0.50	0.42	0.56
Tan Chi Shan	0.77	0.50	0.88	0.93	0.50	0.62	0.59	0.50	0.86	0.57	0.47	0.67	0.86	0.53	0.50	0.47
Tham Ve Shan	0.71	0.46	0.67	0.88	0.36	0.44	0.43	0.38	0.80	0.53	0.38	0.67	0.78	0.60	0.59	0.57
To Hieu	0.63	0.35	0.56	0.67	0.53	0.63	0.60	0.50	0.59	0.56	0.50	0.52	0.71	0.44	0.62	0.30
Purple-leaved Suoi Giang	0.67	0.55	0.56	0.71	0.56	0.53	0.53	0.56	0.63	0.63	0.63	0.62	0.63	0.59	0.71	0.74
Suoi Giang 2	0.47	0.61	0.56	0.63	0.63	0.59	0.50	0.50	0.67	0.53	0.40	0.67	0.56	0.44	0.50	0.70

Table A3.3 (cont.)

	TRI2025	TRI777	Ngoc Thuy	No1	No3	No4	No5	No6	No7	No8	No10	No11	No13	No17	Chat Tien	Den Sang
Tua Chua TST	0.59	0.45	0.32	0.63	0.44	0.45	0.43	0.44	0.67	0.53	0.38	0.57	0.64	0.54	0.42	0.54
Tua Chua PCO	0.63	0.54	0.39	0.67	0.55	0.64	0.47	0.36	0.70	0.67	0.31	0.63	0.64	0.69	0.48	0.56
1A	0.63	0.34	0.43	0.56	0.48	0.38	0.38	0.40	0.59	0.33	0.25	0.61	0.57	0.56	0.67	0.58
6A	0.77	0.42	0.63	0.80	0.60	0.59	0.57	0.42	0.86	0.57	0.32	0.57	0.71	0.53	0.63	0.53
A18	0.80	0.59	0.80	0.82	0.75	0.80	0.82	0.63	0.88	0.59	0.47	0.70	0.88	0.67	0.60	0.63
A42	0.59	0.33	0.53	0.63	0.40	0.47	0.48	0.38	0.67	0.42	0.57	0.57	0.56	0.56	0.62	0.57
CDPxTD	0.59	0.32	0.59	0.63	0.67	0.71	0.63	0.44	0.67	0.63	0.63	0.55	0.56	0.67	0.55	0.56
F16	0.63	0.59	0.55	0.67	0.61	0.63	0.58	0.58	0.60	0.57	0.42	0.65	0.70	0.64	0.71	0.58
F35	0.74	0.36	0.52	0.76	0.55	0.50	0.47	0.41	0.80	0.48	0.43	0.70	0.67	0.64	0.50	0.64
Large-leaved Nam Ngat	0.44	0.48	0.60	0.60	0.57	0.56	0.45	0.27	0.63	0.40	0.36	0.62	0.53	0.60	0.29	0.55
LDP1	0.73	0.36	0.38	0.73	0.33	0.38	0.42	0.42	0.73	0.36	0.20	0.53	0.67	0.48	0.30	0.43
LDP2	0.67	0.37	0.30	0.67	0.26	0.27	0.35	0.43	0.67	0.44	0.21	0.55	0.57	0.50	0.42	0.45
Minh Rong 1	0.71	0.39	0.48	0.74	0.52	0.55	0.57	0.44	0.78	0.53	0.31	0.63	0.73	0.62	0.40	0.46
Minh Rong 2	0.70	0.59	0.43	0.73	0.47	0.48	0.45	0.53	0.67	0.64	0.41	0.74	0.80	0.64	0.45	0.69
PH1	0.82	0.50	0.57	0.84	0.78	0.73	0.83	0.55	0.89	0.74	0.55	0.74	0.82	0.78	0.67	0.67
Suoi Giang 6	0.63	0.43	0.56	0.67	0.53	0.63	0.60	0.50	0.71	0.56	0.60	0.59	0.59	0.63	1.00	0.70
France tea	0.71	0.43	0.64	0.74	0.58	0.48	0.48	0.42	0.78	0.42	0.43	0.63	0.57	0.61	0.63	0.70
Ho Nam 3	0.50	0.26	0.32	0.44	0.44	0.45	0.43	0.30	0.47	0.56	0.31	0.46	0.45	0.31	0.40	0.46
Phuc An 1	0.40	0.18	0.20	0.35	0.38	0.30	0.45	0.32	0.38	0.38	0.40	0.40	0.40	0.43	0.47	0.42
Phuc Dinh 3	0.75	0.26	0.40	0.67	0.44	0.45	0.50	0.44	0.59	0.56	0.38	0.57	0.64	0.46	0.50	0.46
Phuc Van 10	0.71	0.50	0.56	0.75	0.53	0.56	0.60	0.59	0.67	0.80	0.56	0.52	0.67	0.46	0.53	0.47
Manipur Messai	0.77	0.40	0.42	0.71	0.46	0.56	0.52	0.38	0.71	0.62	0.33	0.61	0.71	0.62	0.60	0.54
Sri Lanka 1	0.67	0.40	0.55	0.60	0.67	0.52	0.64	0.43	0.63	0.50	0.43	0.57	0.52	0.67	0.59	0.55
Darjeeling 2	0.63	0.21	0.35	0.56	0.42	0.38	0.48	0.35	0.59	0.35	0.26	0.44	0.57	0.48	0.40	0.43

Table A3.3 (cont.)

	TRI2025	TRI777	Ngoc Thuy	No1	No3	No4	No5	No6	No7	No8	No10	No11	No13	No17	Chat Tien	Den Sang
TB18BL	0.61	0.39	0.33	0.56	0.50	0.52	0.48	0.38	0.58	0.48	0.39	0.60	0.59	0.76	0.58	0.58
Ngoc Thuy BL	0.50	0.35	0.36	0.55	0.40	0.31	0.39	0.40	0.57	0.36	0.41	0.58	0.46	0.59	0.64	0.62
PH1BL	0.77	0.30	0.50	0.67	0.63	0.77	0.71	0.50	0.71	0.57	0.47	0.50	0.71	0.63	0.77	0.47
1ABL	0.63	0.39	0.40	0.57	0.44	0.43	0.43	0.44	0.60	0.38	0.31	0.63	0.61	0.64	0.63	0.62
Small-leaved China tea BL	0.56	0.44	0.45	0.50	0.43	0.44	0.42	0.33	0.53	0.30	0.25	0.58	0.53	0.64	0.35	0.50
Kim Tuyen BL	0.57	0.23	0.41	0.61	0.32	0.40	0.38	0.39	0.64	0.35	0.33	0.51	0.56	0.43	0.45	0.47
No9	0.67	0.41	0.50	0.70	0.48	0.57	0.54	0.46	0.73	0.43	0.46	0.61	0.64	0.73	0.59	0.62
No15	0.63	0.44	0.45	0.57	0.52	0.53	0.50	0.42	0.60	0.38	0.33	0.58	0.60	0.67	0.59	0.58
No21	0.75	0.35	0.56	0.67	0.63	0.75	0.70	0.50	0.71	0.56	0.50	0.44	0.71	0.53	0.62	0.50
Phuc Dinh 1	0.63	0.44	0.50	0.56	0.59	0.63	0.67	0.59	0.59	0.56	0.56	0.48	0.59	0.62	0.73	0.59
Japan 6	0.62	0.17	0.30	0.53	0.48	0.71	0.45	0.27	0.57	0.50	0.43	0.57	0.57	0.40	0.29	0.38
Nam Ngat 2	0.80	0.33	0.20	0.67	0.38	0.50	0.36	0.55	0.73	0.62	0.45	0.59	0.63	0.67	0.62	0.50
LD97BL	0.73	0.21	0.16	0.62	0.31	0.42	0.30	0.37	0.67	0.57	0.37	0.63	0.63	0.64	0.44	0.48
TB11BL	0.67	0.22	0.26	0.71	0.33	0.44	0.32	0.32	0.77	0.53	0.48	0.60	0.56	0.63	0.40	0.52

Table A3.3 (cont.)

	Lung Phin	Minh Rong 3	Shan tuyet	Tan Chi Shan	Tham Ve Shan	To Hieu	Purple-leaved Suoi Giang	Suoi Giang 2
Lung Phin	1.00							
Minh Rong 3	0.50	1.00						
Shan tuyet	0.46	0.40	1.00					
Tan Chi Shan	0.53	0.31	0.56	1.00				
Tham Ve Shan	0.61	0.42	0.52	0.89	1.00			
To Hieu	0.48	0.46	0.50	0.75	0.67	1.00		
Purple-leaved Suoi Giang	0.57	0.53	0.70	0.56	0.60	0.56	1.00	
Suoi Giang 2	0.48	0.46	0.80	0.50	0.56	0.33	0.67	1.00

Table A3.3 (cont.)

	Lung Phin	Minh Rong 3	Shan tuyet	Tan Chi Shan	Tham Ve Shan	To Hieu	Purple-leaved Suoi Giang	Suoi Giang 2
Tua Chua TST	0.52	0.50	0.52	0.44	0.43	0.48	0.50	0.57
Tua Chua PCO	0.55	0.38	0.42	0.43	0.48	0.46	0.48	0.54
1A	0.56	0.53	0.48	0.59	0.64	0.63	0.63	0.53
6A	0.57	0.47	0.57	0.75	0.70	0.75	0.56	0.63
A18	0.53	0.60	0.71	0.77	0.71	0.67	0.53	0.63
A42	0.36	0.57	0.48	0.59	0.63	0.53	0.53	0.53
CDPxTD	0.35	0.33	0.42	0.53	0.50	0.47	0.59	0.56
F16	0.64	0.47	0.67	0.70	0.73	0.82	0.73	0.55
F35	0.41	0.36	0.41	0.60	0.56	0.43	0.55	0.52
Large-leaved Nam Ngat	0.43	0.27	0.55	0.56	0.60	0.30	0.50	0.70
LDP1	0.24	0.21	0.43	0.40	0.50	0.24	0.24	0.35
LDP2	0.33	0.22	0.45	0.46	0.42	0.27	0.27	0.40
Minh Rong 1	0.44	0.48	0.59	0.53	0.50	0.48	0.38	0.57
Minh Rong 2	0.47	0.52	0.73	0.48	0.54	0.42	0.61	0.67
PH1	0.60	0.50	0.52	0.71	0.67	0.71	0.63	0.56
Suoi Giang 6	0.57	0.62	0.60	0.63	0.67	0.56	0.78	0.67
France tea	0.50	0.44	0.58	0.71	0.67	0.53	0.74	0.74
Ho Nam 3	0.37	0.29	0.30	0.22	0.25	0.30	0.40	0.40
Phuc An 1	0.42	0.44	0.27	0.25	0.30	0.50	0.44	0.24
Phuc Dinh 3	0.44	0.38	0.44	0.56	0.50	0.70	0.60	0.30
Phuc Van 10	0.59	0.38	0.60	0.67	0.56	0.80	0.71	0.50
Manipur Messai	0.59	0.38	0.48	0.56	0.61	0.53	0.60	0.40
Sri Lanka 1	0.48	0.44	0.50	0.50	0.50	0.56	0.67	0.53
Darjeeling 2	0.32	0.38	0.40	0.42	0.42	0.42	0.38	0.32

Table A3.3 (cont.)

	Lung Phin	Minh Rong 3	Shan tuyet	Tan Chi Shan	Tham Ve Shan	To Hieu	Purple-leaved Suoi Giang	Suoi Giang 2
TB18BL	0.56	0.40	0.50	0.42	0.50	0.44	0.54	0.44
Ngoc Thuy BL	0.47	0.50	0.47	0.48	0.52	0.35	0.61	0.61
PH1BL	0.53	0.46	0.44	0.63	0.56	0.75	0.56	0.38
1ABL	0.52	0.50	0.52	0.53	0.61	0.55	0.57	0.55
Small-leaved China tea BL	0.48	0.35	0.50	0.40	0.45	0.27	0.55	0.45
Kim Tuyen BL	0.39	0.33	0.32	0.52	0.52	0.46	0.48	0.38
No9	0.37	0.44	0.54	0.57	0.61	0.42	0.52	0.50
No15	0.48	0.47	0.58	0.50	0.55	0.45	0.55	0.55
No21	0.38	0.46	0.50	0.50	0.44	0.56	0.56	0.44
Phuc Dinh 1	0.50	0.55	0.56	0.43	0.40	0.50	0.63	0.47
Japan 6	0.36	0.27	0.29	0.24	0.21	0.38	0.44	0.30
Nam Ngat 2	0.50	0.50	0.40	0.33	0.35	0.40	0.33	0.43
LD97BL	0.32	0.48	0.32	0.27	0.27	0.33	0.27	0.35
TB11BL	0.35	0.32	0.43	0.40	0.40	0.33	0.40	0.47

Table A3.3 (cont.)

	Tua Chua TST	Tua Chua PCO	1A	6A	A18	A42	CDPxTD	F16	F35	Large-leaved Nam Ngat	LDP1	LDP2	Minh Rong 1
Tua Chua TST	1.00												
Tua Chua PCO	0.59	1.00											
1A	0.46	0.56	1.00										
6A	0.48	0.52	0.80	1.00									
A18	0.63	0.67	0.67	0.92	1.00								
A42	0.45	0.44	0.60	0.71	0.63	1.00							
CDPxTD	0.33	0.60	0.47	0.53	0.63	0.78	1.00						
F16	0.48	0.67	0.78	0.80	0.67	0.52	0.50	1.00					

Table A3.3 (cont.)

	Tua Chua TST	Tua Chua PCO	1A	6A	A18	A42	CDPxTD	F16	F35	Large-leaved Nam Ngat	LDP1	LDP2	Minh Rong 1
F35	0.53	0.61	0.71	0.61	0.67	0.58	0.60	0.52	1.00				
Large-leaved Nam Ngat	0.35	0.57	0.48	0.56	0.59	0.57	0.63	0.50	0.56	1.00			
LDP1	0.32	0.39	0.33	0.32	0.80	0.35	0.60	0.38	0.44	0.53	1.00		
LDP2	0.50	0.40	0.35	0.33	0.75	0.40	0.50	0.42	0.46	0.35	0.81	1.00	
Minh Rong 1	0.57	0.53	0.46	0.55	0.88	0.45	0.56	0.48	0.53	0.61	0.80	0.67	1.00
Minh Rong 2	0.58	0.49	0.48	0.50	0.63	0.40	0.38	0.57	0.48	0.54	0.52	0.46	0.65
PH1	0.64	0.72	0.57	0.71	0.88	0.56	0.67	0.70	0.67	0.53	0.75	0.67	0.82
Suoi Giang 6	0.48	0.54	0.63	0.75	0.67	0.74	0.71	0.64	0.61	0.50	0.33	0.50	0.48
France tea	0.48	0.52	0.78	0.84	0.75	0.80	0.67	0.70	0.74	0.67	0.36	0.38	0.48
Ho Nam 3	0.29	0.41	0.31	0.27	0.53	0.29	0.59	0.33	0.47	0.27	0.40	0.42	0.43
Phuc An 1	0.40	0.35	0.40	0.32	0.40	0.35	0.35	0.42	0.55	0.11	0.46	0.50	0.48
Phuc Dinh 3	0.29	0.41	0.54	0.45	0.67	0.38	0.59	0.67	0.40	0.27	0.48	0.42	0.57
Phuc Van 10	0.44	0.57	0.44	0.59	0.71	0.38	0.50	0.78	0.50	0.35	0.33	0.36	0.53
Manipur Messai	0.44	0.55	0.54	0.57	0.83	0.60	0.77	0.70	0.47	0.45	0.64	0.67	0.59
Sri Lanka 1	0.35	0.54	0.64	0.74	0.71	0.63	0.63	0.67	0.56	0.50	0.63	0.53	0.61
Darjeeling 2	0.40	0.39	0.50	0.45	0.63	0.40	0.44	0.43	0.44	0.38	0.67	0.50	0.69
TB18BL	0.55	0.62	0.58	0.54	0.55	0.50	0.50	0.58	0.57	0.48	0.50	0.44	0.55
Ngoc Thuy BL	0.52	0.49	0.55	0.48	0.53	0.58	0.48	0.52	0.55	0.56	0.54	0.56	0.52
PH1BL	0.44	0.52	0.71	0.75	0.77	0.59	0.67	0.70	0.60	0.33	0.38	0.43	0.53
1ABL	0.50	0.59	0.85	0.67	0.67	0.61	0.50	0.69	0.67	0.50	0.42	0.43	0.50
Small-leaved China tea BL	0.40	0.47	0.52	0.50	0.59	0.43	0.42	0.46	0.52	0.58	0.48	0.42	0.48
Kim Tuyen BL	0.50	0.42	0.53	0.48	0.60	0.52	0.55	0.47	0.53	0.43	0.52	0.46	0.50
No9	0.44	0.50	0.56	0.67	0.60	0.64	0.55	0.50	0.62	0.46	0.48	0.53	0.52
No15	0.48	0.53	0.70	0.70	0.67	0.61	0.50	0.62	0.59	0.50	0.50	0.56	0.48

Table A3.3 (cont.)

	Tua Chua TST	Tua Chua PCO	1A	6A	A18	A42	CDPxTD	F16	F35	Large-leaved Nam Ngat	LDP1	LDP2	Minh Rong 1
No21	0.38	0.46	0.53	0.63	0.80	0.53	0.71	0.55	0.52	0.40	0.56	0.50	0.57
Phuc Dinh 1	0.47	0.53	0.50	0.57	0.67	0.47	0.59	0.53	0.63	0.33	0.55	0.67	0.59
Japan 6	0.36	0.52	0.40	0.35	0.67	0.45	0.80	0.35	0.50	0.48	0.42	0.33	0.45
Nam Ngat 2	0.55	0.54	0.42	0.40	0.67	0.50	0.55	0.38	0.64	0.40	0.33	0.42	0.48
LD97BL	0.52	0.45	0.33	0.30	0.60	0.42	0.50	0.32	0.52	0.33	0.36	0.52	0.38
TB11BL	0.56	0.48	0.36	0.33	0.55	0.63	0.77	0.32	0.64	0.56	0.53	0.70	0.50

Table A3.3 (cont.)

	Minh Rong 2	PH1	Suoi Giang 6	France tea	Ho Nam 3	Phuc An 1	Phuc Dinh 3	Phuc Van 10	Manipur Messai	Sri Lanka 1
Minh Rong 2	1.00									
PH1	0.64	1.00								
Suoi Giang 6	0.50	0.71	1.00							
France tea	0.50	0.57	0.84	1.00						
Ho Nam 3	0.39	0.64	0.50	0.32	1.00					
Phuc An 1	0.35	0.60	0.50	0.30	0.67	1.00				
Phuc Dinh 3	0.52	0.73	0.50	0.40	0.57	0.57	1.00			
Phuc Van 10	0.57	0.67	0.67	0.44	0.53	0.50	0.74	1.00		
Manipur Messai	0.48	0.89	0.67	0.56	0.59	0.67	0.67	0.57	1.00	
Sri Lanka 1	0.46	0.70	0.78	0.73	0.61	0.55	0.61	0.50	0.89	1.00
Darjeeling 2	0.50	0.67	0.42	0.43	0.46	0.52	0.62	0.38	0.61	0.70
TB18BL	0.61	0.67	0.59	0.60	0.48	0.48	0.55	0.43	0.73	0.71
Ngoc Thuy BL	0.53	0.54	0.70	0.64	0.52	0.40	0.39	0.35	0.62	0.59
PH1BL	0.38	0.86	0.75	0.71	0.56	0.50	0.67	0.67	0.67	0.88
1ABL	0.52	0.61	0.64	0.72	0.43	0.48	0.50	0.42	0.62	0.75
Small-leaved China tea BL	0.43	0.53	0.45	0.61	0.42	0.33	0.42	0.35	0.70	0.80

Kim Tuyen BL	0.40	0.56	0.54	0.55	0.50	0.43	0.50	0.38	0.67	0.62
No9	0.53	0.64	0.67	0.72	0.38	0.40	0.46	0.42	0.67	0.78
No15	0.50	0.60	0.64	0.78	0.50	0.42	0.42	0.44	0.73	0.86
No21	0.42	0.82	0.67	0.63	0.60	0.50	0.60	0.67	0.70	0.78
Phuc Dinh 1	0.50	0.71	0.75	0.59	0.88	0.63	0.63	0.80	0.77	0.78
Japan 6	0.33	0.71	0.40	0.40	0.61	0.46	0.55	0.55	0.64	0.67
Nam Ngat 2	0.43	0.67	0.57	0.44	0.45	0.46	0.36	0.36	0.50	0.40
LD97BL	0.57	0.56	0.47	0.35	0.59	0.38	0.37	0.29	0.50	0.44
TB11BL	0.46	0.59	0.59	0.48	0.48	0.40	0.40	0.31	0.64	0.35

Table A3.3 (cont.)

