GENOTYPIC AND PHENOTYPIC CHARACTERIZATION OF SOME SELECTED AND STANDARD CLONES OF TEA [*CAMELLIA SINENSIS* (L) O. KUNTZE]

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By

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DEDICATED TO THE MEMORY

OF

MY DEPARTED MOTHER

DECLARATION

I hereby declare that the whole work submitted as a thesis entitled **"Genotypic and Phenotypic Characterization of Some Selected and Standard Clones of Tea [*Camellia sinensis* (L) O. Kuntze]"** in the Institute of Biological Sciences, Rajshahi University, Rajshahi, for the degree of Doctor of Philosophy is the result of my own investigation and was carried out under the supervision of Professor Dr. M. Monzur Hossain, Department of Botany, Rajshahi University, Rajshahi. The thesis has not already been submitted in the substance for any degree and has not been concurrently submitted in the candidature for any other degree.

June, 2013 **(Md. Abdul Aziz)**

CERTIFICATE

It is my pleasure to certify the thesis entitled **"Genotypic and Phenotypic Characterization of Some Selected and Standard Clones of Tea [*Camellia sinensis* (L) O. Kuntze]"** by Md. Abdul Aziz, Institute of Biological Sciences, Rajshahi University, Bangladesh for the degree of **Doctor of Philosophy.**

We here by certify that (i) the candidate has fulfilled the residential requirements, (ii) the work embodied in the thesis were carried out by the candidate and (iii) the data, to the best of our knowledge are genuine and original. No part of the work has been submitted in the substance for any other degree.

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ABSTRACT

Present investigation was under taken with a view to study and evaluates the extent of genetic variation present among 12 selected tea genotypes. This study also includes the analyses of major biochemical and nutritional components present in the selected tea genotype. In addition, the extent of genetic diversity was also assessed with RAPD markers among the 12 tea genotypes. The 12 tea genotypes selected for this study were MZ/39, E/4, D/13, B2×T1, Br/2/97, SDL/1, BT2, Ph/9/4, Ph/9/25, Ph/9/40, BS-67 and BT5 collected from the germplasm repository of Bangladesh Tea Research Institute (BTRI). All the field experiments were conducted in the experimental field of BTRI at Srimongal in Moulvibazar district, Bangladesh. The laboratory experiments were conducted in the Plant Breeding and Gene Engineering Laboratory of the Department of Botany, University of Rajshahi.

In order to study the extent of variability, 15 quantitative characters viz., angle of the first branch, height, length and breadth of the plucking surface, length, breadth, length and breadth ratio, and leaf angle of the fifth leaf, pluckable shoot length, fresh weight, dry weight and fresh and dry weight ratio of pluckable shoots, leaf area and shoot density of pluckable shoots, and pluckable shoot yield/plant were used as parameters. The data on these characters were statistically analyzed to calculate the variability, coefficients of variability, heritability, genetic advance as % of mean, principal component of variation, correlation coefficient, path coefficient analysis and genetic diversity.

The results of these study reveals that the presence of wide range of variations for almost all the quantitative characters were found. In the present study, the highest genotypic component of variation (GCV) was observed for pluckable shoot yield/plant followed by leaf area of the pluckable shoot, leaf angle of the fifth leaf and shoot density. These results suggest that the greater extent of variability in most of the characters was genetic in nature which are less affected by environment and hence could be improved through selection.

Heritability estimates in broad sense were relatively high for almost all the characters studied. In the present study high heritability estimates for leaf breadth, leaf angle,

leaf length and breadth ratio and the length of the fifth leaf, dry weight, leaf area and fresh weight of pluckable shoots, and angle of the first branch were found associated with high genetic advance as percentage of mean, which suggested that the scope of improvement of these characters through selection.

Out of 14 quantitative characters, five yield contributing characters viz., height of the plucking surface, length of the plucking surface, breadth of the plucking surface, angle of the first branch and shoot density selected through Ridge regression were subjected for correlation coefficient and path coefficient analysis. Correlation coefficient analysis showed that the pluckable shoot yield/plant was positively and significantly correlated with height, length, breadth of the plucking surface and shoot density. Path coefficient analysis using genotypic correlation reveals that height and length of plucking surface and shoot density had direct positive influence on leaf yield. These characters also showed significant positive correlation with yield. Therefore, selection on the basis of these characters should get preference for future improvement of pluckable shoot yield.

The results of principal component analysis (PCA) reveal that the first two principal components accounted for 46.92% of the total variance. Genetic diversity among the 12 genotypes as determined using multivariate analysis shows that the 12 tea genotypes could be grouped into two different clusters (C1 and C2). The maximum numbers of genotypes were included in Cluster 1.