	Darjeeling 2	TB18BL	Ngoc Thuy BL	PH1BL	1ABL	Small-leaved China tea BL	Kim Tuyen BL	No9	No15	No21
Darjeeling 2	1.00									
TB18BL	0.80	1.00								
Ngoc Thuy BL	0.62	0.67	1.00							
PH1BL	0.63	0.67	0.57	1.00						
1ABL	0.64	0.73	0.65	0.74	1.00					
Small-leaved China tea BL	0.61	0.71	0.52	0.50	0.69	1.00				
Kim Tuyen BL	0.69	0.70	0.69	0.61	0.69	0.60	1.00			
No9	0.75	0.79	0.62	0.67	0.71	0.64	0.63	1.00		
No15	0.70	0.77	0.74	0.70	0.85	0.77	0.67	0.79	1.00	
No21	0.63	0.59	0.52	0.75	0.55	0.55	0.54	0.58	0.73	1.00
Phuc Dinh 1	0.59	0.61	0.60	0.86	0.63	0.56	0.57	0.57	0.63	0.75
Japan 6	0.33	0.46	0.36	0.47	0.43	0.52	0.56	0.31	0.43	0.60
Nam Ngat 2	0.44	0.70	0.52	0.55	0.48	0.59	0.50	0.63	0.59	0.57
LD97BL	0.43	0.64	0.57	0.57	0.46	0.60	0.55	0.64	0.70	0.59
TB11BL	0.48	0.69	0.54	0.43	0.42	0.50	0.59	0.73	0.60	0.59

Table A3.3 (cont.)

	Phuc Dinh 1	Japan 6	Nam Ngat 2	LD97BL	TB11BL
Phuc Dinh 1	1.00				
Japan 6	0.62	1.00			
Nam Ngat 2	0.67	0.50	1.00		
LD97BL	0.80	0.61	0.76	1.00	
TB11BL	0.55	0.61	0.74	0.75	1.00

Chapter 4

Revealing genetic diversity in tea grown in Vietnam by using simple sequence repeat (SSR) markers

Abstract

Molecular markers are a useful tool for assaying genetic variation. Revealing genetic diversity by molecular markers has assisted breeders assigning appropriate parents to be used in crosses. Among different classes of molecular markers, SSR markers are useful for many applications in plant genetics and breeding because of their reproducibility, multi-allelic nature, co-dominant inheritance, relative abundance and good genome coverage. In this study, genetic diversity of 69 tea accessions grown in Vietnam was revealed by SSR markers. To exploit the data, expected heterozygosity was calculated and cluster analysis based on Dice similarity coefficient matrix was performed using the unweighted pair-group method with arithmetic average (UPGMA) to group all the studied teas. Analysis of molecular variance (AMOVA) was computed to analyze molecular variance to evaluate the population genetic structure. With 6 SSR primer pairs, a total of 115 different alleles were detected. The resulting dendrogram confirmed that all accessions could be distinguished and clustered into one large group and four smaller groups. AMOVA revealed that most parts of genetic diversity could be attributed to the differences among accessions within the different groups.

4.1 Introduction

In recent years, significant progress has been made in use of molecular approaches for plant breeding. Molecular markers are a useful tool for assessing genetic variation and have greatly enhanced the genetic analysis of plants. Revealing genetic diversity by using molecular markers has assisted breeders assigning appropriate parents to be used in crosses. Since a variety of molecular markers, including restriction fragment length polymorphisms (RFLPs), random amplification of polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs) and simple sequence repeats (SSRs) or microsatellites, has become available nowadays, efforts are also being made to identify the most efficient and cost effective markers that can be used by practicing plant breeders.

Among different classes of molecular markers, SSR markers are suitable for many applications in plant genetics and breeding because of their reproducibility, multi-allelic nature, co-dominant inheritance, relative abundance and good genome coverage (Powell *et al.*, 1996). These markers have been used for genetic mapping (Holton *et al.*, 2002; Yu

et al., 2004a), to analyze functional diversity (Senior *et al*., 1998; Leigh *et al*., 2003; Dreisigacker *et al*., 2004), and for comparative mapping (Yu *et al*., 2004b; Varshney *et al*., 2005).

Simple sequence repeats (SSRs) also termed microsatellites; short tandem repeats (STRs) or sequence-tagged microsatellite sites (STMS) are tandem repeats of short nucleotide sequence motifs (mono-, di-, tri-, tetra- or penta-nucleotide unites). Microsatellites are abundant and are relatively evenly spaced throughout eukaryotic genomes (Tautz and Renz, 1984). The high level of polymorphism, relative to RFLPs and RAPDs, combined with a high interspersion rate make SSRs an abundant source of genetic markers (Gupta *et al*., 1999). The first report of microsatellites in plants was made by Condit and Hubbel (1991), also suggesting their abundance. The polymorphism of these loci can be detected in a simple manner by measuring the size of the PCR-amplified fragments (Navajas and Fenton, 2000).

Identification of SSRs in gene sequences of plants species was carried out as early as 1993 by Morgante and Olivieri (Varshney *et al*., 2005). However, at that time the amount of sequence data available for SSR analysis was limited and therefore only a few genomic SSRs were reported. Recently, the increase in the amount of sequence data generated from expressed sequence tag (EST) projects in several plant species facilitated the identification of genomic SSRs in large numbers (Varshney *et al*., 2005).

In tea, Ueno *et al*. (1999) developed and characterized 4 microsatellite markers in *Camellia japonica*. Later Freeman *et al*. (2004) successfully isolated and characterized 13 highly polymorphic microsatellites in *Camellia sinensis*. Based on these results, in this study, genetic diversity at molecular level of tea grown in Vietnam was revealed by using SSR markers.

4.2 Materials and Methods

4.2.1 Materials

From 144 tea accessions taken from Vietnam, only 96 genomic DNAs which generated polymorphism bands with 3 tested RAPD primers (also see 3.3.1, 3.4.1 and Table A3.1) were used for analyses.

4.2.2 Primers

Seventeen SSR primer pairs (Table 4.1), which were developed by Ueno *et al*. (1999) and Freeman *et al*. (2004) were chosen and supplied by MWG-Biotech AG for revealing the polymorphism of tea accessions.

In order to analyze the PCR products, the M13(-21) tail (5'-TTT CCC AGT CAC GAC GTT-3') was attached to each forward primer. The fluorescent dye labels used in this study were 6-carboxy-fluorescine (FAM) (blue), hexachloro-6-carboxy-fluorescine (HEX) (green) and NED (yellow) (Schuelke, 2000).

4.2.3 PCR analysis

PCR mixture (20 µl), adapted to the *Taq* polymerase commonly used in the Institute (FIREPol® DNA polymerase I, Solis Biodyne), contained 1 X buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.05 µM tfPrimer, 0.05 µM rPrimer, 0.05 µM M13-primer, 25 ng template DNA and 1 U *Taq* polymerase

SSR amplification was implemented on the Whatman Biometra© T1 Thermocycle generally using the protocols of Ueno *et al*. (1999) with a minor modification of the thermal cycles as follows:

- One cycle of pre-denaturation at 94^{o}C for 3 min;

- Forty five cycles of DNA amplification; each cycle consists of three steps, i.e., denaturizing at 94^{o}C for 1 min, then annealing at 55^{o}C for 1 min, and finally extending at 72^{o}C for 2 min;

- One cycle of further extension at 72^{o}C for 10 min; 4^{o}C end.

The PCR products were detected on the ABI PRISM® 3100 Genetic Analyzer, associated by the ABI PRISM 3100 Data Collection Software, using short capillary array (36 cm) and Genescan polymer 3100 POP-6. For being loaded, PCR products were diluted at 1:20, and then 2 µl of diluted product was mixed with 12 µl master solution consisting of GeneScan™ 500 ROX® size standard (6-carboxy-X-rhodamine) and Hi-Di™ Formamide at the ratio of 1: 615, denatured at 95^{o}C for 2 minutes and ice cooled before being loaded.

Table 4.1: Characteristics of SSR primer pairs used in the present study

Code	Sequence	The length (-mer)	GC percentage (%)	Molecular weight (g/mol)	Melting temperature T_m (^oC)
tfCamsinM1	5'-TTT CCC AGT CAC GAC GTT GAA TCA GGA CAT TAT AGG AAT TAA-3'	42	38.1	12920	69.4
rCamsinM1	5'-GGC CGA ATG TTG TCT TTT GT-3'	20	45.0	6145	55.3
tfCamsinM2	5'-TTT CCC AGT CAC GAC GTT CCT CTG GGT GTC CTA CAC CT-3'	38	55.3	11507	74.9
rCamsinM2	5'-AAA GCC TTG ATG CCT TTC G-3'	19	47.4	5779	54.5
tfCamsinM3	5'-TTT CCC AGT CAC GAC GTT GGT GTG GTG TTT TGA AGA AA-3'	38	44.7	11755	70.5
rCamsinM3	5'-TGT TAA GCC GCT TCA ATG C-3'	19	47.4	5779	54.5
tfCamsinM4	5'-TTT CCC AGT CAC GAC GTT ACA TTC AAG CA(AGCT) TCC ACA TAT GTG AAA-3'	45	41.1	13728	71.7
rCamsinM4	5'-CCT G(AGCT)T GCA GGA CTG TCT ATA GAT GA-3'	26	48.1	8006	64.0
tfCamsinM5	5'-TTT CCC AGT CAC GAC GTT AAA CTT CAA CAA CCA GCT CTG GTA-3'	42	45.2	12777	72.4
rCamsinM5	5'-ATT ATA GGA TGC AAA CAG GCA TGA-3'	24	37.5	7434	57.6
tfCamsinM6	5'-TTT CCC AGT CAC GAC GTT TGT TTT CTT AGG GTT GGA TAA AGG-3'	42	42.9	12956	71.4
rCamsinM6	5'-TTT TGT TGT AAT GAC GAA AAT TC-3'	23	26.1	7068	51.7
tfCamsinM7	5'-TTT CCC AGT CAC GAC GTT TGG TAA GGG TCC TAA GAG GTA CAC-3'	42	50.5	12929	74.3
rCamsinM7	5'-TTC CAA TCT TTT TCT ATA ACA TCT GC-3'	26	30.8	7821	56.9
tfCamsinM8	5'-TTT CCC AGT CAC GAC GTT CCA TCA TTG GCC ATT ACT ACA A-3'	40	45.0	12126	71.5
rCamsinM8	5'-CCA TAT GTG TGT GAA TGA TAA AAC C-3'	25	36.0	7689	58.1
tfCamsinM9	5'-TTT CCC AGT CAC GAC GTT CTC ATG GAG TCC AAG GAA GC-3'	38	52.6	11639	73.8
rCamsinM9	5'-AAA GCA GTC TGG AAC CTT GC-3'	20	50.0	6126	57.3

Table 4.1: Characteristics of SSR primer pairs used in the present study (cont.)

Code	Sequence	The length (-mer)	GC percentage (%)	Molecular weight (g/mol)	Melting temperature T_m (^oC)
tfCamsinM10	5'-TTT CCC AGT CAC GAC GTT TTA CAT CTC TTT TGC AGC TGT CGG-3'	42	47.6	12788	73.3
rCamsinM10	5'-CTT CGG GAA CTT CTG CTT CAT C-3'	22	50.0	6652	60.3
tfCamsinM11	5'-TTT CCC AGT CAC GAC GTT GCA TCA TTC CAC CAC TCA CC-3'	38	52.6	11429	73.8
rCamsinM11	5'-GTC ATC AAA CCA GTG GCT CA-3'	20	50.0	6086	57.3
tfCamsinM12	5'-TTT CCC AGT CAC GAC GTT CAT TAT CGT CAC TTG CAA AGA GGT-3'	42	45.2	12839	72.4
rCamsinM12	5'-CGA GAA GAA GAG CTC TAT TGG TT-3'	23	43.5	7128	58.9
tfCamsinM13	5'-TTT CCC AGT CAC GAC GTT CAC ATT GTG GCG TGT TAT TAA TTT-3'	42	40.5	12851	70.4
rCamsinM13	5'-ACA TTG GCT ATC TCT CAT CAT GG-3'	23	43.5	6990	58.9
tfMScjaF25	5'-TTT CCC AGT CAC GAC GTT GGG AAG GTG CAT AAA ATA CT-3'	38	44.7	11702	70.5
rMScjaF25	5'-TGC GAC CTA AGA TTA CTA AA-3'	20	35.0	6109	51.2
tfMSCjaH38	5'-TTT CCC AGT CAC GAC GTT GCT GAG CTT GGA GAT TTT GTT-3'	39	46.2	11986	71.6
rMSCjaH38	5'-CCT ATT GCC TAC GAC CAT TTC-3'	21	47.6	6292	57.9
tfMSCjaF37	5'-TTT CCC AGT CAC GAC GTT CGA GCC TTC CTT TTC CCA TTC-3'	39	51.3	11747	73.7
rMSCjaF37	5'-CGC TCG ACG TAA TGC CAC ACT-3'	21	57.1	6351	61.8
tfMSCjaH46	5'-TTT CCC AGT CAC GAC GTT CAT CGT CCT AAT CCA CTT CAC-3'	39	48.7	11749	72.6
rMSCjaH46	5'-AGG GAG CAT TAT GAG TCG TCT-3'	21	47.6	6501	57.9

4.2.4 Data analysis

The presence or absence of bands were recorded by ABI PRISM® Genotyper® 3.7 NT software (Applied Biosystems) and used to statistically compute:

- Expected heterozygosity (H_e) (Nei, 1978)

$$H_e = 1 - \sum_{i=1}^{l} p_i^2$$

where p_i: frequency of the i^{th} allele.

- The similarity coefficient (Dice, 1945) between two accessions *i* and *j* was computed as follow:

$$\mathbf{S_{ij} = 2a/ (2a + b + c),}$$

where a: number of the common bands,

b: number of bands only present in *i* but not *j*,

c: number of bands only present in *j* but not *i*.

- Cluster analysis based on Dice similarity coefficient matrix was done using the unweighted pair-group method with arithmetic average (UPGMA) to group all the studied teas by using NTSYS-pc 2.1 software (Rohlf, 2000). To test the reliability of clustering, bootstrap analysis was done with 4000 replications using PAUP* 4.0 beta version (Swofford, 1998).

- By using *Arlerquin* 3.1 software (Excoffier *et al*., 2005; Excoffier *et al*., 1992), AMOVA analysis was performed to analyze molecular variance of the population structure.

4.3 Results and Discussion

To estimate the genetic diversity among 96 accessions of tea, 17 SSR primer pairs developed by Ueno *et al*. (1999) and Freeman *et al*. (2004) were screened to choose 6 primer pairs, which generated the clearest polymorphic bands, to be used in the study (Table 4.3).

Unexpectedly, the preliminary results showed that only 69 accessions produced polymorphic bands with SSR primer pairs (Table 4.2). The high amount of tannin in

accessions and the long time of storage of dried samples at room temperature during working time in Vietnam were thought to be the main factors degrading the quality of extracted genomic DNA.

Table 4.2: List of accessions generating polymorphic bands with SSR primer pairs and therefore used for analyzing the genetic diversity

Accession	Type	Currently local taxonomic status
France tea	Local	n/a
Gia Vai	Local	Shan
Green Trung du	Local	Large-leaved
Huong Tich Son	Local	Shan
Lung Phin	Local	Shan
Mixed Trung du	Local	Large-leaved
Moc Chau Shan	Local	Shan
Nam Ngat 2	Local	Shan
No1	Local	n/a
No13	Local	n/a
No14	Local	n/a
No16	Local	n/a
No17	Local	n/a
No18	Local	n/a
Suoi Giang 2	Wild giant	Shan
Suoi Giang 3	Wild giant	Shan
Tua Chua TST	Wild giant	Shan
Tua Chua PCO	Wild giant	Shan
1A	Selected	Assam
6A	Selected	n/a
A18	Selected	n/a
CDPxTD	Selected	Cross
F16	Selected	n/a
F35	Selected	n/a
LDP_1	Selected	Cross
$LD_{97}BL$	Selected	Shan
Manipur	Selected	Assam
Minh Rong 1	Selected	n/a
Minh Rong 2	Selected	n/a
PH_1	Selected	Assam
PH_1BL	Selected	Assam
TB_{14}	Selected	Shan
TB_{18} BL	Selected	Shan
Dai Bach Tra	Imported	S-l China
Darjeeling 2	Imported	Assam
No3	Local	n/a
No5	Local	n/a
No6	Local	n/a
No7	Local	n/a
No8	Local	n/a
No9	Local	n/a
No10	Local	n/a
No11	Local	n/a
No12	Local	n/a
No21	Local	n/a
Phu Tho 10	Local	n/a
Small-leaved China tea BL	Local	Small-leaved China tea (S-l China)
Su Neo	Local	Shan
Tien Phong	Local	n/a
Ho Nam 1	Imported	n/a
Japan 6	Imported	S-l China
Japan 8	Imported	S-l China
Jetinga	Imported	Shan
KimTuyen BL	Imported	S-l China
Macomen	Imported	n/a
Manipur Messai	Imported	Assam
Ngoc Thuy	Imported	S-l China
Ngoc Thuy BL	Imported	S-l China
Phuc An 1	Imported	S-l China
Phuc An 2	Imported	S-l China
Phuc Dinh 1	Imported	S-l China
Phuc Van 10	Imported	S-l China
Sri Lanka 1	Imported	n/a
Sri Lanka 2	Imported	n/a
Swinglaybari	Imported	n/a
TRI2025	Imported	Shan
TRI777	Imported	Shan
Triet Giang 2	Imported	S-l China
Yabukita	Imported	S-l China

The number of alleles per SSR marker varied from 11 to 25 (Table 4.3), with an average of 19.17 and a total of 115 different alleles were detected. The PCR fragment sizes were between 183 bp and 483 bp. Figure 4.1 shows the scored bands of PCR products on the ABI PRISM® 3100 Genetic Analyzer software. The accessions used in Figure 4.1 were chosen from different sources: TB_{14} (selected shan tea), Yabukita (China tea, imported from Japan), Macomen (taxonomic unknown, imported from Laos), Ngoc Thuy (China tea, imported from Taiwan) and Suoi Giang 2 (wild giant tea). The expected heterozygosity was very high, ranging from 0.703 (locus CamsinM4) to 0.928 (locus CamsinM2) (Table 4.3). The fragment sizes in current study were a little bit bigger that those reported by Ueno *et al*. (1999) and Freeman *et al*. (2004).

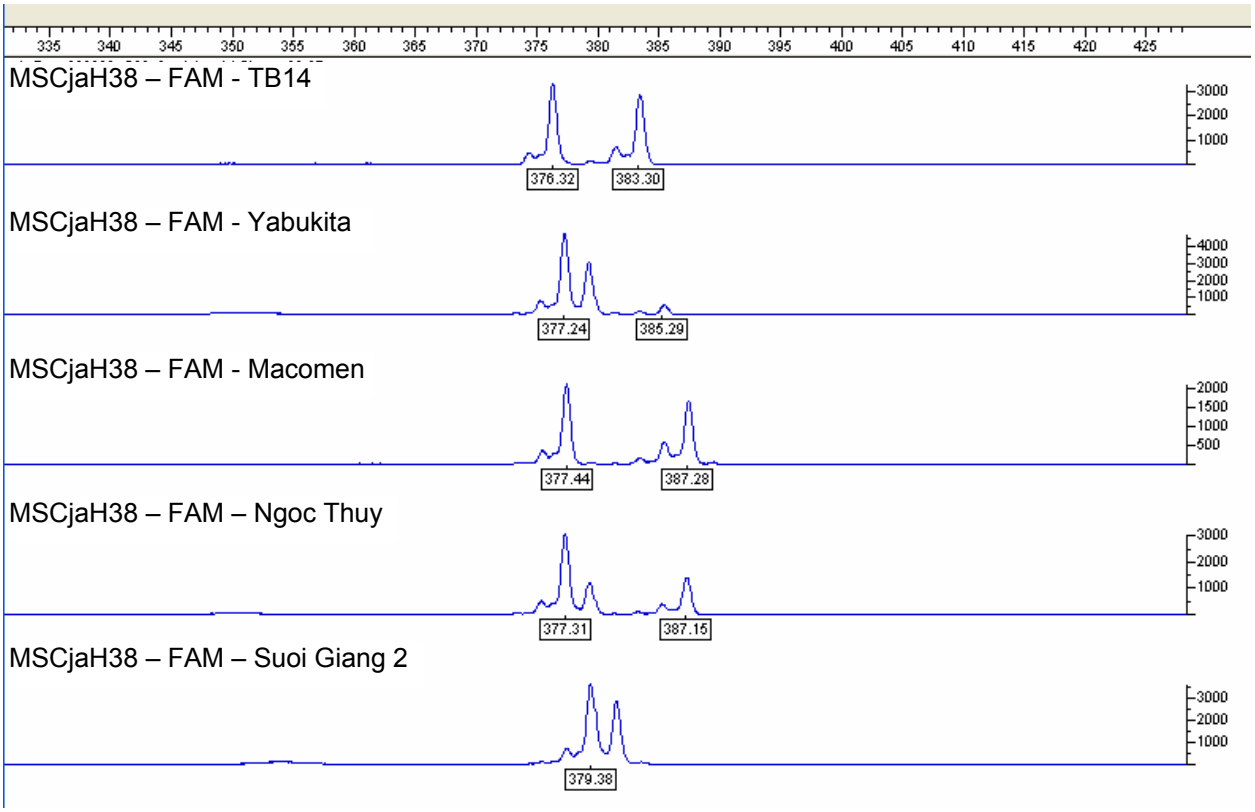


Figure 4.1: PCR products were detected on the ABI PRISM® 3100 Genetic Analyzer

Dice similarity coefficient values were expressed in a similarity matrix table (Table A4.2) and were used for UPGMA cluster analysis (Figure 4.2). The similarity matrix based on the proportion of shared fragments (Dice, 1945) was used to establish the level of relatedness between 69 tested accessions. Estimated similarity ranged from the minimum of 0.00, between the most distant (dissimilar) accessions (326 pairs of accessions with no alleles in common) to the maximum of 0.95, between the closest (most similar) accessions F16

(selected tea) and Ho Nam 1 (imported tea). This variation is larger than the earlier reports (Wachira et al., 1995; Paul et al., 1997; Modal, 2002) and may be explained by the wide variability in origin of the tested accessions.