The evaluation of the tea quality through organoleptic test reveals the significant difference in total tea quality tasting score among the 12 genotypes. According to the tasters' quality score, all genotypes could be categorized as good (above average) quality except Ph/9/40 which was categorized as average.

The results on antioxidant activity reveal that the amount of total phenolic compounds was significantly different among the tea genotypes. Among the 12 genotypes, the highest amount of phenolics was found in BT5. The content of phenolics in the leaf extracts correlates with the antioxidant activity. The total antioxidant capacity determined as equivalent to ascorbic acid of the methanolic extract of fresh leaves of 12 tea genotypes were also significantly different among which BT5 performed the

highest antioxidant capacity. The DPPH (1,1-diphenyl-2-picrylhydrazyl) assay of free radical scavenging activity of the methanolic extract of leaf compared with butylated hydroxytoluene (BHT) at 519 nm shows that the highest antioxidant activity (IC_{50} value of 39 µg/ml) in BT5 genotype.

The biochemical and nutritional components viz., gallic acid, theobromine, theophylline, caffeine, epigallocatechin, catechin, epicatechin, epigallocatechin gallet, gallocatechin gallet, epicatechin gallet and catechin gallet were analyzed in methanolic leaf extracts of 12 different tea genotypes through HPLC. The results reveal that among the 12 genotypes, Ph/9/25 showed the highest percentage of gallic acid, whereas no gallic acid was found in the genotypes D/13, B2×T1 and BS-67. The tea genotypes MZ/39, E/4, Ph/9/25 and Ph/9/40 showed the highest amount of total alkaloid content (theophylline, theobromine and caffeine). The highest amount caffeine content was recorded in Ph/9/40 followed by MZ/39 and Ph/9/25. The caffeine content was the lowest in BS-67. On the other hand, among all the genotypes, total catechin content was the highest in D/13 followed by E/4 and MZ/39.

Randomly amplified polymorphic DNA (RAPD) assay was performed to estimate the genetic diversity among the twelve tea genotypes.The genomic DNA amplified with ten decamer primers (OPA-7, OPA-9, OPA-10, OPB-10, OPB-13, OPC-9, OPC-17, OPD-3, OPD8 and OPD-15) showed total 83 distinct score able bands of which 64 (77.18%) bands were polymorphic. The highest percentage polymorphic loci were found in OPC9 and e the lowest loci (60%) in OPA9. The highest genetic distance was found between the Ph/9/25 and MZ/39 whereas the lowest genetic distance was found in SDL/1 and MZ/39. By The consensus phylogenetic dendogram generated using PAST (PAleontological STatistics) programme based on genetic distances segregated the twelve tea genotype into three different groups as clusters. Genotype E/4, D/13, MZ/39 and SDL/1 formed group A cluster, genotype B2×T1, BT2 and BT5 made group B cluster and Br2/97, Ph/9/4 and genotype Ph/9/40 grouped in C cluster. The result indicates the genetic diversity among the tea genotypes and RAPD marker could be used for improvement of tea cultivars.

ABBREVIATIONS

^{0}C	Degrees Celsius
^{0}E	Degree East
^{0}N	Degrees North
^{0}S	Degrees South
AFB	Angle of the first branch
AFLP	Amplified Fragment Length Polymorphisms
ANOVA	Analysis of variance
BC	Before Christ
bp	Base pairs
BPS	Breadth of the plucking surface
BTRI	Bangladesh Tea Research Institute
BTRP	Bangladesh Tea Rehabilitation Project
CAPS	Cleaved Amplified Polymorphism Sequences
cDNA	Complementary DNA
CHS	Chalcone Synthase
cm	Centimetre
cm^2	Centimetre square
CTAB	Cetyltrimethylammonium bromide
df	Degrees of freedom
DFR	Dihydroflavonol 4-Reductase
DNA	Deoxyribonucleic Acid
dNTP	Deoxyribonucleoside triphosphate
DWPS	Dry weight of the pluckable shoot
ECG	(-)-Epicatechin-3-gallate
EGCG	(-)-Epigallocatechin-3-gallate
EGC	(-)-Epigallocatechin
EMS	Error mean square
EST-SSR	Expressed Sequence Tag-Simple Sequence Repeats