Table 4.3: Repeat motif, product size range, allele number and expected heterozygosity (H_e) estimates of 6 SSR primers

SSR locus	Repeat motif	Product size (bp)	No. of alleles	H_e
CamsinM2	$(GT)_{17}$	245 – 281	25	0.928
CamsinM4	$(GA)_{19}$	348 – 379	11	0.703
CamsinM5	$(GT)_{15}(GA)_8$	183 – 224	25	0.907
CamsinM10	$(GT)_{16}$	190 – 221	20	0.890
MSCjaH38	$(GA)_{14}$	375 – 399	17	0.870
MSCjaH46	$(GA)_{16}$	462 – 483	17	0.890
Average			19.17	0.865

Although the resulting UPGMA dendrogram (Figure 4.2) does not show clearly distinguished groups, at about 20% similarity level, except for some accessions ungrouped, the remaining ones were clustered into one large group (group 2, consisting of 36 accessions) and four small groups (group 1, 3, 4 and 5; possessing 4 to 9 accessions). Notably this cluster is rather different from the results in chapter 3 (see 3.4.3) as well as from the conventional classification. Commercial clones TB_{14} and Ngoc Thuy are conventionally classified as shan and small-leaved China tea, respectively, but in cluster 2 they gathered into one sub-group. Similarly Moc Chau shan (shan tea) closed to PH_1BL and Sri Lanka 1 (assam tea); or Yabukita (small-leaved China tea) closed to TRI777 and Tua Chua TST (shan tea). The wild giant accessions are not clearly separated from the cultivated accessions because tea is freely cross pollinated and it seems that there have been many introgressions from genetic resources of wild giant into cultivated tea. Tran and Nguyen (1998) reported that clone TRI777 was selected at Tea Research Institute (TRI, Sri Lanka) from the collection of Vietnam tea and clone TRI777 is originated from Cho Long Shan (Son La province).

The low values of consensus generated from bootstrap analysis agree with the topology of UPGMA-derived dendrogram: the clusters are not separated clearly.

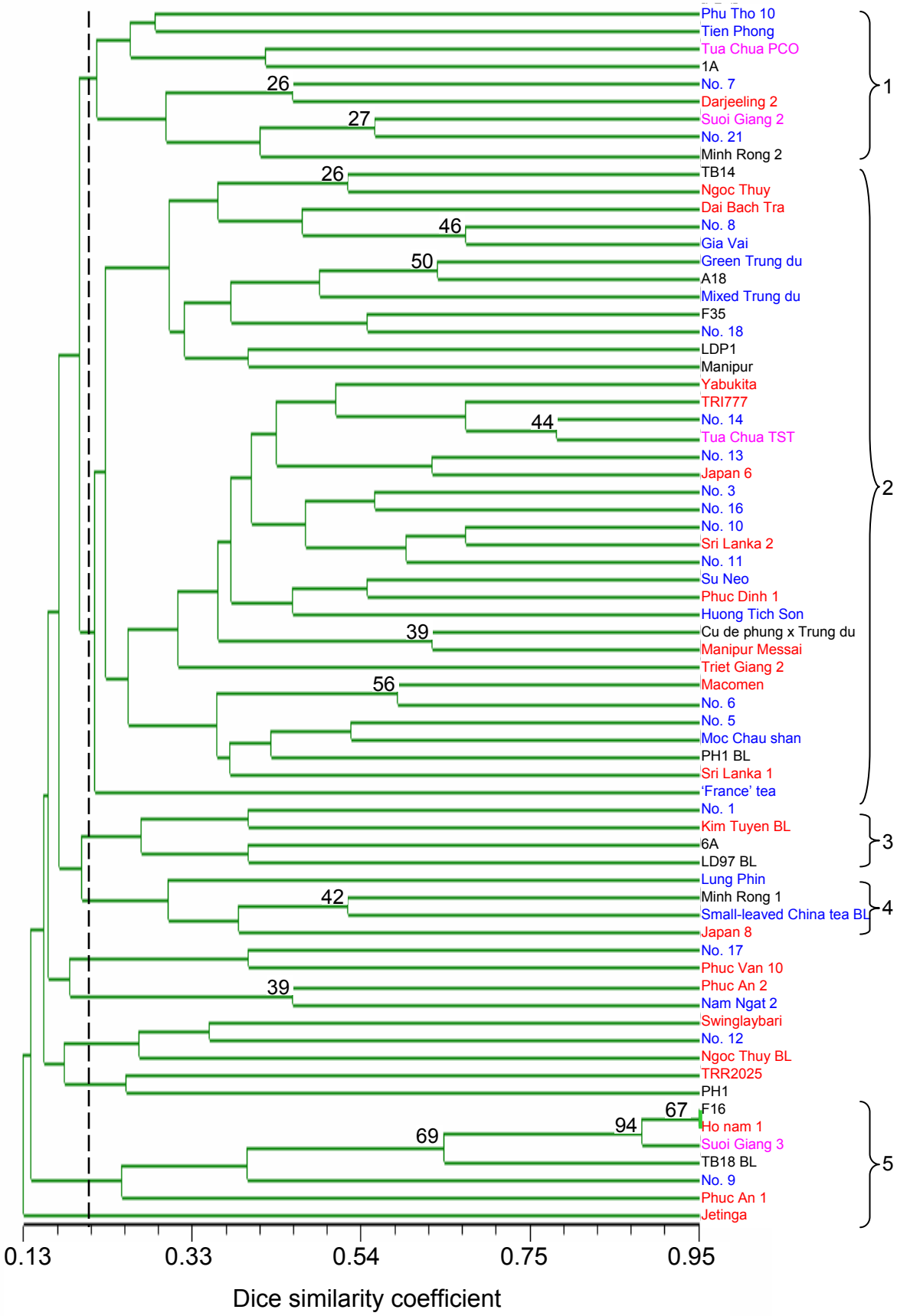


Figure 4.2: Dendrogram illustrating genetic relationships among 69 accessions of local (name in blue), wild (in pink), selected (in black) and imported (in red) teas, generated by UPGMA cluster analysis (NTSYS) calculated from 115 SSR markers produced by 6 primer pairs. The figures attached indicate the bootstrap values based on 4000 replicate analyses.

AMOVA showed no genetic difference between the groups of local, wild, selected and imported tea accessions (Table 4.4). The variance within groups was large and could explain the total variation.

Table 4.4: Analysis of molecular variance (AMOVA) for 69 tea accessions using SSR markers

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	P-value[2]
Among groups	3	14.784	0.00[1]	0.00[1]	ns
Within groups	65	372.404	5.72930	100.90	
Total	68	387.188	5.67829		
Fixation Index FST: -0.00898					ns

[1] Negative estimates, due to estimation errors

[2] Significance tests after 4032 permutations

Statistical comparing pairs of population samples also confirmed the non-significant differences at 0.05 in pairs of populations (data not shown). It could be explained by the highly variation of accessions within populations.

In conclusion, 115 different alleles were detected from 69 tea accessions by 6 SSR primer pairs. Cluster and AMOVA analysis presented the great variation among accessions; 326 pairs of accessions had no common alleles. The separation of clusters was not clear. The dendrogram formed based on SSR markers was quite differed from that based on ISSR markers and from the conventional classification.

Appendices

Table A4.1: The presence and absence of polymorphic bands generated from 69 accessions with 6 SSR primer pairs (1: presence; 0: absence; ?: missing data)

Francetea	000000000000010000010000	00101000000	10000100000000000000000000	00010001000000000000	00100000000000000	00100000000010000
GiaVai	000000000000000010000000000	00000000100	00000100000000010000000000	01000000000000000000	00100000000000000	????????????????
GreenTD	000000000000000010000000000	00000010100	00001001000000000000000000	00000000000000010001	00000000100000000	00000000000000010
HuongTichSon	000000000000000001010000000	00000000100	00000100001000000000000000	00000010010000000000	00100000000000000	00100000001000000
LungPhin	010000000000010000000000	00000010000	00000100000000000000000000	01000000001000000000	00000001000000000	00100000001000000
MixedTD	000000000010000000000000	00000010100	00001000000000010000000000	00000001001000000000	00001000100000000	01000000000000010
MocChauShan	000000000000100000000000	??????????	00000000000000010000000100	00000000000100001000	00010000001000000	01000000000010000
NamNgat2	000000000000100000010000	00000010100	00000000000000010000000001	01000000000000001000	00001000000010000	00000000000001000
No1	000000000001000010000000	??????????	00001000000000000000000000	01000100000000000000	????????????????	00000100000000000
No3	000000100000000000010000	00000010000	00000000101000000000000000	00000000000000010100	00000000001010000	00100000000000000
No5	000000000010100000000000	00000010100	00000000000000001000000010	00000001000001000000	00100000001000000	01000000000010000
No6	000000000101000000000000	00000010100	00000000000100000000001000	00000000000100001000	00000000001010000	00000000000010010
No7	000001000000000000001000000	00010000000	00000000100001000000000000	01000000100000000000	00000000101000000	00000000001000000
No8	000001000000000001000000000	00000000100	00000100000001000000000000	01000000001000000000	00100100000000000	00000100000000000
No9	????????????????????????	00000010000	00000000000001000000000100	01000000000100000000	10000000000010000	10000000010000000
No10	000000000000000010100000	00000010000	00000000101000000000000000	00000101000000000000	00000000101000000	00100000000000001
No11	000000000000000000001000100	??????????	00000000101000000000000000	00000001000000000000	00000000001000000	00001000000000000
No12	000000100010000000000000000	00010000000	00000000001000000000000000	01000001000000000000	00000001000000000	00000000011000000
No13	010000000010000000000000000	00000010100	00000100100000000000000000	00000001000001000000	00000010100000000	00000100000000000
No14	000000000010000000010000	00000000100	00000100100000000000000000	00000001000000000000	00100000001000000	00100000000000000
No16	000000000000100000010000	??????????	00000000101000000000000000	00000001001000000000	00000000001000000	00100000001000000
No17	000000000000000000000001000	01000000100	00000100000000000000000000	00100000100000000000	????????????????	00010000000000000
No18	000000000000010000010000	00000000100	00001000000000000010000000	00000001000000001000	00101000000000000	00000000001000010
No21	000000000001000000000000	00000000100	00000001100000000000000000	01000000000010000000	00000001001000000	????????????????
PhuTho10	000010000000000010000000	00100000000	00000000100000001000000000	00000100001000000000	00000100100000000	00100000000010000
SChinaBL	000000000000000101000000	00000010010	00001000000000000000000100	01000000100000000000	00010000010000000	00100001000000000
SuNeo	100000000000000000001000000	??????????	00000000001000000000000000	00000001100000000000	00000000001000000	00100000000000000
TienPhong	000000000001000100000000	00000010000	00000000001001000000000000	00000100001000000000	00000000100000000	00000010000000001
SuoiGiang2	000000000000100000100000	00000010100	00000001100000000000000000	01000001000000000000	00000100001000000	00010000000000000
SuoiGiang3	000000000000000100100000	00000000100	00000000000001000000000100	00000000000010000100	00001000000010000	00000000010010000
TuaChuaPCO	000001000010000000000000	00000000100	00011000000000000000000000	01000000001000000000	00000010100000000	00010000000000100
TuaChuaTST	000000000010000000000100	00000010100	00000000100000000000000000	00000001000000000000	00100000001000000	00100000000000000
1A	000000000010001000000000	??????????	00000100001000000000000000	01000000001000000000	00000100100000000	00000100000000000
6A	000000000010000010000000	00000000100	00011000000000000000000000	00100000100000000000	00001100000000000	00000000000010000

A18	000000000000000010000000000	00000000100	000010010000000000000000	000000010001000000000	000000001010000000	000000000000100010
CDPxTD	000000000010000000000000	00000010100	000000001010000000000000	000001000000000010000	000000100010000000	000000000010010000
F16	000000000000000000000100000	00000000001	000000000000010000000100	000000000000010000100	000010000000010000	000000000010010000
F35	001000000000000010000000000	00000000100	000010000010000000000000	000000010010000000000	001010000000000000	001000000001000000
LD97BL	000000000000000000100100000	???????????	000010000000000000000001	000000001000010000000	??????????????????	001000000010000000
LDP1	000001000000000010000000000	00100000100	000000010010000000000000	000000010001000000000	000000001000010000	001000000000000001
Manipur	000000000000000010001000000	00000000100	000000010000010000000000	000000000000000000010	001000000000000000	001000000000000000
MinhRong1	000000000100000001000000000	00000000010	000010000010000000000000	010000000010000000000	000100010000000000	001000000000010000
MinhRong2	000000000000000010001000000	00000100000	000000001000000010000000000	010000001000000000000	000001000010000000	000000100000000000
PH1	010000000000000000000000000	00000100000	000010000010000000000000000	000000100100000000000	000001000000000000	??????????????????
PH1BL	000000000000100000000000100	00000000100	010000001000000000000000000	000000010001000000000	000000100010000000	000000001000100000
TB14	000000000001000100000000000	00001000100	000001000000001000000000000	010001000000000000000	000010001000000000	000000000000010000
TB18BL	000100000000000000100000000	00000000100	000000000000010000000100	000000000000100001000	000000100010000000	??????????????????
DaiBachTra	000000000000010100000000000	00000010100	000000000000000001000000000	010000001000000000000	001000001000000000	000000010000000000
Darjeeling2	010000010000000000000000000	01000000001	000000001000010000000000000	010000000010000000000	000001001000000000	000010000010000000
HoNam1	000000000000000000000100000	00000000101	000000000000010000000100	000000000000100001000	000010000000100000	000000000010010000
Japan6	010000000000000010000000000	00000000100	000001001000000000000000000	??????????????????????	000000001000000000	001000000000000000
Japan8	000000000000000010000000000	00000010000	000010000010000000000000000	000000000000100000000	000010000100000000	001000000000100000
Jetinga	000001000000000000000000010	00000010010	000000000000000000100000010	000000000000001001000	000100000010000000	000000000001000010
KimTuyenBL	000000000000000000000100000	00000001000	000010000010000000000000000	000001010000000000000	010000000001000000	??????????????????
Macomen	000000000001000100000000000	00000010100	000000000000000000000101000	000000000000100001000	001000000010000000	010000000100000000
ManipurMessai	000000000010000000000000000	00000000100	000000001010000000000000000	000001000000000000000	001010000000000000	??????????????????
NgocThuy	000000001000000010000000000	10000000100	000001000000001000000000000	000000010000000001000	000010001000000000	001000000010000000
NgocThuyBL	000001000000000010000000000	00000100100	001000000010000000000000000	000010000000000000000	000000000001000001	010000000100000000
PhucAn1	000000000000000000000000001	00000000100	000000000000000000001000100	100000010000000000000	001000000001000000	000100000100000000
PhucAn2	000000000000010000000100000	00000000010	000000000000000000000010001	010000010000000000000	000101000000000000	000001000000010000
PhucDinh1	010000000000000000000000000	00000000100	000000000010000000000000000	??????????????????????	001000000001000000	011000000000000000
PhucVan10	000000000000010000001000000	00000000100	000001001000000000000000000	000000100000000000000	000100000000000000	000100000000010000
SriLanka1	000000100000010000000000000	10000000100	000000000000001000000000001	000000010000000001000	001000000010000000	000000000000100000
SriLanka2	??????????????????????????	00000010100	000000001000010000000000000	000000010000100000000	000000001010000000	??????????????????
Swinglaybari	000000000000001000000000000	???????????	000000000010000000000000010	000000010000000100000	000010000000000100	010000000100000000
TRI2025	010000000000000000010000000	00000000100	000000100000000000000000010	000000010000100000000	000001000000000010	000000000010000010
TRI777	000000000000000010000000000	00000010100	000011000000000000000000000	000000010000000000000	001000000010000000	001000000000000000
TrietGiang2	000000000010000000000000100	00000000010	000000001000000000000000000	000000010000000000000	000100000010000000	000100000000000000
Yabukita	000000000010000010000000000	10000000100	000010000100000000000000000	000000010000000001000	000010000010000000	001000000000000001

Table A4.2: Dice similarity coefficient matrix of 69 tested accessions based on the presence or absence of SSR markers generated with 6 primer pairs

	PhuTho10	TienPhong	TB14	DaiBachTra	Swinglaybari	Yabukita	Macomen
Phu Tho 10	1.00						
Tien Phong	0.29	1.00					
TB14	0.27	0.38	1.00				
Dai Bach Tra	0.19	0.30	0.38	1.00			
Swinglaybari	0.00	0.11	0.11	0.00	1.00		
Yabukita	0.17	0.09	0.17	0.09	0.21	1.00	
Macomen	0.00	0.27	0.26	0.36	0.21	0.25	1.00
Jetinga	0.00	0.09	0.00	0.09	0.11	0.17	0.25
TRI2025	0.09	0.10	0.09	0.10	0.21	0.26	0.09
TRI777	0.20	0.11	0.20	0.32	0.13	0.57	0.38
Ngoc Thuy	0.17	0.18	0.52	0.27	0.21	0.50	0.25
No1	0.29	0.29	0.46	0.17	0.00	0.29	0.14
No3	0.19	0.20	0.00	0.10	0.11	0.18	0.18
No5	0.09	0.09	0.17	0.27	0.32	0.33	0.42
No6	0.09	0.18	0.26	0.18	0.00	0.25	0.58
No7	0.19	0.20	0.19	0.30	0.00	0.09	0.09
No8	0.19	0.20	0.38	0.40	0.00	0.09	0.27
No10	0.45	0.48	0.18	0.19	0.21	0.43	0.17
No11	0.12	0.13	0.00	0.00	0.25	0.24	0.12
No12	0.00	0.11	0.10	0.11	0.35	0.19	0.10
No13	0.18	0.19	0.27	0.29	0.11	0.26	0.17
No16	0.32	0.22	0.00	0.00	0.22	0.32	0.11
No14	0.20	0.00	0.20	0.21	0.12	0.48	0.29
No17	0.00	0.00	0.25	0.27	0.00	0.12	0.12
Gia Vai	0.00	0.14	0.50	0.53	0.00	0.13	0.38
Green TD	0.10	0.32	0.30	0.42	0.00	0.19	0.29
Lung Phin	0.20	0.21	0.20	0.32	0.00	0.10	0.10
Mixed TD	0.18	0.29	0.27	0.29	0.33	0.43	0.26
Moc Chau Shan	0.11	0.00	0.11	0.00	0.11	0.21	0.42
Su Neo	0.12	0.13	0.00	0.13	0.25	0.35	0.12
Suoi Giang 2	0.18	0.10	0.18	0.29	0.11	0.26	0.26
Tua Chua TST	0.20	0.11	0.10	0.32	0.13	0.48	0.38
Tua Chua PCO	0.18	0.19	0.27	0.29	0.00	0.26	0.09
1A	0.32	0.33	0.33	0.24	0.22	0.11	0.00
6A	0.29	0.00	0.29	0.20	0.11	0.45	0.09
A18	0.10	0.20	0.29	0.30	0.11	0.36	0.36
CDPxTD	0.27	0.29	0.27	0.19	0.22	0.26	0.35
F16	0.10	0.10	0.19	0.00	0.22	0.09	0.09

Table A4.2 (cont.)

	PhuTho10	TienPhong	TB14	DaiBachTra	Swinglaybari	Yabukita	Macomen
F35	0.18	0.29	0.27	0.29	0.32	0.43	0.26
LDP1	0.26	0.36	0.26	0.27	0.21	0.33	0.25
Manipur	0.11	0.22	0.21	0.33	0.00	0.20	0.30
Minh Rong 1	0.27	0.19	0.18	0.10	0.11	0.17	0.00
Minh Rong 2	0.19	0.20	0.19	0.30	0.00	0.09	0.18
PH1	0.13	0.13	0.00	0.00	0.15	0.12	0.00
France tea	0.27	0.00	0.27	0.19	0.11	0.17	0.09
Suoi Giang 3	0.18	0.10	0.27	0.10	0.21	0.26	0.17
Ho Nam 1	0.09	0.10	0.27	0.10	0.22	0.17	0.17
Phuc An 1	0.00	0.00	0.10	0.20	0.22	0.18	0.27
Phuc An 2	0.09	0.00	0.09	0.10	0.11	0.09	0.00
Phuc Van 10	0.10	0.00	0.20	0.11	0.00	0.10	0.10
Triet Giang 2	0.11	0.00	0.00	0.00	0.13	0.30	0.10
Manipur Messai	0.25	0.27	0.35	0.25	0.31	0.35	0.24
Sri Lanka 1	0.09	0.00	0.27	0.29	0.11	0.43	0.35
Darjeeling 2	0.35	0.27	0.17	0.18	0.00	0.00	0.00
TB18 BL	0.00	0.12	0.11	0.11	0.00	0.21	0.21
Ngoc Thuy BL	0.00	0.19	0.18	0.19	0.33	0.17	0.43
PH1 BL	0.18	0.00	0.18	0.10	0.11	0.26	0.26
SChina BL	0.09	0.18	0.17	0.45	0.00	0.17	0.17
Kim Tuyen BL	0.12	0.25	0.11	0.00	0.29	0.33	0.11
No9	0.00	0.24	0.11	0.24	0.13	0.00	0.32
No18	0.00	0.00	0.18	0.29	0.21	0.43	0.26
No21	0.12	0.13	0.33	0.24	0.00	0.22	0.33
Huong Tich Son	0.19	0.10	0.19	0.20	0.11	0.27	0.18
Phuc Dinh 1	0.13	0.13	0.13	0.27	0.31	0.35	0.47
Japan 6	0.50	0.13	0.38	0.27	0.00	0.35	0.12
Japan 8	0.10	0.32	0.20	0.21	0.24	0.29	0.19
SriLanka 2	0.27	0.43	0.25	0.40	0.17	0.38	0.38
Nam Ngat 2	0.00	0.10	0.36	0.29	0.11	0.26	0.26
LD97 BL	0.25	0.00	0.00	0.14	0.13	0.38	0.13

Table A4.2 (cont.)