EtBr	Ethidium Bromide
F1	First generation
Fig.	Figure
FWPS	Fresh weight of the pluckable shoot
FW/DWPS	Fresh weight/dry weight ratio of pluckable shoot
HPS	Height of the plucking surface
IPGRI	International Plant Genetic Resources Institute
ISSR	Inter Simple Sequence Repeats
ITC	International Tea Committee
kbp	Kilo base pair
kg/ha	Kilogram per hectare
km	Kilo meter
L	Litre
LA	Leaf Area
LAI	Leaf Area Index
LAFL	Leaf angle of the fifth leaf
LBFL	Leaf breadth of the fifth leaf
LLFL	Leaf length of the fifth leaf
LL/LBFL	Leaf length/breadth ration
LPS	Length of the plucking surface
LSD	Least significant difference
MAS	Marker-assisted selection
mg	Mili grams
min	Minutes
mM	Mili molar
mm	mili meter
mm^2	mili meter square
mRNA	Mature Ribonucleic Acid
n	Number of samples
ng	Nanograms
nm	Nanometer

ng/ml	Nanogram per milliliter
PAL	Phenylalanine Ammonia-Lyase
PCR	Polymerase Chain Reaction
PCR-RFLP	Polymerase Chain Reaction-Restriction Fragment
pH	Negative logarithm of hydrogen ion (H^+)
PPO	Polyphenol Oxidase
PO	Peroxidase
PSL	Pluckable shoot length
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphisms
RNA	Ribonucleic Acid
RNase	Ribonuclease
SD	Shoot density
SSR	Simple sequence repeats
STS-RFLP	Sequence Tagged Site-Restriction Fragment Length Polymorphism
TAE	Tris-acetate EDTA buffer
TRFCA	Tea Research Foundation of Central Africa
UPASI	United Planters' Association of South India
UPGMA	Unweighted Pair Group Method with Arithmetic Average
UV	Ultra Violet
Y	Total pluckable shoot yield/plant.

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Chapter 1
GENERAL INTRODUCTION

1.1. TEA: A GENERAL ACCOUNT

Tea plant *Camellia sinensis* (L.) O. Kuntze has been used to produce the oldest and most popular non-alcoholic, caffeine containing soft beverage across 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) for the past 3000 years (Eden, 1958). The word tea is derived from T'e of the Chinese Fukien dialect and tea is known as ch'a in Cantonese language. Being indigenous to South-East Asia, in an area stretching from Assam in the West to China in the East and down to Vietnam in the South (Mathews and Stephen, 1998), tea spontaneously grows widely from tropical to temperate regions in Asia. The first tea to reach Europe came by the way of the Dutch in the early part of the 17th century (Weatherstone, 1992).

1.1.1. Botanical Description of Tea

Tea plant is an evergreen, perennial, cross-pollinated plant and grows naturally as tall as 15 m. However, under cultivation, the bush height of 60 – 100 cm is maintained through pruning for harvesting the tender leaves that may continue even more than 100 years. The main morphological features of tea plants were summarized in **Table 1.1.** The flowers are white in color and borne singly or in 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, 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 oolong tea (semi-fermented tea).

Leaf is the main criterion by which three types of tea plants are classified. Briefly,

- Assam type has biggest leaves
- China type has smallest leaves and
- Cambod leaves size are in-between of Assam and China type.

Table 1.1 Variability in vegetative characteristics of tea

Characteristics	Range of variability
Mean of leaf angle	$50 – 120^0$
Laminar angle	$110 – 125^0$
Internodal length	15 – 70 mm
Individual leaf area	120 – 200 mm^2
Leaf area index (LAI)	3.5 – 8.5
Leaf length/breadth ratio	2.0 – 2.8
Height	184 – 539 cm
Girth at collar	25 – 42 cm
Branching habit	Acutely orthotropic to plagiotropic
Thickness of branches at 60 cm from ground level	1.4 – 4.4 cm
Length of internode between the second and the third leaves from the apical bud of flush shoot	0.9 – 3.2 cm
Length of the third leaf from the apical bud of growing shoot	2.0 – 6.0 cm
Breadth of the third leaf from the apical bud of flush shoot	1.5 – 3.8 cm
Angle between the third leaf of flush shoot and the internode above	$35 – 65^0$
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)	60 – 350 mg

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

In its natural habitat, China tea is a dwarf tree (shrub) 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 colour varies, but the green is usually lighter than that of China type and is sometimes even very light, young banana leaf. 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 1.1**) with high degree of plasticity (Willson and Clifford, 1992; Yamamoto *et al*., 1997).

1.1.2. Origin and Distribution of Tea

Tea had its genesis in China untold centuries ago. The origin of tea plants perhaps lost in antiquity. No one will possibly ever know when tea was discovered as a palatable drink and how the cultivation of tea began. Tea cultivation had first started in China, in the province of Szechwan about 350 A.D. and gradually extended to other provinces during the T'ang dynesty, A.D. 620-907 (Sana, 1989). The cultivation of tea in China attracted the travelers from different parts of the world and China became the fountainhead whence tea culture spread to other countries of the world.

Tea was introduced into Japan in 805 A.D. by Buddhist Monks. Subsequently the royal patronage of Japanese rulers had brought that glorification of tea. It has been used as a medicine, rather than a beverage, for next five hundred years made tea so popular that tea was used to combat the plague in Japan in A.D. 951 (Sana, 1989).
Outside China and Japan, the glamour of tea-drinking and the search for tea trade had been continuing till late 17^{th} century. Organized tea cultivation had started with ardent zeal and adventures throughout the hemisphere since early seventeenth century. It started at Java in 1690, Formosa in 1818, Turkey in 1839, Russia in 1847, South Africa (Durban) in 1850, India (Darjeeling) in 1855, Assam (Cachar) and Bangladesh (Sylhet) in 1857, Sri Lanka in 1860, Indonesia in 1872, and India (Doors) in 1874. By 1887, South Africa started tea cultivation which had eventually put a momentum for a rapid spread in Kenya, Uganda, Tanzania, Malawi, South Zimbabwe, Cameroon, the Congo and Mozambique (Sana, 1989). During the latter part of 19^{th} century, tea has been growing in Peru, Brazil and Argentina (South America). The fervency of tea

cultivation is still pervading during the fag end of 20th century in many countries like Pakistan, Iran etc.

Therefore, the original home or 'the primary center of origin' of tea was South-East Asia i.e. at the point of intersection between the 29^0 N (latitude) and 98^0 E (longitude) near the source of the Irrawaddy River at the confluence of North-East India, North Burma, South-West China and Tibet provinces (Wight, 1958). Tea thrives well within the latitudinal ranges between 45^0 N to 34^0 S that cross about 52 countries. Tea occupies about 2.7 million hectares of cultivable land of the world with an annual production of about 2.2 million tones (Mondal *et al.*, 2004).

1.1.3. Genetic Diversity in Tea

Taxonomically economic tea plants belong to the genus *Camellia* of the family Theaceae. 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 arised mainly due to inter specific hybridization. At present time, over 600 cultivated tea varieties are available worldwide, of which many have unique traits such as high caffeine content, blister blight disease tolerance, drought resistance, frost tolerance etc (Mondal, 2002a). Besides a few natural triploids, the chromosome number for all varieties of *C. sinensis* studied is 2n=30 (Bezbaruah, 1971; Kondo, 1977).

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. sinensis* (L.) O.

Kuntze or China type, *C. assamica* (Masters) or Assam type and *C. assamica* sub spp *lasiocalyx* (Planchon ex Watt.) or Cambod or Southern type and to some extent *C. irrawadiensis* have mainly contributed to the genetic pool of tea. The term 'tea' should therefore covers progenies of these taxa and the hybrids thereof between them. Bush architecture and leaf are the main criteria by which three types of tea are classified (**Table 1.2**).

Table 1.2 Comparison of bush architecture and leaf of different taxa.

Parameters	**Assam**	**China**	**Cambod**
Growth habit	Under trees with virgate branches	Vigorous shrub with compound branches	Erect, openly branched shrubs
Branching pattern	Semi-orthotropic arise above the ground, excellent spread	Semi-plagiotropic compact, arise from the ground	Semi-orthotropic and semi-plagiotropic with profuse branching, arise from the base
No. of branches in each order	2.6 to 6.8	3.4 to 8.6	2.6 to 6.4
Branch length (cm)	11 to 34	7 to 28	8 to 31
Branch angle (^{0})	51 to 72	40 to 45	51 to 73
Internode length (cm)	2 to 7	1 to 4	1 to 5
Leaves	Large, horizontal, broad, light green	Small, narrow, erect, dark green	medium size, broad, light green, semi-erect
Leaf angle type	Planophile (>70^0)	Erectophile (<50^0)	Oligophile (50-70^0)

Source: Ponmurugan *et al.* (2000).

1.1.4. Economic Importance, Health and Other Benefits

Tea was initially used as medicine and subsequently as beverage and is potential an important raw material for the pharmaceutical industry. 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). It is estimated that more than 3 billion cups of tea are consumed every day, just less than water consumption (Chen, 1994). Nowadays, consumption of tea is part of people's daily routine, as an everyday drink and as a therapeutic aid in many illnesses. Worldwide, 80% of the tea consumed

is black tea, which is also the most popular drink in Asia, Europe, North America, and North Africa (except Morocco), whereas green tea is drunk throughout South East Asia; oolong tea is popular in China and Taiwan (Wu *et al.* 2002). Approximately 76-78% of the tea produced and consumed worldwide is black tea, 20-22% is green tea, and <2% is oolong tea (Costa *et al.* 2002; Zuo *et al.* 2002; Yang and Landau, 2000).