	Jetinga	TRI2025	TRI777	NgocThuy	No1	No3	No5	No6	No7	No8	No10
Jetinga	1.00										
TRI2025	0.17	1.00									
TRI777	0.19	0.20	1.00								
Ngoc Thuy	0.17	0.26	0.38	1.00							

Table A4.2 (cont.)

	Jetinga	TRI2025	TRI777	NgocThuy	No1	No3	No5	No6	No7	No8	No10
No1	0.00	0.00	0.36	0.00	1.00						
No3	0.18	0.00	0.32	0.09	0.00	1.00					
No5	0.33	0.26	0.48	0.17	0.00	0.18	1.00				
No6	0.33	0.09	0.29	0.17	0.14	0.27	0.33	1.00			
No7	0.27	0.19	0.11	0.18	0.15	0.20	0.09	0.09	1.00		
No8	0.09	0.19	0.32	0.27	0.31	0.00	0.18	0.09	0.20	1.00	
No10	0.17	0.27	0.50	0.26	0.29	0.48	0.26	0.17	0.38	0.00	1.00
No11	0.12	0.24	0.29	0.12	0.00	0.38	0.24	0.12	0.38	0.00	0.59
No12	0.10	0.20	0.11	0.19	0.15	0.21	0.19	0.00	0.32	0.11	0.20
No13	0.17	0.27	0.40	0.35	0.15	0.19	0.43	0.17	0.19	0.29	0.36
No16	0.21	0.21	0.38	0.32	0.00	0.56	0.32	0.11	0.33	0.11	0.53
No14	0.10	0.20	0.67	0.38	0.00	0.42	0.48	0.19	0.21	0.32	0.40
No17	0.00	0.13	0.29	0.24	0.00	0.00	0.12	0.12	0.13	0.27	0.00
Gia Vai	0.00	0.13	0.43	0.38	0.22	0.00	0.38	0.13	0.13	0.67	0.00
Green TD	0.19	0.10	0.33	0.29	0.17	0.21	0.19	0.29	0.11	0.21	0.20
Lung Phin	0.19	0.20	0.33	0.29	0.15	0.21	0.10	0.10	0.21	0.32	0.20
Mixed TD	0.17	0.18	0.40	0.35	0.15	0.10	0.52	0.26	0.10	0.19	0.27
Moc Chau Shan	0.32	0.00	0.13	0.11	0.00	0.11	0.53	0.42	0.11	0.00	0.11
Su Neo	0.12	0.24	0.43	0.24	0.00	0.38	0.24	0.12	0.38	0.00	0.59
Suoi Giang 2	0.17	0.27	0.40	0.17	0.15	0.29	0.43	0.26	0.29	0.29	0.36
Tua Chua TST	0.19	0.20	0.67	0.29	0.00	0.42	0.57	0.29	0.21	0.21	0.50
Tua Chua PCO	0.09	0.09	0.20	0.17	0.29	0.00	0.17	0.09	0.29	0.38	0.09
1A	0.00	0.11	0.13	0.21	0.31	0.11	0.11	0.00	0.22	0.56	0.21
6A	0.00	0.19	0.32	0.18	0.31	0.00	0.27	0.18	0.10	0.20	0.10
A18	0.18	0.19	0.42	0.36	0.15	0.10	0.27	0.36	0.20	0.20	0.29
CDPxTD	0.17	0.09	0.30	0.09	0.15	0.48	0.43	0.35	0.19	0.10	0.45
F16	0.00	0.10	0.00	0.09	0.00	0.20	0.09	0.18	0.10	0.00	0.00
F35	0.09	0.27	0.50	0.52	0.14	0.19	0.26	0.09	0.10	0.38	0.27
LDP1	0.08	0.26	0.29	0.42	0.00	0.18	0.17	0.17	0.18	0.27	0.43
Manipur	0.00	0.11	0.35	0.30	0.00	0.11	0.20	0.10	0.11	0.33	0.11
Minh Rong 1	0.17	0.00	0.20	0.09	0.29	0.19	0.09	0.17	0.10	0.19	0.18
Minh Rong 2	0.09	0.10	0.11	0.09	0.15	0.20	0.18	0.09	0.40	0.30	0.19
PH1	0.00	0.25	0.13	0.00	0.20	0.13	0.00	0.00	0.00	0.13	0.13
France tea	0.00	0.09	0.40	0.26	0.00	0.19	0.26	0.09	0.00	0.19	0.18
Suoi Giang 3	0.00	0.18	0.20	0.17	0.14	0.19	0.17	0.26	0.10	0.10	0.09
Ho Nam 1	0.00	0.18	0.10	0.17	0.00	0.19	0.17	0.26	0.10	0.10	0.00
Phuc An 1	0.00	0.19	0.32	0.18	0.00	0.00	0.27	0.09	0.00	0.20	0.10
Phuc An 2	0.17	0.18	0.10	0.09	0.29	0.10	0.17	0.00	0.10	0.29	0.09
Phuc Van 10	0.10	0.10	0.22	0.19	0.00	0.11	0.19	0.10	0.11	0.21	0.10

Table A4.2 (cont.)

	Jetinga	TRI2025	TRI777	NgocThuy	No1	No3	No5	No6	No7	No8	No10
Triet Giang 2	0.30	0.11	0.24	0.10	0.00	0.22	0.30	0.10	0.22	0.00	0.32
Manipur Messai	0.00	0.13	0.27	0.24	0.22	0.25	0.35	0.12	0.13	0.25	0.38
Sri Lanka 1	0.17	0.18	0.40	0.43	0.00	0.19	0.43	0.35	0.10	0.19	0.18
Darjeeling 2	0.08	0.26	0.00	0.17	0.14	0.09	0.00	0.00	0.45	0.27	0.17
TB18 BL	0.11	0.22	0.24	0.11	0.00	0.22	0.21	0.21	0.22	0.11	0.11
Ngoc Thuy BL	0.17	0.09	0.20	0.17	0.00	0.19	0.26	0.17	0.19	0.29	0.18
PH1 BL	0.09	0.18	0.30	0.17	0.00	0.19	0.43	0.35	0.19	0.10	0.27
SChina BL	0.25	0.00	0.29	0.17	0.29	0.18	0.08	0.08	0.18	0.18	0.17
Kim Tuyen BL	0.11	0.12	0.38	0.11	0.40	0.24	0.22	0.11	0.12	0.00	0.47
No9	0.11	0.00	0.12	0.00	0.20	0.24	0.11	0.32	0.24	0.12	0.11
No18	0.35	0.27	0.40	0.43	0.14	0.10	0.26	0.26	0.10	0.19	0.09
No21	0.11	0.24	0.25	0.11	0.40	0.24	0.22	0.33	0.35	0.24	0.24
Huong Tich Son	0.09	0.29	0.53	0.36	0.14	0.20	0.18	0.09	0.20	0.30	0.38
Phuc Dinh 1	0.12	0.25	0.53	0.24	0.00	0.40	0.47	0.24	0.13	0.27	0.38
Japan 6	0.00	0.25	0.53	0.47	0.22	0.27	0.12	0.12	0.27	0.27	0.50
Japan 8	0.10	0.10	0.33	0.29	0.17	0.32	0.10	0.10	0.00	0.11	0.30
SriLanka 2	0.25	0.40	0.53	0.38	0.00	0.40	0.50	0.38	0.53	0.13	0.67
Nam Ngat 2	0.17	0.09	0.20	0.35	0.15	0.29	0.26	0.35	0.10	0.19	0.09
LD97 BL	0.13	0.00	0.46	0.13	0.29	0.13	0.13	0.00	0.13	0.00	0.25

Table A4.2 (cont.)

	No11	No12	No13	No16	No14	No17	GiaVai	GreenTD	LungPhin	MixedTD
No11	1.00									
No12	0.27	1.00								
No13	0.25	0.20	1.00							
No16	0.50	0.35	0.22	1.00						
No14	0.40	0.22	0.50	0.59	1.00					
No17	0.00	0.00	0.25	0.00	0.29	1.00				
Gia Vai	0.00	0.15	0.25	0.00	0.43	0.36	1.00			
Green TD	0.00	0.00	0.30	0.00	0.11	0.13	0.29	1.00		
Lung Phin	0.00	0.33	0.30	0.35	0.22	0.13	0.31	0.11	1.00	
Mixed TD	0.13	0.20	0.45	0.22	0.30	0.13	0.27	0.50	0.20	1.00
Moc Chau Shan	0.13	0.00	0.00	0.22	0.12	0.00	0.17	0.00	0.00	0.22
Su Neo	0.57	0.27	0.13	0.50	0.40	0.18	0.00	0.00	0.13	0.13
Suoi Giang 2	0.38	0.20	0.36	0.44	0.40	0.25	0.25	0.30	0.20	0.27
Tua Chua TST	0.57	0.22	0.50	0.50	0.78	0.14	0.29	0.22	0.22	0.40
Tua Chua PCO	0.00	0.20	0.36	0.11	0.20	0.25	0.27	0.30	0.20	0.45
1A	0.13	0.35	0.44	0.22	0.24	0.17	0.31	0.13	0.35	0.33

Table A4.2 (cont.)

	No11	No12	No13	No16	No14	No17	GiaVai	GreenTD	LungPhin	MixedTD
6A	0.00	0.11	0.19	0.00	0.21	0.40	0.13	0.21	0.00	0.38
A18	0.25	0.11	0.29	0.22	0.32	0.13	0.29	0.63	0.00	0.48
CDPxTD	0.38	0.30	0.45	0.33	0.40	0.13	0.13	0.30	0.10	0.27
F16	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10
F35	0.24	0.30	0.18	0.53	0.40	0.13	0.40	0.30	0.30	0.45
LDP1	0.24	0.19	0.26	0.32	0.29	0.12	0.25	0.38	0.10	0.26
Manipur	0.00	0.00	0.11	0.13	0.35	0.14	0.46	0.35	0.12	0.11
Minh Rong 1	0.12	0.30	0.00	0.32	0.10	0.00	0.13	0.10	0.40	0.18
Minh Rong 2	0.25	0.11	0.10	0.22	0.21	0.13	0.40	0.11	0.11	0.10
PH1	0.17	0.14	0.12	0.15	0.00	0.00	0.00	0.13	0.14	0.13
France tea	0.13	0.10	0.18	0.33	0.50	0.12	0.27	0.00	0.30	0.09
Suoi Giang 3	0.00	0.10	0.09	0.00	0.10	0.13	0.13	0.10	0.00	0.18
Ho Nam 1	0.00	0.10	0.09	0.00	0.10	0.13	0.13	0.10	0.00	0.18
Phuc An 1	0.13	0.21	0.19	0.11	0.32	0.27	0.29	0.11	0.00	0.19
Phuc An 2	0.12	0.20	0.18	0.32	0.20	0.00	0.13	0.00	0.10	0.09
Phuc Van 10	0.13	0.00	0.30	0.24	0.33	0.40	0.31	0.11	0.11	0.10
Triet Giang 2	0.57	0.24	0.32	0.38	0.47	0.15	0.00	0.00	0.00	0.21
Manipur Messai	0.33	0.29	0.35	0.31	0.53	0.18	0.31	0.13	0.00	0.38
Sri Lanka 1	0.25	0.20	0.18	0.22	0.40	0.13	0.25	0.10	0.10	0.18
Darjeeling 2	0.24	0.19	0.26	0.32	0.10	0.12	0.13	0.10	0.38	0.17
TB18 BL	0.14	0.00	0.21	0.13	0.24	0.15	0.13	0.12	0.00	0.11
Ngoc Thuy BL	0.25	0.20	0.09	0.22	0.20	0.13	0.27	0.20	0.00	0.18
PH1 BL	0.47	0.10	0.36	0.42	0.40	0.13	0.13	0.10	0.00	0.18
SChina BL	0.00	0.10	0.09	0.11	0.10	0.12	0.25	0.29	0.29	0.17
Kim Tuyen BL	0.46	0.27	0.11	0.43	0.25	0.00	0.00	0.13	0.00	0.24
No9	0.00	0.25	0.11	0.00	0.00	0.00	0.17	0.12	0.25	0.11
No18	0.12	0.20	0.18	0.32	0.40	0.13	0.27	0.30	0.20	0.45
No21	0.31	0.27	0.22	0.29	0.38	0.17	0.29	0.25	0.27	0.12
Huong Tich Son	0.25	0.21	0.19	0.33	0.42	0.25	0.43	0.11	0.32	0.10
Phuc Dinh 1	0.33	0.14	0.25	0.46	0.53	0.20	0.40	0.14	0.29	0.25
Japan 6	0.17	0.00	0.63	0.31	0.53	0.36	0.36	0.29	0.43	0.25
Japan 8	0.13	0.11	0.10	0.24	0.11	0.00	0.15	0.33	0.22	0.30
SriLanka 2	0.60	0.15	0.63	0.55	0.57	0.18	0.15	0.40	0.15	0.50
Nam Ngat 2	0.00	0.10	0.18	0.22	0.20	0.13	0.25	0.20	0.20	0.27
LD97 BL	0.00	0.13	0.13	0.13	0.14	0.15	0.00	0.14	0.13	0.13

Table A4.2 (cont.)

	MocChauShan	SuNeo	SuoiGiang2	TuaChuaTST	TuaChuaPCO	1A	6A
Moc Chau Shan	1.00						
Su Neo	0.13	1.00					
Suoi Giang 2	0.22	0.25	1.00				
Tua Chua TST	0.13	0.43	0.50	1.00			
Tua Chua PCO	0.00	0.00	0.27	0.20	1.00		
1A	0.00	0.13	0.22	0.13	0.42	1.00	
6A	0.11	0.13	0.19	0.21	0.38	0.22	1.00
A18	0.22	0.25	0.38	0.32	0.29	0.11	0.20
CDPxTD	0.22	0.25	0.36	0.50	0.27	0.22	0.29
F16	0.22	0.00	0.10	0.00	0.00	0.00	0.20
F35	0.00	0.35	0.18	0.40	0.27	0.21	0.29
LDP1	0.11	0.35	0.26	0.29	0.26	0.21	0.09
Manipur	0.00	0.14	0.32	0.35	0.11	0.00	0.11
Minh Rong 1	0.21	0.24	0.09	0.10	0.27	0.32	0.19
Minh Rong 2	0.22	0.25	0.48	0.21	0.10	0.22	0.20
PH1	0.00	0.17	0.12	0.00	0.13	0.29	0.25
France tea	0.11	0.25	0.09	0.30	0.00	0.11	0.10
Suoi Giang 3	0.21	0.00	0.18	0.10	0.09	0.00	0.38
Ho Nam 1	0.22	0.00	0.18	0.10	0.09	0.00	0.29
Phuc An 1	0.11	0.13	0.29	0.32	0.19	0.00	0.10
Phuc An 2	0.21	0.12	0.36	0.10	0.09	0.32	0.10
Phuc Van 10	0.24	0.00	0.50	0.22	0.20	0.12	0.11
Triet Giang 2	0.25	0.29	0.42	0.59	0.21	0.13	0.11
Manipur Messai	0.00	0.17	0.24	0.53	0.25	0.29	0.38
Sri Lanka 1	0.33	0.25	0.27	0.40	0.09	0.00	0.19
Darjeeling 2	0.00	0.00	0.26	0.10	0.26	0.42	0.09
TB18 BL	0.27	0.14	0.32	0.24	0.22	0.00	0.11
Ngoc Thuy BL	0.22	0.25	0.18	0.20	0.18	0.11	0.10
PH1 BL	0.42	0.24	0.45	0.50	0.18	0.00	0.19
SChina BL	0.21	0.24	0.17	0.19	0.17	0.11	0.18
Kim Tuyen BL	0.14	0.46	0.33	0.25	0.12	0.13	0.12
No9	0.25	0.00	0.22	0.13	0.11	0.13	0.00
No18	0.11	0.12	0.18	0.30	0.18	0.00	0.29
No21	0.14	0.15	0.56	0.38	0.24	0.13	0.12
Huong Tich Son	0.00	0.38	0.10	0.32	0.10	0.22	0.20
Phuc Dinh 1	0.31	0.55	0.25	0.53	0.13	0.15	0.13
Japan 6	0.00	0.18	0.25	0.40	0.25	0.31	0.27
Japan 8	0.00	0.27	0.10	0.22	0.10	0.12	0.21
SriLanka 2	0.17	0.40	0.63	0.71	0.27	0.17	0.13

Nam Ngat 2	0.22	0.00	0.36	0.20	0.18	0.11	0.19
LD97 BL	0.00	0.29	0.13	0.15	0.13	0.00	0.40

Table A4.2 (cont.)

	A18	**CDPxTD**	**F16**	**F35**	**LDP1**	**Manipur**	**MinhRong1**	**MinhRong2**	**PH1**
A18	1.00								
CDPxTD	0.19	1.00							
F16	0.00	0.19	1.00						
F35	0.38	0.18	0.10	1.00					
LDP1	0.55	0.17	0.00	0.43	1.00				
Manipur	0.33	0.11	0.22	0.42	0.40	1.00			
Minh Rong 1	0.10	0.18	0.10	0.36	0.17	0.11	1.00		
Minh Rong 2	0.20	0.19	0.10	0.10	0.09	0.22	0.10	1.00	
PH1	0.13	0.13	0.00	0.25	0.12	0.00	0.25	0.25	1.00
France tea	0.10	0.09	0.10	0.27	0.26	0.21	0.18	0.00	0.00
Suoi Giang 3	0.10	0.27	0.86	0.18	0.09	0.32	0.09	0.10	0.00
Ho Nam 1	0.10	0.27	0.95	0.18	0.09	0.32	0.09	0.10	0.00
Phuc An 1	0.20	0.19	0.20	0.29	0.18	0.22	0.00	0.00	0.00
Phuc An 2	0.10	0.00	0.00	0.09	0.09	0.00	0.27	0.19	0.13
Phuc Van 10	0.11	0.20	0.11	0.10	0.10	0.24	0.10	0.21	0.14
Triet Giang 2	0.22	0.32	0.00	0.11	0.10	0.00	0.21	0.22	0.00
Manipur Messai	0.13	0.63	0.13	0.50	0.24	0.29	0.13	0.13	0.14
Sri Lanka 1	0.29	0.27	0.10	0.27	0.17	0.21	0.09	0.10	0.00
Darjeeling 2	0.09	0.09	0.18	0.17	0.08	0.10	0.17	0.27	0.24
TB18 BL	0.24	0.33	0.59	0.11	0.11	0.38	0.00	0.22	0.00
Ngoc Thuy BL	0.29	0.36	0.10	0.27	0.35	0.21	0.09	0.29	0.25
PH1 BL	0.38	0.45	0.10	0.18	0.26	0.11	0.09	0.19	0.00
SChina BL	0.18	0.09	0.09	0.26	0.17	0.20	0.52	0.27	0.12
Kim Tuyen BL	0.38	0.35	0.13	0.35	0.22	0.13	0.24	0.24	0.27
No9	0.11	0.21	0.44	0.00	0.11	0.13	0.11	0.12	0.00
No18	0.38	0.09	0.10	0.55	0.17	0.21	0.09	0.00	0.13
No21	0.38	0.35	0.13	0.12	0.22	0.27	0.24	0.35	0.00
Huong Tich Son	0.10	0.19	0.00	0.48	0.27	0.33	0.19	0.00	0.40
Phuc Dinh 1	0.27	0.38	0.00	0.50	0.35	0.43	0.25	0.13	0.40
Japan 6	0.27	0.25	0.00	0.25	0.35	0.29	0.13	0.13	0.18
Japan 8	0.32	0.20	0.21	0.50	0.29	0.24	0.30	0.11	0.29
SriLanka 2	0.53	0.50	0.27	0.27	0.38	0.31	0.00	0.27	0.00
Nam Ngat 2	0.10	0.18	0.19	0.18	0.09	0.11	0.09	0.10	0.00
LD97 BL	0.13	0.13	0.27	0.25	0.13	0.29	0.25	0.27	0.18

Table A4.2 (cont.)