Tea has been considered a medicine and a healthful beverage since ancient times, but recently it has received a great deal of attention because tea polyphenols are strong antioxidants. Drinking tea can yield the positive health effects such as improving the growth of beneficial microflora in the intestine, 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. Oxidative stress has been shown to be involved in the pathogenesis of numerous diseases, including cancer (Feng *et al.*, 2001 and Embola *et al.*, 2002). Moreover, some epidemiological studies have associated the consumption of tea with a lower risk of several types of cancers, including stomach, oral cavity, esophagus, and lung cancers (Xie *et al.*, 1998; Amantana *et al.*, 2002 and Kondo *et al.*, 2002). Tea appears, therefore, to be an effective chemopreventive agent for toxic chemicals and carcinogens (Embola *et al.*, 2002). Numerous studies have also demonstrated that the aqueous extract of the major tea polyphenols possesses antimutagenic, antidiabetic, antibacterial, anti-inflammatory, and hypocholesterolemic qualities (Feng *et al.*, 2001; Xie *et al.*, 1998; Amantana *et al.*, 2002 and Kondo *et al.*, 2002). Beneficial effects in oral diseases including dental caries, periodontal disease, and tooth loss, which may significantly affect a person's overall health, have been also described (Wu *et al.*, 2002). Among all tea polyphenols, especially catechins and gallic acid have been considered to be the main players in these beneficial effects on the human health. The major tea catechins are (-)-epigallocatechin gallate (EGCG), (-)-epigallocatechin

(EGC), (-)-epicatechin gallate (ECG), and (-)-epicatechin (EC) (Ho *et al.*, 1992). Tea also contains a certain amount of caffeine, and caffeine has attracted much scientific and public attention in recent years due to its stimulatory effects (Zuo *et al.*, 2002). The composition of catechins, gallic acid, and caffeine in commercial teas varied depending on species, season, and horticultural conditions and, particularly, with degree of fermentation during the manufacturing process (Fernández *et al.*, 2001 and Fernández *et al.*, 2002).

On the economic aspect, at the household level, tea plant is so-called the crop of the poor, especially in the tropical hilly 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 national 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.

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 (Mondal *et al*., 2004). Planting tea plant on the remote mountainous areas is considered as an effective method to cover the spare sloping lands.

1.1.5. 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 averagely up to 83% of world tea production in the year from 2001 to 2010 (ITC, 2011). China and India contributed shares of 28.9% and 26.2% respectively, to global supply (**Table 1.3**).

Table 1.3 World tea *vis-à-vis* Bangladesh tea – an over view. Average data of 10 years (2001 - 2010) has been compiled.

Country	Area			Production			Yield (kg/ha)	Export		
	000ha	%	Position	Mkg	%	Position		Mkg	%	Position
China	1467.92	46.8	1st	1024.47	28.9	1st	697.90	280.71	18.1	3rd
India	550.46	17.6	2nd	930.39	26.2	2nd	1690.21	190.73	12.3	4th
Sri Lanka	188.40	6.0	3rd	309.08	8.7	4th	1640.57	293.98	19.0	2nd
Kenya	144.84	4.6	4th	326.27	9.2	3rd	2252.62	331.40	21.4	1st
Indonesia	137.28	4.4	5th	150.72	4.2	6th	1097.90	94.36	6.1	5th
Vietnam	121.96	3.9	6th	129.37	3.6	7th	1060.74	90.56	5.9	6th
Turkey	77.44	2.5	7th	151.59	4.3	5th	1957.54	5.09	0.3	17th
Myanmer	76.41	2.4	8th	18.11	0.5	16th	237.01	-	-	-
Bangladesh	52.19	1.7	9th	57.47	1.6	10th	1101.26	8.90	0.6	13th
Japan	48.25	1.5	10th	93.70	2.6	8th	1941.86	1.39	0.1	27th
Argentina	37.49	1.2	11th	77.30	2.2	9th	2061.84	69.91	4.5	7th
Georgia	36.53	1.2	12th	3.89	0.1	27th	106.61	0.84	0.1	29th
Iran	24.56	0.8	13th	32.09	0.9	13th	1306.59	6.04	0.4	15th
Uganda	22.66	0.7	14th	40.88	1.2	12th	1804.52	37.58	2.4	9th
Others	148.39	4.7	-	203.64	5.7	-	1372.33	135.63	8.8	-
Total	3134.78	100	-	3548.98	100	-	-	1547.11	100	-

Source: ITC, 2011.