	Francetea	SuoiGiang3	HoNam1	PhucAn1	PhucAn2	PhucVan10	TrietGiang2
France tea	1.00						
Suoi Giang 3	0.09	1.00					
Ho Nam 1	0.09	0.91	1.00				
Phuc An 1	0.19	0.29	0.29	1.00			
Phuc An 2	0.18	0.00	0.00	0.10	1.00		
Phuc Van 10	0.10	0.20	0.20	0.21	0.30	1.00	
Triet Giang 2	0.11	0.00	0.00	0.22	0.32	0.35	1.00
Manipur Messai	0.13	0.25	0.25	0.27	0.00	0.29	0.29
Sri Lanka 1	0.36	0.18	0.18	0.29	0.18	0.10	0.21
Darjeeling 2	0.00	0.09	0.17	0.00	0.17	0.10	0.10
TB18 BL	0.00	0.67	0.67	0.24	0.00	0.25	0.13
Ngoc Thuy BL	0.00	0.18	0.18	0.19	0.00	0.10	0.11
PH1 BL	0.18	0.18	0.18	0.19	0.18	0.30	0.42
SChina BL	0.09	0.09	0.09	0.09	0.26	0.10	0.20
Kim Tuyen BL	0.12	0.12	0.12	0.13	0.12	0.13	0.27
No9	0.00	0.44	0.42	0.22	0.11	0.00	0.00
No18	0.36	0.18	0.18	0.29	0.18	0.10	0.11
No21	0.00	0.24	0.24	0.13	0.12	0.27	0.27
Huong Tich Son	0.29	0.19	0.10	0.20	0.00	0.32	0.00
Phuc Dinh 1	0.25	0.13	0.13	0.27	0.00	0.13	0.14
Japan 6	0.25	0.25	0.13	0.13	0.00	0.40	0.14
Japan 8	0.10	0.20	0.20	0.00	0.00	0.00	0.00
SriLanka 2	0.13	0.40	0.38	0.27	0.13	0.31	0.46
Nam Ngat 2	0.09	0.27	0.27	0.10	0.45	0.30	0.00
LD97 BL	0.13	0.38	0.27	0.13	0.13	0.13	0.00

Table A4.2 (cont.)

	ManipurMessai	SriLanka1	Darjeeling2	TB18BL	NgocThuyBL	PH1BL
ManipurMessai	1.00					
SriLanka1	0.24	1.00				
Darjeeling2	0.12	0.00	1.00			
TB18BL	0.13	0.21	0.11	1.00		
NgocThuyBL	0.25	0.18	0.00	0.22	1.00	
PH1BL	0.25	0.36	0.09	0.33	0.18	1.00
SChinaBL	0.00	0.00	0.08	0.11	0.09	0.00
KimTuyenBL	0.27	0.22	0.00	0.24	0.24	0.24
No9	0.00	0.00	0.21	0.29	0.11	0.11
No18	0.38	0.45	0.09	0.11	0.09	0.18

No21	0.27	0.22	0.22	0.35	0.24	0.35
HuongTichSon	0.40	0.19	0.09	0.12	0.19	0.10
PhucDinh1	0.55	0.38	0.12	0.33	0.47	0.25
Japan6	0.33	0.13	0.35	0.15	0.12	0.25
Japan8	0.29	0.00	0.00	0.13	0.20	0.00
SriLanka2	0.29	0.38	0.38	0.53	0.27	0.53
NamNgat2	0.24	0.36	0.09	0.11	0.09	0.18
LD97BL	0.00	0.13	0.00	0.17	0.13	0.00

Table A4.2 (cont.)

	SChinaBL	**KimTuyenBL**	**No9**	**No18**	**No21**	**HuongTichSon**	**PhucDinh1**
SChinaBL	1.00						
KimTuyenBL	0.11	1.00					
No9	0.32	0.00	1.00				
No18	0.09	0.24	0.00	1.00			
No21	0.11	0.13	0.14	0.12	1.00		
HuongTichSon	0.09	0.13	0.00	0.29	0.13	1.00	
PhucDinh1	0.12	0.36	0.00	0.25	0.36	0.53	1.00
Japan6	0.12	0.00	0.00	0.13	0.33	0.53	0.43
Japan8	0.48	0.27	0.12	0.20	0.13	0.21	0.27
SriLanka2	0.13	0.27	0.27	0.27	0.53	0.14	0.40
NamNgat2	0.17	0.00	0.33	0.36	0.22	0.10	0.13
LD97BL	0.38	0.36	0.17	0.13	0.00	0.25	0.20

Table A4.2 (cont.)

	Japan6	**Japan8**	**SriLanka2**	**NamNgat2**	**LD97BL**
Japan6	1.00				
Japan8	0.13	1.00			
SriLanka2	0.60	0.29	1.00		
NamNgat2	0.13	0.20	0.25	1.00	
LD97BL	0.36	0.29	0.00	0.13	1.00

Chapter 5

Assessment of genetic diversity of tea grown in Vietnam by combined analysis of ISSR and SSR and general conclusions

As reported in chapters 3 and 4, due to the large variation of quality of extracted DNA, not all tested DNA could produce clear polymorphic bands with ISSR and SSR markers. From a total of 96 tested accessions, with ISSR markers, 71 accessions could be analyzed (chapter 3); and with SSR markers, 69 accessions gave clear bands (chapter 4). For 51 accessions, results from both ISSR and SSR are available. They generated 65 and 115 polymorphic bands with both 7 ISSR primer and 6 SSR primer pairs, respectively. The data were analysis in the same way as described in chapters 3 and 4.

UPGMA-derived dendrogram (Figure 5.1) did not clearly separate all 51 tested accessions; the groups of local, selected and imported tea accessions were hardly differentiated. At the value of 0.39 of Dice similarity coefficient, except for the accessions of Japan 6, Phuc Van 10, Ngoc Thuy, Yabukita, Jetinga, small-leaved China tea BL, LD_{97}BL, Nam Ngat 2, Macomen, No6 and Phuc An 1 remaining distinct and ungrouped, 25 accessions of local, selected and imported tea were all grouped into one large cluster (cluster 2), the rest was distributed in 3 small clusters (cluster 1, 3 and 4). In each cluster, some sub-groups agreed with the known taxonomy system. For example, in cluster 1, accessions of 1A, 6A, F35 (India tea) formed sub-group with ‘France’ tea (unknown); or accessions of No17 and No8 (thought to be originated from shan tea population of the North of Vietnam) sub-grouped with Tua Chua PCO (wild giant shan tea). However the results also showed some differences to the conventional taxonomy system. All known cultivated shan tea accessions were scattered in different clusters: TB_{18}BL (in cluster 3), TB_{14} (in cluster 4), LD_{97}BL (ungrouped). The group of wild giant tea accessions does not clearly separate from other groups.

The low bootstrap values (Figure 5.1) produced from bootstrapping analysis using PAUP* software implied that no clear clusters could be identified.

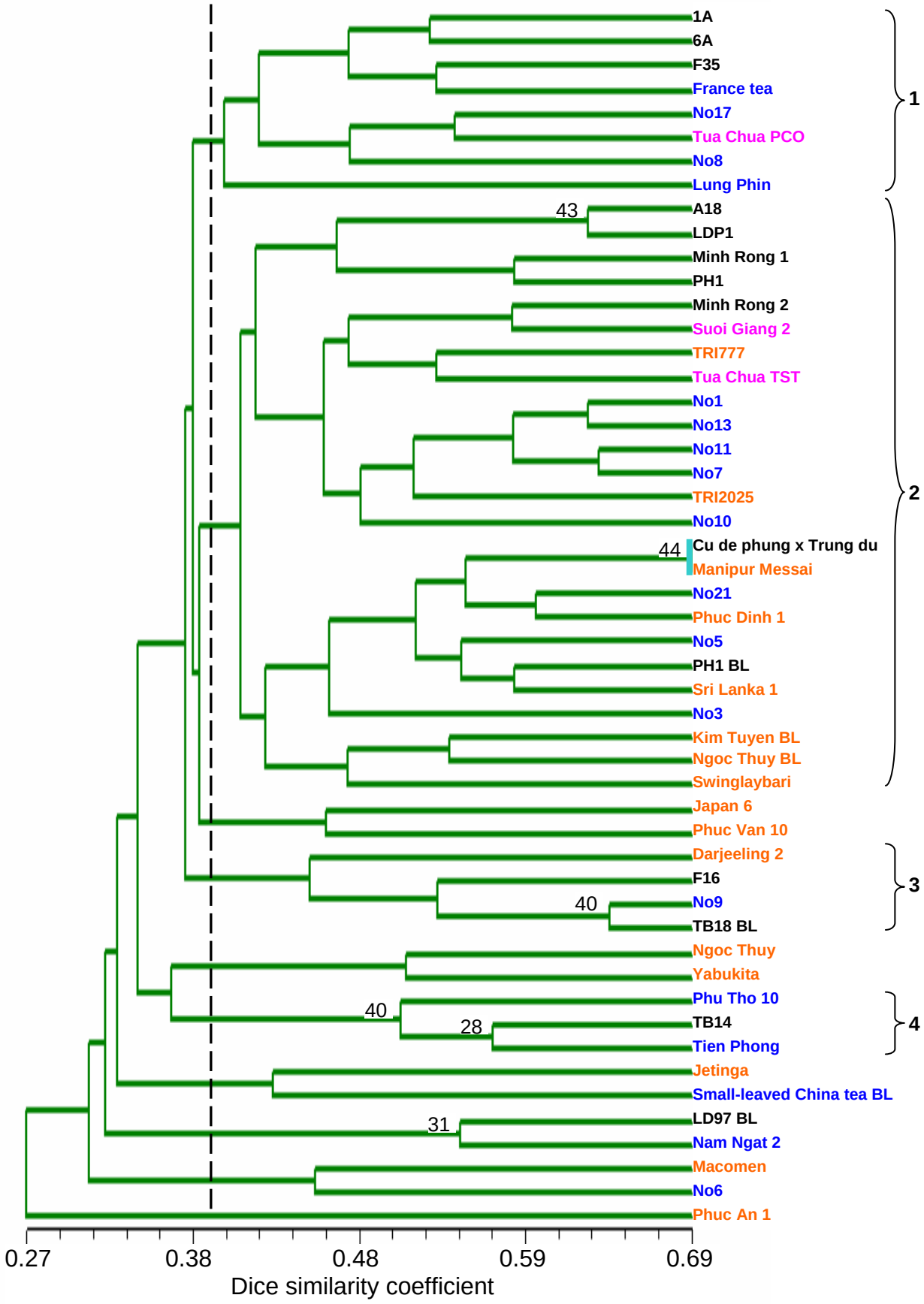


Figure 5.1: UPGMA derived dendrogram portraying genetic relationships among 51 accessions of local (in blue), wild (in pink), selected (in black) and imported (in red) teas, calculated from 65 ISSR markers and 115 SSR markers. Figures attached indicate bootstrap values based on 4000 replicate analyses.

The PCoA-derived diagram (Figure 5.2) presented the similar result with the dendrogram. The first two principal co-ordinates only contributed 13.5% of the total variation. The separation of 51 tea accessions was not clear; however, some sub-groups of known classified accessions were identified. Sub-group 1 (TB_{14} and Suoi Giang 2), 2 (Lung Phin, Tua Chua PCO and Minh Rong 1) and 3 (LD_{97}BL and TB_{18}BL) involve some known shan tea; sub-group 4 (Kim Tuyen BL and Ngoc Thuy BL) belongs to small-leaved China tea; and sub-group 5 with Swinglaybari, PH_1 and Sri Lanka 1 is originated from India tea.

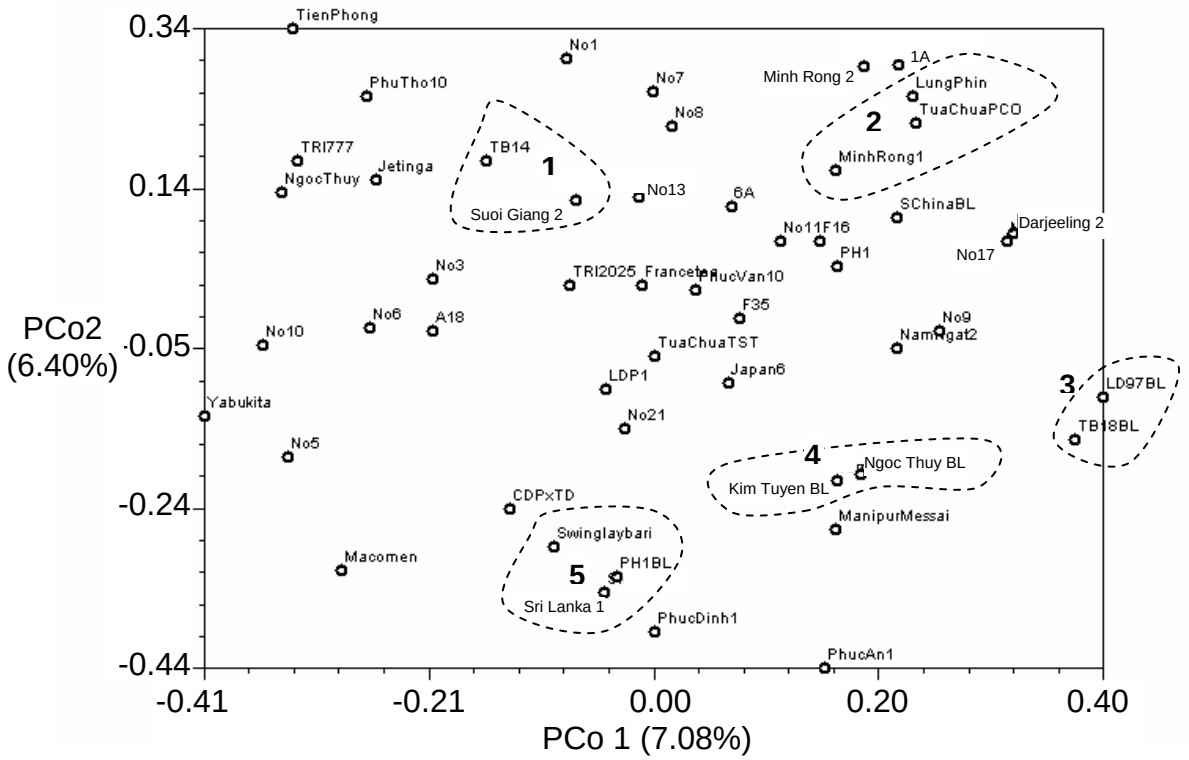


Figure 5.2: Plot of the first and second principal co-ordinates scores for 51 tea accessions based on 180 polymorphic bands generated by 7 ISSR primers and 6 SSR primer pairs.

There are two main points to be discussed to explain why the tested accessions could not be clearly distinguished. First, too few markers have been used to distinguish all tested accessions. The results showed that there were many polymorphic bands (180 bands) generated, but from only few loci. Second, there could be some variations within some tea accessions. However these variations could not be detected because for each accession the sample was taken from only one plant.

In conclusion, the main results of the study can be summarized as follow:

- In chapter 2, high genetic diversity was revealed in investigated tea accessions at Lam Dong province based on the morphological characteristics. Although the dendrogram failed in clearly separating the tested accessions, all known China, India and Shan teas

were clearly separated in sub-groups. The results of this study generally meet with the reputed taxa currently accepted in local tea production.

- In chapter 3, 65 ISSR polymorphic markers, generated from 71 accessions and 7 ISSR primers, revealed high variation among tested accessions. However, no clear cluster was identified. Most accessions including widely cultivated teas gathered in one large group.

- In chapter 4, a total of 115 different alleles were detected from 69 tea accessions by 6 SSR markers. Cluster and AMOVA analysis presented the great variation among accessions; 326 pairs of accessions had no common alleles. The separation of clusters was not clear. UPGMA dendrogram formed based on SSR markers was quite different from that based on ISSR markers and from the conventional classification.

- The dendrogram generated by combining analysis of ISSR and SSR markers also could not give clearly distinguished clusters. The group of wild giant tea accession could not separate from the other groups.

Summary

Tea (*Camellia sinensis* (L.) O. Kuntze) is probably the most important beverage worldwide and gains further popularity as an important ‘health drink’. It is served as daily morning drink for two third of the world population. Although demand of tea increases yearly, the available land for tea cultivation is limited and an increasing productivity with reduced production costs is required. Therefore, tea breeding will be of increasing importance.

Known as a cross pollinated plant, tea can not be separated into discrete groups to identify various taxa. Since hybridization is used and clonal propagation is recommended, the widespread cultivation of clonal tea can diminish genetic diversity if care is not taken to use clones of dispersed origin. Information on taxonomic characteristics, genetic diversity and biogeography of tea in the living collections may help in identifying genotypes with high production potential which could be used as genetics resources to improve the commercially grown tea.

With a longstanding history of cultivating and consuming tea, Vietnam is believed to be highly rich in genetic diversity of tea. The main objectives of this study are: (1) assessing the morphological diversity of tea grown at Lam Dong province – the main tea producing province of Vietnam; (2) collecting accessions of wild giant shan tea, local tea, selected/improved tea, and imported tea to assess the genetic diversity on molecular level by using inter simple sequence repeat (ISSR) and simple sequence repeat (SSR) markers. This will help to identify parents for hybridization and to reduce the number of accessions needed to maintain a broad range of genetic variability.

In Chapter 1, the background of this study is reviewed, i.e., the main properties and types of molecular markers presently used; an overview of tea biology and the world tea production; the recent advances of assessing tea diversity; and tea production and research in Vietnam. Vietnam is one of the top producing tea countries, but tea yield is still lower than the world average. Tea production has an important role in the agricultural sector, particularly at the highlands and the mountainous areas.

Chapter 2 reports the genetic diversity of 31 tea accessions which are commercially planted or new promising selections in Lam Dong province based on 34 quantitative and qualitative morphological characteristics. The stem, the 4th leaf, young shoot and flower were described following the IPGRI's guidelines. UPGMA-derived dendrogram and principal co-ordinates (PCo) plot were produced based on Euclidean distances for 16 quantitative morphological data. Results showed the high diversity of Lam Dong tea. All tested accessions clustered into 4 groups and all known China, India and Shan teas were clearly separated in sub-groups. The results of this study generally meet with the reputed taxa currently accepted in local tea production.

The assessment of genetic diversity in Vietnam tea using molecular markers is presented in chapters 3 and 4. In chapter 3, 144 tea accessions growing in Vietnam were screened by inter-simple sequence repeat (ISSR) markers to reveal the genetic diversity at molecular level. Genomic DNAs of all 144 accessions were extracted and primarily checked by 3 RAPD markers. Due to the low DNA quality, only 71 accessions were used in the study. The data were statistically exploited via cluster analysis based on Dice similarity coefficient matrix and AMOVA analysis. Among 15 ISSR primers tested, only 7 primers generated 64 polymorphic bands. The number of polymorphic bands generated by a primer varied between 2 and 16 and the size of the polymorphic amplified fragments scored ranged from around 325 to 2500 bp. Estimated Dice similarity values ranged from 0.09, between the most distant accessions LD97 BL (selected tea) and Yabukita (imported tea), to 1.00, between the most similar accessions Chat Tien (local tea) and Suoi Giang 6 (wild giant tea). The tested accessions exhibited more variation than in earlier reports. This great variation may be attributed to the wide variability in origin of the tested accessions. Except for some accessions remaining distinct and ungrouped, at about 50% similarity level, the dendrogram using UPGMA formed 4 clusters. The results showed the high variation between and within local, wild, selected and imported accessions.

When using simple sequence repeat (SSR) markers (chapter 4), 6 out of 17 primer pairs detected 115 different alleles from 69 accessions. The number of alleles per SSR marker varied from 11 to 25. The expected heterozygosity was very high, ranging from 0.703 to 0.928. 326 pairs of accessions had no alleles in common. The maximum value of estimated similarity was 0.95, between the most similar accessions F16 (selected tea) and Ho Nam 1 (imported tea). Cluster and AMOVA analysis showed the high variation between and within local, wild, selected and imported accessions.

For 51 accessions, results from both ISSR and SSR markers are available (chapter 5). A total of 180 polymorphic bands were generated with 7 ISSR and 6 SSR markers. UPGMA-derived dendrogram, bootstrap values and the PCo plot all showed that no clear clusters could be identified. At the value of 0.39 of Dice similarity coefficient, except for some accessions remaining distinct and ungrouped, 25 accessions of wild giant, local, selected and imported tea were all grouped into one large cluster; the others were clustered into 3 small groups. In each cluster, some sub-groups agreed with the known taxonomy system. However the results also showed some differences to the conventional taxonomy system.

Although no clearly distinct clusters could be identified, in general the results met the conventional classification (with some exception). The variations between and within local, wild, selected and imported accessions were very high due to the wide range of origin of the tested accessions. The wild giant accessions are not clearly separated from the other ones, probably because tea is freely cross pollinated and there have been many introgressions from genetic resources of wild giant tea into cultivated tea.

Zusammenfassung

Tee (*Camellia sinensis* (L.) O. Kuntze) ist wahrscheinlich das weltweit wichtigste Getränk – zwei Drittel der Weltbevölkerung trinken allmorgendlich Tee –, und es gewinnt als „gesundes Getränk“ weiter an Beliebtheit. Während also die Nachfrage nach Tee stetig steigt, ist die für den Anbau zur Verfügung stehende Fläche begrenzt, was eine Produktivitätssteigerung bei gleichzeitiger Senkung der Produktionskosten erforderlich macht. In diesem Zusammenhang kommt der Züchtung von Tee eine wachsende Bedeutung zu.