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).

1.1.6. Tea Breeding: A Brief Review of Important Research

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 in 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). 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.

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 micropropagated 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).

1.2. 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 their 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; Hajra, 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, based on these markers tea cannot be separated into discrete groups for identification (Wickremaratne, 1981).

1.3. TEA PRODUCTION AND RESEARCH IN BANGLADESH

Bangladesh has a longstanding tradition and customs of growing and drinking tea. Bangladesh is one of the main tea producers in the world. In recent decade the harvested area occupied 1.7% of the world acreage and contributes only 1.6% in world tea production as well as 0.6% in exports in world trade which are the 9th, 10th and 13th position respectively (**Table 1.3**).

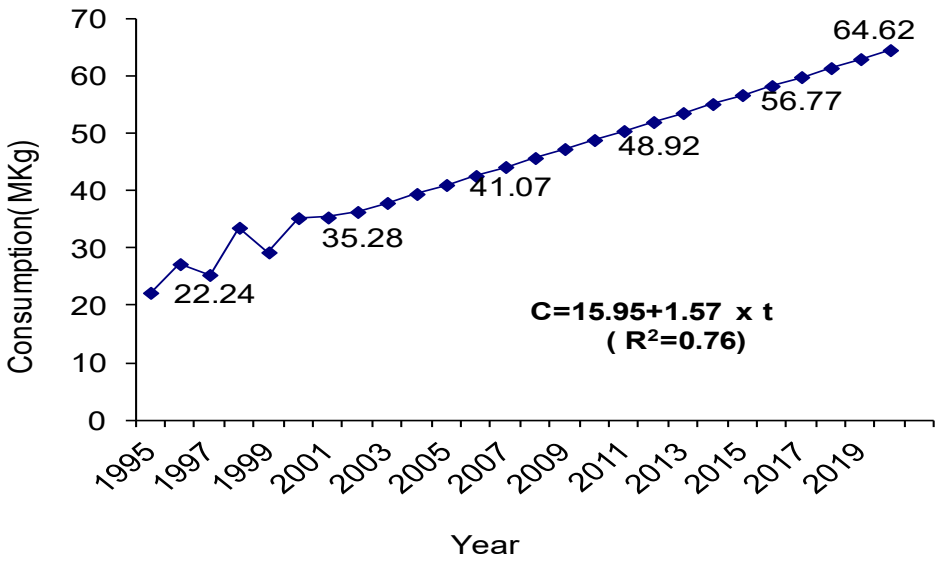


Figure 1.1 Trends of domestic consumption of Bangladesh up to 2020

Since the early time of Bangladesh tea industry (around 1857) until the end of 1970s, there have been many tea types planted mainly seed grown tea plants which were not well selected or documented. Importance of improved clones and bi and polyclonal seeds was duly realized only in post liberation days and substantial proportion of our tea is now with such clones (21%) and improved seeds (4%) (Alam, 2002). BTRI (Bangladesh Tea Research Institute) clones, few indigenous gardens and some other exotic clonal varieties have helped Bangladesh tea industry in raising productivity from 735 kg/ha in 1970 to 1300 kg/ha in 2009 (**Table 1.4**).

Table 1.4 Tea in Bangladesh at different periods.

Year	Area (ha)		Production (MKg)		Yield (kg/ha)**		Remarks
	Total	Increase/ Decrease*	Total	Increase/ Decrease*	Total	Increase/ Decrease*	
1947	30353	-	18.88	-	622	-	At partition of India
1957	31287	+ 934	25.55	+ 6665	817	+ 195	Pre-mandatory extension period
1970	42688	+ 11401	31.38	+ 5832	735	- 82	Up to pre-liberation period
1980	43732	+ 1044	40.04	+ 8657	916	+ 181	Pre-BTRP*** status
1992	47781	+ 4049	48.93	+ 8892	1040	+ 124	Closing of BTRP*** status
1998	48735	+ 954	55.82	+ 6894	1145	+ 105	BTRP impact
2001	50974	+ 2239	56.82	+ 996	1115	- 30	
2003	52202	+ 1228	59.21	+ 2390	1247	+ 132	
2006	53350	+ 100	53.10	- 6510	1001	- 284	Drought year
2007	53500	+ 150	57.25	+ 4152	1109	+ 235	
2008	53570	+ 70	58.65	+ 1398	1236	+ 75	Drought year
2009	53000	- 570	60.00	+ 1340	1300	+ 64	Drought year

Source: Ahmed (2011)
*Difference from previous total denoted by (+) or (-) sign
**Calculated on the basis of Production/Total tea area
***BTRP – Bangladesh Tea Rehabilitation Project

However, this achievement is not enough to meet the demand while domestic consumption is increasing rapidly (**Figure 1.1**). So, step should be taken for the development and improvement of our existing tea varieties.

1.3.1. Research Activities on Tea Improvement in Bangladesh

Tea, being an out breeding perennial crop is extremely heterozygous. Improvement through breeding, mutation and polyploidization is, therefore, slow and laborious. Nevertheless, there exists a wide range of variability in the seedling population which can profitably be exploited by selection and vegetative propagation. Through selection, the institute so far released 18 outstanding clones having high yield and quality potential (Chakrabarty *et al.* 1975; Rashid *et al.* 1983; Alam *et al.* 1992; Alam and Haque, 2001) in its BT series for commercial use of the tea industry of

Bangladesh. Of them, BT2 possesses a touch of flavour which is unique for a low elevation tea like Bangladesh.

Ahmed (1963) was attempted to distinguish the tea population of East Pakistan into seven eco and genocategories, viz. Manipuri, Burma, large-leaved Assam, China, Hybrid I, II and III. Reference had also been made to the important role played by selection, vegetative propagation and line breeding leading to the clonal source of seeds in the improvement of tea.

The Institute, since 1964, has undertaken a breeding programme and from several hundred combinations, ♀ BT1 × ♂ TV1, ♀ B207/39 × ♂ TV1, ♀ BT1 × ♂ TV19 and ♀ B207/39 × ♂ TV19 have been found to produce highly uniform seedlings with uniform branching habit (Alam *et al.*, 1974, Dutta *et al.*, 1999 and Dutta, 2000). Progeny from ♀ BT1 × ♂ TV1 combination produces large plucking shoots with average to near above average cup quality while ♀ B207/39 × ♂ TV1 combination produces medium plucking shoots with average cup quality. Biclonal seedbaries with BT1 and TV1 have been suggested for the tea estates (Alam, 1984 and Dutta *et al.*, 2000) and this is gaining popularity (Alam, 2002). On the other hand, further study has to be needed to confirm the yield and quality potential for the progeny of ♀ BT1 × ♂ TV19 combination.

Alam *et al.* (1990) was under taken a study to determine the advantages of BTRI biclonal seed stock BTS1 (♀ BT1 × ♂ TV1), in terms of yield and quality over an average Bangladesh seed jats, a Tocklai camboid hybrid seed jat TS450 and BT1 as clonal standard. It was observed that in per plant green leaf, BTS1 gave statistically significant higher yield to the local seed jat and BT1 throughout their early four years of harvest, while Tocklai stock TS450 had a slightly higher trend, the difference being insignificant. Four years average per hectare yield from 3rd to 6th year of planting showed BTS1 yielding higher by about 38% than local seed and 27% than BT1 but by only 2% less than TS450. Cup quality of BTS1 was persistently above average and equally comparable to that of clone BT1. TS450 was average and the other seed jat was below average in the cup.

Ahmed *et al.* (1965) studied on the viability of pollen grains of three ecotypes of *Camellia sinensis*, namely China, Manipuri and Assam type, under normal breeding procedure. Their results indicated that pollen grains from China plant had longer viability and also higher germination percent than those from Assam and Manipuri plants.

Ahmed *et al.* (1966) determined the influence of low temperature on the viability of pollen grains collected from three contrasting agrotypes of tea plant – China, Manipuri and Assam. They observed that viability of pollen grains gradually decreased in all samples from the day of storage. Percentage of viability came down to below 5% level in 11^{th} week in Manipuri plants, 13^{th} week in Assam plants and 15^{th} week in China plants.

Rashid *et al.* (1980) observed that there was significant variation on flushing pattern and leaf compositions of three BTRI Clones, namely, BT1, BT2 and BT3. Flush expressed as the incidence of two leaves and a bud was found best in BT3 followed by BT2 and BT1. BT2 produced least numbers of banji. Results indicated that those traits are controlled synergistically by environmental and genetical factors.

A detailed study of morphology, cytology and hybridization was undertaken by Rashid *et al.* (1982) in order to evaluate the possible gene exchange between the tetraploid and existing diploid agrotypes. Comparative morphologyical studies between diploid agrotypes and tetraploid tea plant TR1 revealed that the tetraploid plant is an intermediate form between China and Burma type. Tetraploid plant TR1 is cross incompatible in the cross ♀ 4X × ♂ 2X and only successful when it was used as donor parent. Hybridization between diploid clone BT1 and Benehomali jat with tetraploid TR1 met with success but seeds obtained from them were found to be abortive while hybridization between diploid TV1 and tetraploid tea produced viable seed.