Aufgrund der Kreuzbestäubung können Teepflanzen nicht in klar getrennte Gruppen eingeteilt werden, um verschiedene Taxa zu identifizieren. Da Kreuzungen üblich sind und Klonen empfohlen wird, kann der verbreitete Anbau von nur wenigen Klonen die genetische Diversität einschränken, wenn nicht Sorge getragen wird, daß Klone verschiedenster Abstammung eingesetzt werden. Informationen zu taxonomischen Charakteristika, genetischer Diversität und der Herkunft von Tee können dabei helfen, Genotypen mit hohem Ertragspotential zu identifizieren, die dann als genetische Ressource zur Verbesserung von kommerziell angebautem Tee dienen können.

Vietnam gilt aufgrund seiner langen Tradition sowohl im Anbau als auch im Konsum von Tee als ein Land, dessen Tee eine besonders reiche genetische Diversität besitzt. Die Hauptziele der vorliegenden Untersuchung sind: (1) die Bestimmung der morphologischen Diversität des Tees, der in Vietnams Hauptanbaugebiet, der Provinz Lam Dong, wächst; (2) das Sammeln von Akzessionen wilden Riesen-Shantees, lokalen Tees, ausgewählten bzw. veredelten Tees und importierten Tees, um die genetische Diversität auf molekularer Ebene durch ISSR- sowie SSR-Marker zu bestimmen. Dies soll dazu beitragen, Elternpflanzen für Kreuzungen zu identifizieren und die Anzahl von Akzessionen zu reduzieren, die benötigt werden, um eine große Breite an genetischer Vielfalt zu bewahren.

Im ersten Kapitel werden die Grundlagen dieser Studie beleuchtet: Die Haupteigenschaften und -typen der gegenwärtig gebräuchlichen molekularen Marker werden erläutert; es wird ein Überblick über die Biologie des Tees und die weltweite Teeproduktion gegeben; die neuesten Fortschritte bei der Bestimmung von Diversität bei Tee werden vorgestellt; und die Teeproduktion und Teeforschung in Vietnam wird dargestellt. Vietnam gehört zu den führenden teeproduzierenden Länder, dennoch liegt der Ertrag immer noch unter dem weltweiten Durchschnitt. Der Anbau von Tee spielt eine wichtige Rolle im Agrarsektor, besonders im Hochland und den bergigen Regionen.

Im zweiten Kapitel wird die genetische Diversität von 31 Akzessionen von Tee aus dem kommerziellen Anbau oder von neuen vielversprechenden Tees aus der Provinz Lam Dong anhand von 34 quantitativen und qualitativen morphologischen Charakteristika dargestellt. Entsprechend der Richtlinien des IPGRI (*International Plant Genetic Resources Institute*) wurden jeweils der Stamm, das vierte Blatt, ein junger Trieb und die Blüte beschrieben. Ein UPGMA-Dendrogramm und ein Hauptkoordinatendiagramm

wurden, basierend auf Euklidischen Distanzen, für 16 quantitative morphologische Datensätze erzeugt. Die Resultate zeigten die große Diversität der Tees in Lam Dong. Alle untersuchten Akzessionen gruppierten sich in vier Gruppen, und alle bekannten chinesischen, Assam- und Shantees waren deutlich als Untergruppen erkennbar. Die Ergebnisse dieser Untersuchung stimmen insgesamt mit den Taxa überein, die derzeit in der lokalen Teeproduktion verwendet werden.

Die Bestimmung der genetischen Diversität des vietnamesischen Tees durch molekulare Marker wird in den beiden folgenden Kapiteln vorgestellt. Das dritte Kapitel beschäftigt sich mit der ISSR-Methode, mit der 144 Akzessionen von in Vietnam wachsenden Tees untersucht wurden, um durch ISSR-Marker die genetische Diversität auf molekularer Ebene zu analysieren. Aus allen 144 Akzessionen wurde genomische DNS extrahiert und zunächst mit drei RAPD-Marker untersucht. Aufgrund der geringen Qualität der DNS konnten nur 71 Akzessionen für die Untersuchung genutzt werden. Die Daten wurden statistisch in einer Clusteranalyse beruhend auf einer Dice-Ähnlichkeitsmatrix und einer Analyse der molekularen Varianz (AMOVA) ausgewertet. Die Anzahl polymorpher Banden lag zwischen zwei und sechzehn je Primer, und die Größe der Fragmente reichte von etwa 325 bp bis 2500 bp. Die geschätzten Werte des Dice-Ähnlichkeitskoeffizienten lagen zwischen 0,09 für die Akzessionen mit der größten Distanz – LD97 BL (veredelter Tee) und Yakubita (importierter Tee) – und 1,00 für die Akzessionen mit der größten Ähnlichkeit, Chat Tien (lokaler Tee) und Suoi Giang 6 (wilder Riesentee). Die untersuchten Akzessionen wiesen eine größere Variation als in früheren Untersuchungen auf, was auf die großen Unterschiede hinsichtlich des Ursprungs der getesteten Akzessionen zurückgeführt werden kann. Abgesehen von einigen Akzessionen, die isoliert blieben und keiner Gruppe zugeordnet werden konnten, bildeten sich bei einem Ähnlichkeitsgrad von etwa 50 Prozent im UPGMA-Dendrogramm vier Gruppen heraus. Die Ergebnisse zeigten sowohl große Unterschiede jeweils zwischen den Akzessionen von lokalen, wilden, veredelten und importierten Tees als auch große Unterschiede der einzelnen Akzessionen untereinander.

Bei der Anwendung von SSR-Markern (Kapitel 4) zeigten sechs von 17 Primerpaaren 115 verschiedene Allele aus 69 Akzessionen. Die Anzahl der Allele pro SSR-Marker variierte von 11 bis 25. Die erwartete Heterozygosität war sehr hoch, sie lag zwischen 0,703 und 0,928. Von den Akzessionen hatten 326 Paare keine gemeinsamen Allele. Der Höchstwert der geschätzten Ähnlichkeit lag bei 0,95, zwischen den ähnlichsten Akzessionen F16 (veredelter Tee) und Ho Nam 1 (importierter Tee). Die Clusteranalyse und die molekulare Varianzanalyse (AMOVA) zeigten eine große Vielfalt sowohl zwischen als auch innerhalb der Akzessionen von lokalen, wilden, veredelten und importierten Tees.

Für 51 Akzessionen liegen Ergebnisse hinsichtlich ISSR- wie auch SSR-Markern vor (Kapitel 5). Insgesamt konnten mit sieben ISSR- und sechs SSR-Markern 180 polymorphe Banden generiert werden. Weder durch ein UPGMA-Dendrogramm, noch über die Bootstrap-Werte oder die Hauptkoordinatenanalyse war eine Identifizierung eindeutiger Cluster möglich. Abgesehen von einigen Akzessionen, die vereinzelt und ungruppiert blieben, gruppierten sich bei einem bei 0,39 liegenden Wert des Dice-Ähnlichkeitskoeffizienten 25 Akzessionen von wilden Riesentees, von lokalen, von veredelten und von importierten Tees in nur ein einziges großes Cluster; die verbleibenden gruppierten sich in drei kleine Cluster. In jedem der Cluster waren einige Untergruppen mit dem bestehenden Taxonomiesystem vereinbar, dennoch zeigten die Ergebnisse auch einige Unterschiede zum konventionellen System.

Obwohl keine eindeutigen Cluster klar identifizierbar waren, entsprachen die Ergebnisse allgemein – mit einigen Ausnahmen – der üblichen Klassifikation. Die sehr großen Unterschiede zwischen den Akzessionen von lokalen, wilden, veredelten und importierten Tees als auch die der einzelnen Akzessionen untereinander können auf die breite Herkunft der untersuchten Akzessionen zurückgeführt werden. Die Akzessionen von wildem Riesentee konnten von den übrigen nicht klar unterschieden werden, wahrscheinlich weil es aufgrund der starken Fremdbefruchtung bei Tee häufig zur Introgression aus genetischer Ressourcen des wilden Riesentees in gezüchteten Tee gekommen ist.

References

Akagi, H., Yokozeki, Y., Inagaki, A., Nakamura, A. & Fujimura, T., 1996. A co-dominant DNA marker closely linked to the rice nuclear restorer gene, *Rf*-1, identified with inter-SSR fingerprinting. *Genome* 39: 1205 – 1209.

Amersham Bioscience, 2003. Nucleon extraction and purification protocols – Plant tissue. Amersham Bioscience Corp.

Anne, C., 2006. Choosing the right molecular genetic markers for studying biodiversity: from molecular evolution to practical aspects. *Genetica* 127: 101 – 120.

Assefa, K., Merker, A. & Tefera, H., 2003. Inter simple sequence repeat (ISSR) analysis of genetic diversity in tef [*Eragrostis tef* (Zucc.) Trotter]. *Hereditas* 139: 174 – 183.

Balasaravanan, T., Pius, P.K., Kumar, R. R., Muraleedharan, N. & Shasany, A.K., 2003. Genetic diversity among south Indian tea germplasm (*Camellia sinensis*, *C*. *assamica* and *C. assamica spp*. *lasiocalyx*) using AFLP markers. *Plant Science* 165: 365 - 372.

Banerjee, B., 1987. Can leaf aspect affect herbivory? A case study with tea. *Ecology* 86: 839 – 843.

Banerjee, B., 1988. Dry matter production and partitioning by tea varieties under differential pruning. *Applied Agricultural Research* 3: 226 - 228.

Banerjee, B., 1992. Botanical classification of tea. In: Willson, K. C. and Clifford, M. N. (eds). *Tea: Cultivation to consumption*: 24 – 51. Chapman & Hall, London.

Bao, J., Corke, H. & Sun, M., 2006. Analysis of genetic diversity and relationships in waxy rice (*Oryza sativa* L.) using AFLP and ISSR markers. *Genetic Resources and Crop Evolution 53*: 323 – 330.

Barua, D. N., 1958. Leaf scleroids in the taxonomy of the *Thea camellias*. I. Wilson's and related camellias. *Phytomorphology* 8: 257 – 264.

Barua, D. N. & Dutta, A. C., 1959. Leaf scleroids in taxonomy of *Thea camellias* II. *Camellia sinensis* L. *Phytomorphology* 9: 372 – 382.

Becker, J. & Heun, M., 1995. Mapping of digested and undigested random amplified microsatellite polymorphisms in barley. *Genome 38*: 991- 998.

Bedrook, J. R. & Kolodner, R., 1979. The structure of chloroplast DNA. *Annual Review of Plant Physiology* 30: 593 – 620.

Bezbaruah, H. P., 1971. Cytological investigation in the family Theacea - I. Chromosome numbers in some Camellia species and allied genera. *Carylogia* 24: 421 – 426.

Borthakur, S., Mondal, T. K., Borthakur, A., & Deka, P. C., 1995. Variation in peroxidase and esterase isoenzymes in tea leaves. *Two and a Bud* 42: 20 – 23.

Borthakur, S., Mondal, T. K., Parveen, S. S., Guha, A., Sen, P., Borthakur, A. & Deka, P. C., 1998. Isolation of chloroplast DNA from tea, Camellia sp. *Indian Journal of Experimental Biology* 36: 1165 – 1167.

Botstein, D., White, R. L., Skolnick, M. & Davis, R. W., 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics* 32: 314 – 331.

Brown, J. S., 1991. Principle component and cluster analyses of cotton cultivar variability across the U.S. cotton belt. *Crop Science* 31: 915 – 922.

Charcosset, A. & Moreau, L. (2004). Use of molecular markers for the development of new cultivars and the evaluation of genetic diversity. *Euphytica* 137: 81 – 94.

Charters, Y. M. & Wilkinson, M. J., 2000. The use of self-pollinated progenies as 'in-groups' for the genetic characterization of cocoa collection. *Theoretical and Applied Genetics* 100: 160 – 166.

Chen, C., 1996. Analysis on the isozymes of tea plants F1 hybrids. *Journal of Tea Science* 16: 31 – 33.

Chen, J., Wang, P., Xia, Y., Xu, M. & Pei, S., 2005. Genetic diversity and differentiation of *Camellia sinensis* L. (cultivated tea) and its wild relatives in Yunnan province of China, revealed by morphology, biochemistry and allozyme studies. *Genetic Resources and Crop Evolution* 52: 41 - 52.

Chen, L., Yang, Y., Yu, F., Gao, Q. & Chen, D., 1998. Genetic diversity of 15 tea [*Camellia sinensis* (L.) O. Kuntze] cultivars using RAPD markers. *Journal of Tea Science* 18: 25 - 33.

Chen, L. & Yu, F., 2001. Distribution, diversity, utilization and conservation of indigenous wild tea *camellias* (*Camellia* sp.) in China. Unpublished article.

Chen, L. & Yamaguchi, S., 2002. Genetic diversity and phylogeny of tea plant (*Camellia sinensis*) and its related species and varieties in the section *Thea* genus *Camellia* determined by randomly amplified polymorphic DNA analysis. *The Journal of Horticulture Science and Biotechnology* 77: 729 – 732.

Chen, Z., 1999. Pharmalogical functions of tea. In: Jain, N. K. (ed). *Global advances in tea science*. Aravali Books International (P) Ltd. India. 333 – 358.

Chengyin, L., Weihua, L. & Mingjun, R., 1992. Relationship between the evolutionary relatives and the variation of esterase isozymes in tea plant. *Journal of Tea Science* 12: 15 – 20.

Chu, D. C., 1997. Green tea – Its cultivation, processing of the leaves for drinking materials, and kinds of green tea. In: Yamamoto, T., Juneja, L. R., Chu, D.–C. and Kim, M. *Chemistry and Applications of green tea*: 1 - 11. CRC Press.

Chu, H. C. & Vo, T. D., 2001. Rooting capacity of tea cutting – Biochemical components in tea shoot and sensorial testing quality of green tea at Lam Dong. The University of Agriculture and Forestry (UAF), Viet Nam. Undergraduate thesis. (in Vietnamese)

Condit, R. & Hubbel, S., 1991. Abundance and DNA sequence of two-base repeat regions in tropical tree genomes. *Genome* 34: 66 – 71.

Dang, T. D., Nguyen, D. T. & Vo, T. D., 2003. The growth capacity of TB14 tea clone grafted onto the seed-developed old tea. Nong Lam University. Undergraduate thesis. (in Vietnamese)

Das, A., Gosal, S. S., Sidhu, J. S. & Dhaliwal, H. S., 2002. Biochemical characterization of induced variants of potato (*Solanum tuberosum* L.). *Indian Journal of Genetics* 62: 146 – 148.

Demesure, B., Sodzi, N. & Petit, R. J., 1995. A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. *Molecular Ecology* 4: 129 – 131.

Devarumath, R.M., Nandy, S., Rani, V., Marimuthu, S., Muraleedharan, N. & Raina, S.N., 2002. RAPD, ISSR and RFLP fingerprints as useful markers to evaluate genetic integrity of micro-propagated plants of three diploid and triploid elite tea clones representing *Camellia sinensis* (china type) and *C. assamica* ssp. *assamica* (Assam-India type). *Plant Cell Reports* 21: 166 - 173.

Dice, L. R., 1945. Measures of the amount of ecologic association between species. *Ecology 26*: 297 – 302.

Djemukhatze. K. M. (translated by Nguyen, N. K. from Russian), 1981. Tea plant in the northern of Vietnam. Agriculture Press. (in Vietnamese).

Do, V. N. et al., 1998. Investigation and selection of Shan tea at the high mountainous areas of the north of Vietnam – Its potential developments. In: Chu, X. A., Do, T. B., Nguyen, V. H., Do, V. N., Nguyen, V. T., Doan, H. T. and Nguyen, V. T. (eds). *Tea researches during 1988 – 1997*: 179 - 190. Agricultural Publisher, Ha Noi, Vietnam. (in Vietnamese)

Doan, H. T. & Do, V. N., Vietnam Tea Research Institute – 10 year of establishment, research and development. In: Chu, X. A., Do, T. B., Nguyen, V. H., Do, V. N., Nguyen, V. T., Doan, H. T. and Nguyen, V. T. (eds). *Tea researches during 1988 – 1997*: 23 - 41. Agricultural Publisher, Ha Noi, Vietnam. (in Vietnamese)

Domon, E., Yanagisawa, T., Saito, A. & Takeda, K., 2004. Single nucleotide polymorphism genotyping of the barley waxy gene by polymerase chain reaction with confronting two-pair primers. *Plant Breeding* 123: 225 – 228.

Dong, C. V., Pham, S & Vo, T. D., 1999. The productivity and Quality of some promising tea clones *Camellia sinensis* (L.) O. Kuntze at Bao Loc Town, Lam Dong Province. The University of Agriculture and Forestry (UAF), Viet Nam. (in Vietnamese)

Dreisigacker, S., Zhang, P., Warburton, M. L., Van Ginkel, M., Hoisington, D., Bohn, M. & Melchinger, A. E., 2004. SSR and pedigree analyses of genetic diversity among CIMMYT wheat lines targeted to different mega-environments. *Crop Science* 44: 381 – 388.

Eden, T., 1958. The development of tea culture. In: Eden, T. (ed) *Tea*: 1 – 4. Longman, London.

Edwards, K. J., Barker, J. H. A. & Daly, A., 1996. Microsatellite libraries enriched for several microsatellites in plants. *Biotechniques* 20: 758 – 759.

Excoffier, L., Laval, G. and Schneider, S., 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online 1*: 47 - 50. Available at http://cmpg.unige.ch/software/arlequin3.

Excoffier, L., Smouse, P. E. & Quattro, J. M., 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction sites. *Genetics* 131: 479 – 491.

FAO, 2006. FAOSTAT-Agriculture. *Statistical databases*. Internet resources http://www.fao.org/waicent/portal/statistics_en.asp.

Fang, D. Q & Roose, M. L., 1997. Identification of closely related citrus cultivars with inter-simple sequence repeat markers. *Theoretical and Applied Genetics* 95: 408 – 417.

Fang, D. Q., Roose, M. L. Krueger, R. R. & Federici, C. T., 1997. Fingerprinting trifoliate orange collection accessions with isozymes, RFLPs, and inter-simple sequence repeat markers. *Theoretical and Applied Genetics* 95: 211 – 219.

Fernández, M. E., Figueiras, A. M. & Benito, C., 2002. The use of ISSR and RAPD markers for detecting DNA polymorphism, genotype identification and genetic diversity among barley cultivars with known origin. *Theoretical and Applied Genetics* 104: 845 – 851.

Forrest, G. I., 1969. Studies on the polyphenol metabolism of tissue culture derived from the tea plant (*C. sinensis* L.). *Biochemistry Journal* 113: 765 – 772.

Freeman, S., West, J., James, C., Lea, V. & Mayes, S., 2004. Isolation and characterization of highly polymorphic microsatellites in tea (*Camellia sinensis*). *Molecular Ecology Notes* 4: 324 – 326.

Fujiki, H., 1999. Two stages of cancer prevention with green tea. *Journal of Cancer Research and Clinical Oncology* 125: 589 – 597.

Galván, M.Z., Bornet, B., Balatti, P. A. & Branchard, M., 2003. Inter simple sequence repeat (ISSR) markers as a tool for the assessment of both genetic diversity and gene pool origin in common bean (*Phaseolus vulgaris* L.). *Euphytica* 132: 297 – 301.

Gemas, V. J. V., Almadanim, M. C., Tenreiro, R., Martins, A. & Fevereiro, P., 2004. Genetic diversity in the Olive tree (*Olea europaea* L. subsp. *europaea*) cultivated in Portugal revealed by RAPD and ISSR markers. *Genetic Resources and Crop Evolution* 51: 501 – 511.

Ghosh-Hazra, N., 2001. Advances in selection and breeding of tea - a review. *Journal of Plant Crop* 29: 1 – 17.

Giancola, S., McKhann, H. I., Bérard, A., Camilleri, C., Durand, S., Libeau, P., Roux, F., Reboud, X., Gut, I. G. & Brunel, D., 2006. Utilization of the three high-throughput SNP genotyping methods, the GOOD assay, Amplifluor and TaqMan, in diploid and polyploid plants. *Theoretical and Applied Genetics* 112: 1115 – 1124.

Gilbert, J. E., Lewis, R. V., Wilkinson, M. J. & Caligari, P. D. S., 1999. Developing an appropriate strategy to assess genetic variability in plant germplasm collections. *Theoretical and Applied Genetics* 98: 1125 – 1131.

Gillet, E. M., 1999. DNA markers – concepts and characteristics. In: Gillet, E. M. (ed). *Programme Molecular tools for biodiversity: Which DNA marker for which purpose?* URL: http://webdoc.sub.gwdg.de/ebook/y/1999/whichmarker/index.htm

Guohua, Z., Zhu, L., Ren, T., Zhang, L. & Gu, J., 1995. Geochemical characteristics affecting the cultivation and quality of Longjing Tea. *Journal of Geochemical Exploration* 55: 183 - 191.

Gupta, M., Chyi, Y.-S., Romero-Severson, J. & Owen, J. L., 1994. Amplification of DNA markers from evolutionarily diverse genomes using single primers of simple-sequence repeats. *Theoretical and Applied Genetics 89*: 998 – 1006.

Gupta, P. K., Varshney, R. K., Sharma, P. C. & Ramesh, B., 1999. Molecular markers and their applications in wheat breeding. *Plant Breeding* 118: 369 – 390.

Gupta, P. K. & Varshney, R. K., 2000. The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. *Euphytica* 113: 163 – 185.

Gustavo, C. –A. & Gresshoff, P. M., 1997. DNA Markers: Protocols, Applications and Overviews. Wiley-VCH.

Ha, Q. T. & Vo, T. D., 2003. Standardization of the Oolong tea processing at Lam Dong province. Nong Lam University. Undergraduate thesis. (in Vietnamese)

Ha, V. N., Nguyen, D. T. & Vo, T. D., 2002. Renewal the old seed-developed tea plantation using top-grafting method. Nong Lam University. Undergraduate thesis. (in Vietnamese)

Haddrill, P. R., Majerus, M. E. N. & Mayes, S., 2002. Isolation and characterization of highly polymorphic microsatellite loci for 2-spot ladybird (*Adalia bipunctata*). *Molecular Ecology Notes* 2: 316 – 319.

Hairong, X., Qiqing, T. & Wanfanz, Z., 1987. Studies on the genetic tendency of tea plant hybrid generation using isozyme technique. *Proc. Inter. Symp. on Tea Quality and Human Health, China*, November 4th–9th: 21 – 25.

Hazarika, M. & Mahanta, P.K., 1984. Composition changes in chlorophylls and carotenoids during the four flushes of tea in north-east India. *Journal of the Science of Food and Agriculture* 35: 298 – 303.

Henry, R. J., 1997. Practical applications of Plant molecular biology. Chapman & Hall.

Hoang, A. T., Pham, T. T. & Vo, T. D., 2005. Investigating the influence of different methods of exterminating enzymes materials to the whole procedure of green tea processing – Improving green tea quality. Nong Lam University. Undergraduate thesis. (in Vietnamese)

Holton, T. A., Christopher, J. T., McClure, L., Harker, N. & Henry, R. J., 2002. Identification and mapping of polymorphic SSR markers from expressed gene sequences of barley and wheat. *Molecular Breeding* 9: 63 – 71.

Hooykass, P. J. J. & Schilerpoort, R. A., 1992. Agrobacterium and plant genetic engineering. *Plant Molecular Biology* 19: 15 – 38.

Hour, T. C., Liang, Y. C., Chu, I. S. and Lin. J. K., 1999. Inhibition of eleven mutagens by various tea extracts, (-)Epigallocatechin-3-gallate, Gallic Acid and Caffeine. *Food and Chemical Toxicology* 37: 569 – 579.

http://www.246.ne.jp/~takeru/chalk-less/lifesci/images/pcr.gif

Huang, J. C. & Sun, M., 2000. Genetic diversity and relationships of sweet potato and its wild relatives in *Ipomoea* series *Batatas* (Convolvullaceae) as revealed by inter-simple sequence repeat (ISSR) and restriction analysis of chloroplast DNA. *Theoretical and Applied Genetics 100*: 1050 – 1060.

Ikeda, N., Kawada, M. & Takeda, Y., 1991. Isozymic analysis of *Camellia sinensis* and its interspecific hybrids *Proc. Inter. Symp. of Tea Science*, Shizuoka, Japan, Aug. 26th– 28th: 98.

IPGRI, 1997. *Descriptors for tea* (*Camellia sinensis*). International Plant Genetic Resources Institute, Rome, Italy.

Iruela, M., Rubio, J., Cubero, J. I., Gil, J. and Millán, T., 2002. Phylogenetic analysis in the genus *Cicer* and cultivated chickpea using RAPD and ISSR markers. *Theoretical and Applied Genetics* 194: 643 - 651.

Jarne, P. & Lagoda, P. J. L, 1996. Microsatellites, from molecules to populations and back. *Trends in Ecology and Evolution* 11: 424 – 429.

Jeong, S. C. & Saghai Maroof, M. A., 2004. Detection and genotyping of SNPs tightly linked to two disease resistance loci, Rsv1 and Rsv3, of soybean. *Plant Breeding* 123: 305 – 310.

Jorgensen, R. A. & Cluster, P. D., 1989. Modes and tempos in the evolution of nuclear ribosomal DNA: new character for evolutionary studies and new markers for genetic and population studies. *Annual Molecular Botany Garden* 75: 1238 – 1247.

Joshi, S. P., Gupta, V. S., Aggarwal, R. K., Ranjekar, P. K. & Brar, D. S., 2000. Genetic diversity and phylogenetic relationship as revealed by inter simple sequence repeat (ISSR) polymorphism in the genus *Oryza*. *Theoretical and Applied Genetics* 100: 1311 – 1320.

Kang, W. S., Lim, I. H., Yuk, D. Y., Chung, K. H., Park, J. B., Yoo, H. S. and Yun, Y. P., 1999. Antithrombotic Activities of Green Tea Catechin and (2)-Epigallocatechin Gallate. *Thrombosis research* 96: 229 – 237.

Kantety, R. V., Zeng, X., Bennetzen, J. L. & Zehr, B. E., 1995. Assessment of genetic diversity in dent and popcorn (*Zea mays* L.) inbred lines using inter-simple sequence repeat (ISSR) amplification. *Molecular Breeding* 1: 365 – 373.

Kato, M., 1985. Regeneration of plantlets from tea stem callus. *Japan Journal Breeding* 35: 317 – 322.

Katoh, Y., Katoh, M., Takeda, Y. and Omori M., 2003. Genetic diversity within cultivated teas based on nucleotide sequence comparison of ribosomal RNA maturase in chloroplast DNA. *Euphytica* 134: 287 - 295.

Kaundun, S. S., Zhyvoloup, A. & Park, Y. G., 2000. Evaluation of the genetic diversity among elite tea (*Camellia sinensis* var. *sinensis*) accessions using RAPD markers. *Euphytica* 115: 7 - 16.

Kaundun, S. S. & Park, Y. G., 2002. Genetic structure of six Korean tea populations as revealed by RAPD-PCR markers. *Crop Science* 42: 594 - 601.

Kaundun, S. S. & Matsumoto, S., 2003. Development of CAPS markers based on three key genes of the phenylpropanoid pathway in Tea, *Camellia sinensis* (L.) O. Kuntze, and differentiation between assamica and sinensis varieties. *Theoretical and Applied Genetics* 106: 375 - 383.

Khlestkina, E. K., Huang, X. Q., Quenum, F. J. –B., Chebotar, S., Roeder, M. S. & Boerner, A., 2004. Genetic diversity in cultivated plants – loss or stability? *Theoretical and Applied Genetics* 108: 1466 - 1472

Kim, D. H., Zur, G., Danin-Poleg, Y., Lee, S. W., Shim, K. B., Kang, C. W. & Kashi, Y., 2002. Genetic relationships of sesame collection collection as revealed by inter-simple sequence repeats. *Plant Breeding* 121: 259 – 262.

Kitamura, S., 1950. Acta phytotaxonomy and Geobotany. Kyoto 14: 56.

Klee, H. J., Horsch, R. & Rogers, S., 1987. Agrobacterium-mediated plant transformation and its further application to plant biology. *Annual Review of Plant Physiology* 38: 467 – 486.

Kochieva, E. Z., Ryzhova, N. N., Khrapalova, I. A. & Pukhalskyi, V. A., 2002. Genetic diversity and phylogenetic relationships in the genus *Lycopersicon* (Tourn.) Mill. as revealed by inter-simple sequence repeat (ISSR) analysis. *Russian Journal of Genetics* 38: 958 – 966.

Kondo, K., 1977. Chromosome numbers in the genus Camellia. *Biotropica* 9: 86 – 94.

Kondo, K., 1978. Cytological studies in cultivated species of *Camellia*. In: Encyclopedia of *Camellia* (ed). Japan Camellia Society 2. Kodansha Publisher Co., Tokyo.

Kondo, K., 1979. Cytological studies in cultivated species of *Camellia.* V. Intraspecific variation of karyotypes in two species of sect. *Thea. Jap J. Breed.* 29: 205 – 210.

Kondo, K. & Parks, C. R., 1979. Giemsa C-banding and karyotype of *Camellia* (-banded karyotypes can tell more detail on inter and intra-specific relationships in *Camellia*). *American Camellia Yearbook* 34: 40 – 47.

Kondo, K. & Parks, C. R., 1981. Cytological studies in cultivated species of *Camellia*. VI. Giemsa C-banded karyotypes of seven accessions of *Camellia japonica* L. *sensu* lato. *Jap. J Breed*. 31: 25 – 34.

Kuhlemeiere, C., Green, P. J. & Chua, N. H., 1987. Regulation of gene expression in higher plants. *Annual Review of Plant Physiology* 38: 221 – 257.

Kulasegaram, S., 1980. Technical developments in tea production. *Tea Quarterly* 49: 157 – 183.

Kung, S. D., 1977. The expression of chloroplast genomes in higher plants. *Annual Review of Plant Physiology* 28: 401 – 437.

Lai, J. A., Yang, W. C. and Hsiao, J. Y., 2001. An assessment of genetic relationships in cultivated tea clones and native wild tea in Taiwan using RAPD and ISSR markers. *Botanical Bulletin of Academia Sinica* 42: 93-100.

Lam Dong Center for Research and Transfer technology in Industrial crops and Fruit tree Production, 2004. Yearly report. (in Vietnamese)

Leigh, F., Lea, V., Law, J., Wolters, P., Powell, W., & Donini, P., 2003. Assessment of EST- and genomic microsatellite markers for variety discrimination and genetic diversity studies in wheat. *Euphytica* 133: 359 – 366.

Liang, Y. R., Tanaka, J. C. & Takeda, Y. Y., 2000. Study on diversity of tea germplasm by RAPD method. *JI Zejiang Forestry College* 17: 215 – 218.

Linnaeus, C., 1752. Species Plantarum 1st edition.

Lopez, C., Piégu, B., Cooke, R., Delseny, M., Tohme, J. & Verdier, V., 2005. Using cDNA and genomic sequences as tools to develop SNP strategies in cassava (*Manihot esculenta* Crantz). *Theoretical and Applied Genetics* 110: 425 – 431.

Magoma, G.N., Wachira, F.N., Obanda, M., Imbuga M. & Agong, S.G., 2000. The use of catechins as biochemical markers in diversity studies of tea (*Camellia sinensis*). *Genetic Resources and Crops Evolution* 47: 107-114.

Mahipal, S., Bandana and Ahuja, P.S., 1999. Isolation and PCR Amplification of genomic DNA from market samples of dry tea. *Plant Molecular Biology Reporter* 17: 171 – 178.

Manimekalai, R. & Nagarajan, P., 2006. Assessing genetic relationships among coconut (*Cosos nucifera* L.) accessions using inter simple sequence repeat markers. *Scientia Horticulturae* 108: 49 – 54.

Martín, J. P. & Sánchez-Yélamo, M. D., 2000. Genetic relationships among species of genus *Diplotaxis* (Brassicaceae) using inter-simple sequence repeat markers. *Theoretical and Applied Genetics* 101: 1234 – 1241.

Matsumoto, S., Takeuchi, A., Hayatsu, M. & Kondo, S., 1994. Molecular cloning of phenyllanine ammonia-lyase cDNA and classification of varieties and cultivars of tea plants (*Camellia sinensis*) using the tea PAL cDNA probe. *Theoretical and Applied Genetics* 89: 671-675.

Matsumoto, S., Kiriiwa, Y. & Takeda, Y., 2002. Differentiation of Japanese green tea cultivars as revealed by RFLP analysis of phenylalanine ammonia-lyase DNA. *Theoretical and Applied Genetics* 104: 998-1002.

Matsumoto, S. Kiriiwa, Y. & Yamaguchi, S., 2004. The Korean tea plant (*Camellia sinensis*): RFLP analysis of genetic diversity and relationship to Japanese tea. *Breeding Science* 54: 231 – 237.

Matthews, R. B. & Stephens, W., 1998. CUPPA-TEA: A simulation model describing seasonal yield variation and potential production of tea. 1. Shoot development and extension. *Experimental Agriculture*, 34, 345 - 367.

Meksem, K., Leister, D., Peleman, J., Zabeau, M., Salamini, F. & Gebhardt, C., 1995. A high resolution map of the vicinity of the R1 locus on chromosome V of potato based on RFLP and AFLP markers. *Molecular and General Genetics* 249: 74 – 81.

Meyer, W., Mitchell, T. G., Freedman, E. Z. & Vilgays, R., 1993. Hybridization probes for conventional DNA fingerprinting used as single primers in the polymerase chain reaction to distinguish strains of Cryptococcus neoformans. *Journal of Clinic Microbiology* 31: 2274 – 2280.

Métais, I., Aubry, C., Hamon, B., Jalouzot, R. & Peltier, D., 2000. Description and analysis of genetic diversity between commercial bean lines (*Phaseolus vulgaris* L.). *Theoretical and Applied Genetics* 101: 1207 – 1214.

Mondal, T. K., 2002a. Camellia biotechnology: A bibliographic search. *International Journal of Tea Sciences* 1: 28 – 37.

Mondal, T. K., 2002b. Assessment of genetic diversity of tea (*Camellia sinensis* (L.) O. Kuntze) by inter-simple sequence repeat polymerase chain reaction. *Euphytica* 128: 307 - 315.

Mondal, T.K., Singh, H. P. & Ahuja, P. S., 2000. Isolation of genomic DNA from tea and other phenol rich plants. *Journal of Plantation Crops* 28: 30 - 34.

Mondal, T.K. & Chand, P. K., 2002. Detection of genetic instability among the micropropagated tea (*Camellia sinensis*) plants. *In Vitro Cell Developmental Biology - Plant* 37: 1 – 5.

Mondal, T. K., Bhattacharya, A., Laxmikumaran, M. & Ahuja, P. S., 2004. Recent advances of tea (*Camellia sinensis*) biotechnology. *Plant Cell, Tissue and Organ Culture* 76: 195 – 254.

Moreno, S., Martin, J. P. & Ortiz, J. M., 1998. Inter-simple sequence repeats PCR for characterization of closely related grapevine collection. *Euphytica* 101: 117 – 125.

Navajas, M. & Fenton, B., 2000. The application of molecular markers in the study of diversity in acarology: a review. *Experimental and Applied Acarology* 24: 751 – 774.

Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583–590.

Nelson, N., Melson, H. & Schatz, G., 1980. Biosynthesis and assembly of the protein-translocating adenosine triphosphete complex from chloroplasts. *Natl. Acad. Sci. 77*: 1361 – 1364.

Nguyen, H. L., Do, V. N., Nguyen, M. H. & Le, T. B., 2004. Assessing the polymorphism of some Shan tea clones at Phu Ho using RAPD markers. *Agriculture and Rural Development* 10/2004: 1331 – 1333.

Nguyen, N. K., 1998. Introduction. In: Chu, X. A., Do, T. B., Nguyen, V. H., Do, V. N., Nguyen, V. T., Doan, H. T. & Nguyen, V. T. (eds). *Tea researches during 1988 – 1997*: 3 - 6. Agricultural Publisher, Ha Noi, Vietnam. (in Vietnamese)

Nguyen, Q. L. & Vo, T. D., 2002. The influence of the age of the scion, stock and the cutting on vegetative propagation of tea plant. Nong Lam University. Undergraduate thesis. (in Vietnamese)

Nguyen, V. T., Tran, T. L. & Nguyen, V. N., 1998a. Methodology in tea plant selection. In: Chu, X. A., Do, T. B., Nguyen, V. H., Do, V. N., Nguyen, V. T., Doan, H. T. and Nguyen, V. T. (eds). *Tea researches during 1988 – 1997*: 309 - 325. Agricultural Publisher, Ha Noi, Vietnam. (in Vietnamese)

Nguyen, V. T *et al*., 1998b. Methodology in tea plant propagation. In: Chu, X. A., Do, T. B., Nguyen, V. H., Do, V. N., Nguyen, V. T., Doan, H. T. and Nguyen, V. T. (eds). *Tea researches during 1988 – 1997*: 326 - 338. Agricultural Publisher, Ha Noi, Vietnam. (in Vietnamese)

Nguyen, V. T. & Nguyen, T. M. P., 2004. Primary results in the hybridization between some Shan clones and China teas. *Agriculture and Rural Development* 10/2004: 1328 – 1330. (in Vietnamese)

Olmstead, R. G. & Palmer, J. D., 1994. Chloroplast DNA and systematic – a review of methods and data analysis. *American Journal of Botany* 81: 1205 – 1224.

Olson, R. K. & Francis, C. A., 1995. A hierarchical framework for evaluating diversity in agroecosystems. In: Olson, R., Francis, C. & Kaffka, S., (eds). *Exploring the role of diversity in sustainable agriculture*: 5 - 34. American Society of Agronomy, Inc.

Owuor, P. O., 1989. Differentiation of teas by the variations of linalools and geraniols contents. *Bull. Chem. Soc Ethip.* 3: 31 – 35.

Owuor, P.O., Reeves, S.G. & Wanyoko, J.K., 1986. Co-relation of flavins content and valuation of Kenyan black teas. *Journal of the Science of Food and Agriculture* 37: 507 – 513.

Panda, S., Martín, J. P. & Aguinagalde, I., 2003. Chloroplast and nuclear DNA studies in a few members of the *Brassica oleracea* L. group using PCR-RFLP and ISSR-PCR markers: a population genetic analysis. *Theoretical and Applied Genetics* 106: 1122 – 1128.

Paris, H. S., Yonash, N., Portnoy, V., Mozes-Daube, N., Tzuri, G. & Katzir, N., 2003. Assessment of genetic relationships in *Cucurbita pepo* (Cucurbitaceae) using DNA markers. *Theoretical and Applied Genetics* 106: 971 – 978.

Park, Y. G., Kaundun, S. S. and Zhyvoloup, A., 2002. Use of the bulked genomic DNA-based RAPD methodology to assess the genetic diversity among abandoned Korean tea plantations. *Genetic Resources and Crop Evolution*.

Patzak, J., 2001. Comparison of RAPD, STS, ISSR and AFLP molecular methods used for assessment of genetic diversity in hop (*Humulus lupulus* L.). *Euphytica* 121: 9 – 18.

Paul, S., Wachira, F.N., Powell, W. & Waugh, R., 1997. Diversity and genetic differentiation among populations of Indian and Kenyan tea (*Camellia sinensis* (L.) O. Kuntze) revealed by AFLP markers. *Theoretical and Applied Genetics* 94: 255 - 263.

Pham, T. T. & Vo, T. D., 2001. The growth and yield of some tea cultivars/clones *Camellia sinensis* (L.) O. Kuntze at Bao Lam District, Lam Dong Province. The University of Agriculture and Forestry (UAF), Viet Nam. Undergraduate thesis. (in Vietnamese)

Powell, W., Machray, G. C. & Provan, J., 1996. Polymorphism revealed by simple sequence repeats. *Trends in Plant Science* 1: 215 – 222.

Prince, L. M. & Parks, C. R., 2001. Phylogenetic relationships of *theaceae* inferred from chloroplast DNA sequence data. *American Journal of Botany* 88: 2309 – 2320.

Purseglove, J. W., 1968. Tropical Crops. In: Dicotyledons 2. Longmans Green, London.

Qian, W., Ge, S. & Hong, D. –Y., 2001. Genetic variation within and among populations of wild rice *Oryza granulate* from China detected by RAPD and ISSR markers. *Theoretical and Applied Genetics* 102: 440 – 449.

Rajasekaran, P., 1997. Development of molecular markers using AFLP in tea. Varghese, J. P. (ed). Molecular Approaches to Crop Improvement. *Proceedings of National Seminar on Molecular approaches to Crop Improvement* 29th – 31st December: 54 – 58. Kottayam, Kerala, India.

Ratnaparkhe, M. B., Tekeoglu, M. & Muehlbauer, F. J., 1998. Inter simple sequence repeat (ISSR) polymorphisms are useful for finding markers associated with disease resistance gene clusters. *Theoretical and Applied Genetics* 97: 515 – 519.

Reddy, M. P., Sarla, N. & Siddiq, E. A., 2002. Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. *Euphytica* 128: 9 – 17.

Rietveld, A. and Wiseman, S., 2003. Antioxidant Effects of Tea: Evidence from Human Clinical Trials. *Proceedings of the Third International Scientific Symposium on Tea and Human Health: Role of Flavonoids in the Diet*. American Society for Nutritional Sciences.

Roberts, E. A. H., Wight, W. & Wood, D.J., 1958. Paper chromatography as an aid to the identification of *Thea camellias*. *New Phytologist* 57: 211 – 225.

Rohlf, F. J., 2000. NTSYS-pc. Numerical taxonomy and multivariate analysis system. Exeter Software, New York.

Roy, A., Bandyopadhyay, A., Mahapatra, A. K., Ghosh, S. K., Singh, N. K., Bansal, K. C., Koundal, K. R. & Mohapatra, T., 2006. Evaluation of genetic diversity in jute (*Corchorus* species) using STMS, ISSR and RAPD markers. *Plant Breeding* 125: 292 – 297.

Saini, N., Jain, N., Jain, S. & Jain, R. K., 2004. Assessment of genetic diversity within and among Basmati and non-Basmati rice varieties using AFLP, ISSR and SSR markers. *Euphytica* 140: 133 – 146.

Sanderson, G. W., 1964. The chemical composition of fresh tea flush as affected by clone and climate. *Tea Quarterly* 35: 101 – 109.

Sankar, A. A. & Moore, G. A., 2001. Evaluation of inter-simple sequence repeat analysis for mapping in Citrus and extension of genetic linkage map. *Theoretical and Applied Genetics* 102: 206 – 214.

Sasazuki, S., Kodama, H., Yoshimasu, K., Liu, Y., Washio, M., Tanaka, K., Tokunaga, S., Kono, S., Arai, H., Doi, Y., Kawano, T., Nakagaki, O., Takada, K., Koyanagi, S., Hiyamuta, K., Nii, T., Shirai, K., Ideishi, M., Arakawa, K., Mohri, M. and Takeshita, A., 2000. Relation between green tea consumption and the severity of coronary atherosclerosis among Japanese men and women. *Annual Epidemiol* 10: 401 – 408.

Satyanarayana, N. and Sharma, V. S., 1986. Tea (*Camellia* L. spp) germplasm in south India. In: Srivastava, H. C., Vatsya, B. and Menon, K. K. g. (eds). *Plantation Crops: Opportunities and constraints*: 173 - 179. Oxford IBH Publishing Co., New Delhi.

Schuelke, M., 2000. An economic method for the fluorescent labeling of PCR fragments. *Nature Biotechnology* 18: 233 – 234.

Sealy, J., 1958. A revision of the genus *Camellia*. Royal Horticulture Society. London.

Senior, M. L., Murphy, J. P., Goodman, M. M. & Stuber, C.W., 1998. Utility of SSRs for determining genetic similarities and relationships in maize using agarose gel system. Crop Science 38: 1088 – 1098.

Sen, P., Bora, U., Roy, B. K. & Deka, P. C., 2000. Isozyme characterization in Camellia spp. *Crop Reservation* 19: 519 – 524.

Seurei, P., 1996. Tea improvement in Kenya: A review. *Tea* 17: 76 – 81.

Singh, H. P. & Ravindranath, S. D., 1994. Occurrence and distribution of PPO Activity in floral organs of some standard and local cultivars of tea. *Journal of the Science of Food and Agriculture* 64: 117 – 120.

Singh, I. D., 1980. Non conventional approaches to the breeding of tea in north-east India. *Two and a Bud* 27: 3 – 6.

Singh, I. D., 1999. Plant Improvement. Jain, N. K. (ed). *Global Advances in Tea*: 427 – 448. Aravali Book International (P) Ltd. India.

Smith, R. H. & Hood, E. E., 1995. *Agrobacterium tumefaciens* transformation of monocots. *Crop Science* 35: 301 – 309.

Smith, J. S. C, Chin, E. C. L., Shu, H., Smith, O. S., Wall, S. J., Senior, M. L., Mitchell, S. E., Kresovich, S. and Zeigle, J., 1997. An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays* L.): Comparison with data from RFLPs and pedigree. *Theoretical and Applied Genetics* 95: 163 – 173.

Soleimani, V. D., Baum, B. R. & Johnson, D. A., 2006. Analysis of Genetic Diversity in Barley Cultivars Reveals Incongruence Between S-SAP, SNP and Pedigree Data. *Genetic Resources and Crop Evolution* 00: 1 – 15.

Souframanien, J. & Gopalakrishna, T., 2004. A comparative analysis of genetic diversity in blackgram genotypes using RAPD and ISSR markers. *Theoretical and Applied Genetics* 109: 1687 – 1693.

Staub, J. E., Kuhns, L. J., May, B. & Grun, P., 1982. Stability of potato tuber isozymes under different storage regimes. J*. Am. Soc. Hort. Sci*. 107: 405–408

Staub, J. E., Serquen, F. C. & Gupta, M., 1996. Genetic markers, map construction, and their application in plant breeding. *Horticultural Science* 31: 729 – 739.

Suganuma, M., Ohkura, Y. and Okabe, S., 2001. Combination cancer chemoprevention with green tea extract and sulindac shown in intestinal tumor formation in Min mice. *Journal of Cancer Research and Clinical Oncology* 127: 69 – 72.

Swofford, D. L., 1998. Phylogenetic analysis using parsimony (* and other methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.

Taberlet, P., Gielly, L., Pauton, G. & Bouvet, J., 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105 – 1109.

Takeda, Y., 1994. Differences in caffeine and tannin contents between tea cultivars and application to tea breeding. *Japan Agricultural Research Quarterly* 28: 117 – 123.

Takeo, T., 1981. Variations in amounts of linalool and geraniol produced in tea shoots by mechanical injury. *Phytochemistry* 30: 2149 – 2151.

Takeo, T., 1983. Effects of clonal specificity of the monoterpene alcohol composition of tea shoots on black tea aroma profile. *Japan. Agric. Res. Duart*. 17: 120 – 124.

Takeuchi, A., Matsumoto, S. & Hayatsu, M., 1994. Amplification of (-)tubulin cDNA from *Camellia sinensis* by PCR. *Bull. Nat. Res. Inst. Veg. Orna*. 7: 13 – 20.

Talhihas, P., Neves-Martins, J. & Leitão, J., 2003. AFLP, ISSR and RAPD markers reveal high levels of genetic diversity among *Lupinus* spp.. *Plant Breeding* 122: 507 – 510.

Tanaka, J. I. & Yamaguchi, S., 1996. Use of RAPD markers for the identification of parentage of tea cultivars. *Bull. Nat. Res. Inst. Veg. Orna. Plant Tea* 9: 31 – 36.

Tanaka, J., Yamaguchi, N. & Nakamura, Y., 2001. Pollen parent of tea cultivar ‘Sayamakaori’ with insect and cold resistance may not exist. *Breeding Reservation* 3: 43 – 48.

Tanksley, S. D., Yong, N. D., Paterson, A. H. & Bonierbals, M. W., 1989. RFLP mapping in plant breeding: new tools for an old science. *Biotechnology* 7: 257 – 264.

Tautz, D. & RenZ, M., 1984. Simple sequences are ubiquitous repetitive components of eukaryotic genomes. *Nucleic Acids Research* 12: 4127 – 4138.

Terzopoulos, P. J., Kolano, B., Bebeli, P. J., Kaltsikes, P. J. & Metzidakis, I., 2005. Identification of *Olea europaea* L. Cultivar using inter-simple sequence repeat markers. *Scientia Horticulturae* 105: 45 – 51.

Tran, H. K. & Vo, T. D., 2002. Study on the media for tea tissue culture. Estimation of tea production based on leaf structure. Nong Lam University. Undergraduate thesis. (in Vietnamese)

Tran, T. L. & Nguyen, V. N., 1998. Results of ten year study on tea breeding. In: Chu, X. A., Do, T. B., Nguyen, V. H., Do, V. N., Nguyen, V. T., Doan, H. T. and Nguyen, V. T. (eds). *Tea researches during 1988 – 1997*: 50 - 66. Agricultural Publisher, Ha Noi, Vietnam. (in Vietnamese)

Tran, X. G., 2003. Personal communication. Ha Noi. (in Vietnamese)

Tsumura, Y., Ohba, K. & Strauss, S. H., 1996. Diversity and inheritance of Inter-simple sequence repeat polymorphism in douglus fir (*Pseudotsuga menziessi*) and sugi (*Cryptomeria japonica*). *Theoretical and Applied Genetics* 92: 40 – 45.

Ueno, S., Yoshimaru, H., Tomaru, N. & Yamamoto, S., 1999. Development and characterization of microsatellite markers in *Camellia japonica* L. *Molecular Ecology* 9: 335 - 336.

Varghese, J. P., Rudolph, B., Uzumova, M. I. & Ecke, W., 2000. Use of 5'-anchored primers for the enhanced recovery of specific microsatellite markers in *Brassica napus* L. *Theoretical and Applied Genetics* 101: 115 – 119.

Varshney, R. K., Graner, A. & Sorrells, M. E., 2005. Genic microsatellite markers in plants: features and applications. *Trends in Biotechnology* 23: 48 – 55.

Varshney, R. K., Sigmund, R., Börner, A., Korzun, V., Stein, N., Sorrells, M. E., Langridge, P. & Graner, A., 2005. Interspecific transferability and comparative mapping of barley EST-SSR markers in wheat, rye and rice. *Plant Science* 168: 195 – 202.

Vietnam Tea Association, 1999. Tea: Economy and technology. (in Vietnamese)

Vignal, A., Milan, D., Cristobal, M. S. & Eggen, A., 2002. A review on SNP and other types of molecular markers and their use in animal genetics. *Genetics Selection Evolution* 34: 275 – 305.

Virk, P. S., Zhu, J., Newbury, H. J., Bryan, G. J., Jackson, M. T. & Ford-Lloyd, B. V., 2000. Effectiveness of different classes of molecular marker for classifying and revealing variation in rice (*Oryza sativa*) germplasm. *Euphytica* 112: 275 – 284.

Visser, T., 1969. Tea Camellia sinensis (L.) O. Kuntze. Ferwerda, F. P. and Wit, F. *Outlines of Perennial Crop breeding in the Tropics*: 459 – 493. H. Veennan and Zonen, Wageningen.

Vo, T. D., Huynh, T. H. & Becker, H. C., 2001. Evaluating the growth, productivity and quality of six promising tea clones (*Camellia sinensis* (L.) O. Kuntze) at Lam Dong province, Viet Nam. George-August Goettingen University. Master thesis.

Vo, V. N. T., Le, Q. H. & Vo, T. D., 1998. Investigating some promising clones at Lam Dong province. The University of Agriculture and Forestry (UAF), Viet Nam. Undergraduate thesis. (in Vietnamese)

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van de Lee, T., Hornes, M., Freijters, A., Pot, J., Peleman, J., Kuiper, M. & Zabeau, M., 1995. AFLP: A new techniques for DNA fingerprinting. *Nucleic Acids Research* 23: 4407 – 4414.

Wachira, F. N., 1990. Desirable tea plants: an overview of a search for markers. *Tea* 11: 42 – 48.

Wachira, F. N., Waugh, R., Hackett, C.A. and Powell, W., 1995. Detection of genetic diversity in tea (*Camellia sinensis*) using RAPD markers. *Genome* 38: 201-210.

Wachira, F. N., Powell, W. & Waugh, R., 1997. An assessment of genetic diversity among *Camellia sinensis* L. (cultivated tea) and its wild relatives based on randomly amplified polymorphic DNA and organelle-specific STS. *Heredity* 78: 603-611.

Waugh, R., Van de Ven, W. T. G., Phillips, M. S. & Powell, W., 1990. Chloroplasts DNA diversity in the genus Rubus (*Rosaceae*) revealed by southern hybridization. *Plant Systematic and Evolution* 172: 65 – 75.

Weather Station of Bao Loc Technical and Vocational high school, 2004. *Monthly report*. (In Vietnamese)

Weatherstone, J., 1992. Historical introduction. In: Willson, K. C. And Clifford, M. N. (eds), 1992. *Tea: Cultivation to consumption*: 1 – 23. Chapman & Hall, London.

Weising, K., Nybom, H, Wolff, K. & Kahl, G., 2005. Detecting DNA variation by molecular markers. In: *DNA fingerprinting in Plants: Principles, methods, and applications*: 21 – 73. Taylor & Francis groups.

Wickramaratne, M. R. T., 1981. Variation in some leaf characteristics in tea (*Camellia sinensis* L.) and their use in the identification of clones. *Tea Quarterly* 50: 183 – 198.

Wickremasinghe, R. L., 1979. Tea. *Advances in Food research* 24: 229 – 286.

Wight, W., 1958. The agrotype concept in tea taxonomy. *Nature* 181: 893 – 895.

Wight, W., 1962. Tea classification revised. *Current Science* 31: 298 – 299.

Wight, W. & Barua, D. N., 1954. Morphological basis of quality in tea. *Nature* 173: 630 – 631.

Williams, J. G. K., Kubelik, A. R. K., Livak, J. L., Rafalski, J. A. & Tingey, S. V., 1990. DNA polymorphisms amplified by random primers are useful as genetic markers. *Nucleic Acids Research* 18: 6531 – 6535.

Willson, K.C. & Clifford, M.N. (eds), 1992. Tea: Cultivation to Consumption. Chapman & Hall, London.

Winter, P. & Kahl, G., 1995. Molecular marker technologies for plant improvement. *World Journal of Microbiology & Biotechnology* 11: 438 – 448.

Wolfr, A. D., Xiang, Q-Y. & Kephart, S. R., 1998. Diploid hybrid speciation in *Penstemon* (*Scrophulariaceae*). Proceeding of National Academic Science USA 95: 5112 – 5115.

Wolff, K., Zietkiewicz, E. & Hofstra, H., 1995. Identification of chrysanthemun cultivars and stability of DNA fingerprint patterns. *Theoretical and Applied Genetics* 91: 439 – 447.

Wu, K., Jones, R., Dannaeberger, L. & Scolnik, P. A., 1994. Detection of microsatellite polymorphisms without cloning. *Nucleic Acids Research* 22: 3257 – 3258.

Wright, L. P., Apostolides, Z. & Louw, A. I., 1996. DNA fingerprinting of tea clones. Whittle, A. M. & Khumalo, F. R. B. (eds). *Proceeding of the 1st Regional Tea Research seminar*: 44 – 50. Blantyre, Malawi 22nd – 23rd March 1995.

Xu, H., Ton, Q. & Zhuang, W., 1987. Studies on genetic tendency of tea plant hybrid generation using isozyme technique. *Proc. Inter. Tea. Quality and Health Symp*.: 21 – 25.

Yamaguchi, S., Kunitake, T. & Hisatomi, S., 1987. Interspecific hybrid between *Camellia japonica* cv. *choclidori* and *C. chrysantha* produced by embryo culture. *Japan Journal of Breeding* 37: 203 – 206.

Yamaguchi, S., Matsumoto, S. and Tanaka, J. I., 1999. Genetic dispersal of tea plant. In: Jain, N. K. (ed). *Global advances in tea science*: 413 – 425. Aravali Books International (P) Ltd. New Delhi, India.

Yamamoto, T., Juneja, L. R, Chu, D. C. & Kim, M., 1997. Chemistry and application of green tea. CRC Press.

Yanagawa, Y., Yamamoto, Y., Hara, Y. and Shimamura, T., 2003. A Combination Effect of Epigallocatechin Gallate, a Major Compound of Green Tea Catechin, with Antibiotics on *Helicobacter pylori* Growth In Vitro. *Current microbiology* 47: 244 – 249.

Yanagisawa, T., Kiribuchi-Otobe, C., Hirano, H., Suzuki, Y. & Fujita, M., 2003. Detection of single nucleotide polymorphism (SNP) controlling the waxy character in wheat by using a derived

cleaved amplified polymorphic sequence (dCAPS) marker. *Theoretical and Applied Genetics* 107: 84 – 88.

Yang, C. S. and Landau, J. M., 2000. Effects of Tea Consumption on Nutrition and Health. *American Society for Nutritional Sciences*. 2409 – 2412.

Yang, W., de Oliveira, A. C., Godwin, I., Schertz, K. & Bennetzen, J. L., 1996. Comparison of DNA marker technologies in characterizing plant genome diversity: variability in Chinese sorghums. *Crop Science* 36: 1669 – 1676.

Yang, Y. & Sun, T., 1994. Study on the esterase isoenzyme in tea mutagenic breeding. *China Tea* 16: 4 – 9.

Yee, Y., Soon, S. K. & Myong, G. C., 1996. Evaluations of the natural monument populations of *Camellia japonica* (*Theaceae*) in Korea based on allozyme studies. *Botanical Bulletin of Academia Sinica* 37: 141 - 146.

Yockteng, R., Ballard, H. E., Mansion, G., Dajoz, I. & Nadot, S., 2003. Relationship among pansies (*Viola* section *Melanium*) investigated using ITS and ISSR markers. *Plant Systematics and Evolution* 241: 153 – 170.

Yu, F. & Xu, N., 1999. Tea germplasm resources of China. In: Jain, N. K. (ed). *Global advances in tea science*: 393 – 412. Aravali Books International (P) Ltd. New Delhi, India.

Yu, J. K., Dake, T. M., Singh, S., Benscher, D., Li, W., Gill, B. & Sorrells, M. E., 2004a. Development and mapping of EST-derived simple sequence repeat (SSR) markers for hexaploid wheat. *Genome* 47: 805 – 818.

Yu, J.-K., La Rota, M., Kantety, R. V. & Sorrells, M. E., 2004b. EST-derived SSR markers for comparative mapping in wheat and rice. *Molecular Genetic and Genomics* 271: 742 – 751.

Zhijian, G., Lifang, X. and Lishan, X., 1988. Report on the chromosome number of some species of *Camellia* in China. *Acta Botanica Yunnanica* 10: 291 – 296.

Zietkiewicz, E., Rafalski, A. & Labuda, D., 1994. Genome fingerprinting by simple-sequence repeat (SSR) anchored polymerase chain reaction amplification. *Genomics* 20: 176 – 183.

ACKNOWLEDGEMENT

This study owes a lot of institutions and individuals who kindly support and encourage me during my study. Even can not mention all by name here, I wish to express my sincere gratitude to all nameless supporters with equal appreciation.

First of all, I would like to extend my deepest gratitude from my heart to **Prof. Dr. Heiko C. Becker**, a very kind supervisor, the most decent person I ever met, for accepting me as his student as well as for his all constructive advices, guidance and supports fully, carefully and timely throughout my study.

My special thanks go to Prof. Dr Reiner Finkeldey for his willingness to be my co-referee and his useful discussion during my work.

Certainly, I would like to gratefully acknowledge the financial support of Vietnam Ministry of Education and Training (via Project 322) for the scholarship; to the International PhD Program for Agricultural Science (IPAG)/ DAAD (Deutscher Akademischer Austausch Dienst) for partly supporting my field work; to 'Professor Werner Schulze-Stiftung' for in part supporting my lab work and the Institute of Agronomy and Plant Breeding for partially supporting during my research.

My deep gratitude goes to Nong Lam University and its rector board; to the Faculty of Agronomy and its dean board and to the Department of Industrial Crops and its head, Mr. Phan Gia Tan for permitting me to interrupt temporarily my work for PhD study abroad.

I also would like to express my genuine gratitude to the Institute of Agronomy and Plant Breeding and its head; to the Faculty of Agriculture and its Dean and to the Georg-August-University Goettingen and its President for giving me a chance to carry out my PhD study in Goettingen.

Much special thankfulness is presented to IPAG and its officers, especially Dr. Jörg Heinzemann and Mrs. Brigitta Lunderstädt; to the Institute of Agronomy and Plant

Breeding and its staffs for providing me favorable conditions and good guidance during my study at Georg-August-University Goettingen.

Deep appreciation is also expressed to the Institute of Forest Genetics and Forest tree breeding, its head, Prof. Dr. Reiner Finkeldey and staffs, particularly Dr. Oliver Gailing, Thomas Seliger and Nicolas George Eliades, for all enthusiasm and useful help when I worked there; to Lam Dong Center for Research and Transfer technology in Industrial crops and Fruit tree Production and is director board and staffs and to Vietnam Tea Association and Vietnam Tea Research Institute due to the effective support my field work.

Frank thanks are extended to Dr. Sabine v. Witzke-Ehbrecht for her enthusiastic care and support me timely; to PD Dr. Wolfgang Ecke for his helpful technical discussion and support during lab work and to Dr. Mahmoud Zeid for his friendship and his guidance in work lab as well as in further discussions.

My personal gratitude sincerely goes to Prof. Dr. Wolfgang Link, Dr. Christian Möllers, Dr. J. Hippe for all their helps during my study; to Dr. S. Ueno (Laboratory of Forestry Ecology and Physiology, School of Agricultural Sciences, Nagoya University, Japan) and Dr. Tapan Kumar Mondal (Biotechnology laboratory, Research and Development Department, Tata Tea limited, India) for their communication on tea research.

Dr. Stefan Abel, Dr. Adefis Teklewold, Beriso Kebede, Mladen Radoev, Rosi Clemens deserve my thanks for their assistance in lab work.

My special gratefulness is distributed to Ass. Prof. Dr. Huynh Thanh Hung and Mrs. Ngo Thi Sau who strongly encourage me in my study as well as daily work and life; to Mr. Nguyen Duc Thiet, Mr. Pham S, Mr. Phan Quoc Hung for their enthusiastic assistance in my field work, to Nguyen Trung Kien due to his useful help in sampling tea accessions.

Sincere words of gratitude are also deserved to Tran Thanh Tung and his family, Nguyen Khanh Du, Nguyen Thi Tinh, Luu Hong Truong and his family, Nguyen Phi Nga and the Union of Vietnamese Student at Goettingen for the warm friendship.

Last but not least, I am greatly indebted to my parents, brothers, sisters, nephews and nieces who always strongly support and care me as well as to all of my close colleagues, friends and students who all give me continuous encouragement.

Curriculum vitae

Personal data

Full name: VO THAI DAN

Sex: male

Date of birth: 18.04.1972

Place of birth: Khanh Hoa Province, Vietnam

Nationality: Vietnamese

Mailing address: 68/166k Tran Quang Khai Street, Tan Dinh Ward, District 1, HoChiMinh City, Vietnam

E-mail: vthaidan@yahoo.com

Academic background

01.12.2003 – present: PhD student, Georg-August University Goettingen, Goettingen, Germany

19.07.1999 – 30.08.2001: Georg-August University Goettingen, Goettingen, Germany. MSc. in Tropical Agriculture

09.1989 – 04.1994: University of Agriculture and Forestry (UAF), Ho Chi Minh City, Vietnam. BSc. in Agronomy

09.1986 – 05.1989: Marie Curie Highschool, Ho Chi Minh City, Vietnam

09.1982 – 05.1986: Van Minh secondary school, Ho Chi Minh City, Vietnam

Employment record

06.1994 – present: Lecturer, Nong Lam University (NLU), Ho Chi Minh City, Vietnam (formerly The University of Agriculture and Forestry)