A study was undertaken by Shiblee *et al.* (1994) to examine the performance of four agrotypes of tea seedlings namely Hybrid, Manipuri, Assam and China at a nursery site. It was noticed that the agrotypes gave significantly different performance in respect of branch number and plant height at five different plant ages. The highest plant height was found in Hybrid followed by Manipuri, Assam and China. In respect of branch number Hybrid also maintained its superiority over other agrotypes.

Manipuri and China gave more or less similar performance whereas Assam gave lowest performance. Regression analysis showed that branch number of each agrotype increased significantly at a linear rate whereas plant height increased at compound rate with the increase of plant age.

Haque *et al.* (2001) were cultured shoot tip and stem segment explants of tea clone BT1, BT2, BT4, BT5, BT6, BT7, BT8, BT10 and BT11 in order to establish suitable protocol for *in vitro* propagation. Regeneration of plantlets was observed in MS media supplemented with BAP + IAA, Kn + IAA and BAP + NAA. Plantlets obtained in regenerating media were subsequently transferred to rooting media containing several combinations of ½ MS supplemented with IBA and IAA but no root initiation was observed. Stem segments of clone BT1, BT2, BT5, BT6 and BT10 were cultured aseptically *in vitro*. Callus was obtained when MS media were supplemented with 2mg/l 2,4-D, 1mg/l 2,4D+300 CH and 1.5mg/l 2,4-D + 300 CH and 1.5mg/l 2,4-D + 300 CH. Higher concentration of 2,4-D were found to inhibit callus induction. The calli thus obtained were transferred to regenerating media containing several combinations of BAP, NAA and Kinetin but no organogenesis was observed.

Haque *et al.* (2002) also reported that profuse callusing was observed while anthers of several tea clones were cultured aseptically *in vitro* in the MS nutrient salt and vitamins of N6 medium supplemented with various combinations of 2,4-D, BAP and Kinetin. The calli thus obtained were transferred to regenerating media but no organogenesis was recorded.

Molla (1980) identified 12 amino acids such as Aspartic acid, Threonine, Serine, Glutamic acid, Glycine, Alanine, Valine, Isoleucine, Leucine, Tyrosine, Phenyl alanine and Theamine in both green leaves and made tea. Assessment of their concentration in the extract showed that the concentrations of all the amino acids during the course of manufacture except Glycine and Alanine. Glycine and Alanine concentration were reduced during the process of manufacture. Tyrosine was too low to quantify in all the sample except in made tea sample of BT3. Phenylalanine could only be quantified in black tea from the clones, BT2 and BT3.

Molla (1981) conducted thin layer chromatographic study in order to ascertain the nature of polyphenols in BTRI released clones BT1, BT2 and BT3. Results revealed the presence of flavanols, esters glycosides, gallic acid, chlorogenic acid, theogallin para-coumeryl quinic acid, leucoanthocyanins and some other unidentified compounds.

Molla (1984) was used gas liquid chromatography (GLC) in order to estimate the five major flavanols in the green leaf of three BTRI clones BT1, BT2 and BT3. It was revealed from the experiment that the concentration of the total flavanols, gallocatechins and gallate ester of flavanols were highest in BT1 followed by BT2 and BT3 whereas the concentration of simple catechins was highest in BT3 followed by BT1 and BT2. In the corresponding black tea samples the theaflavin levels were greater in BT1 followed by BT2 and BT3.

Islam *et al.* (1985) observed the development of theaflavins during fermentation stage of tea processing. They experimentally found that higher average temperature to the tune of 42.6^0C at CTC cutting point deteriorated the quality of tea over that cut at 31^0C which was evident from theaflavin measurement in black tea samples. This constituent was found to bear a negative correlation with the temperature.

Based on Islam's findings and observations, Molla (1988) was set up a simulated experiment in the laboratory in order to develop an equation and graph to assess the enzyme activity in industrially processed tea leaves to produce the best quality black tea.

Choudhury (1988) was initiated an experiment to explore the relationship of moisture absorption and release of tea with respect to relative humidity and temperature of the encircled air, and have to find out the effect of storing temperature on the tea quality. Results showed that theaflavin (Tf) concentration decreases with the increase of moisture content and storing time. Tea of smaller grain having a moisture content of 15% loses its most of the theaflavin within eight months. Maximum level of moisture absorption of tea is much higher in rainy days than that of dull seasons. Maximum quality benefit may be derived by reducing moisture content upto 3% in post drying condition.

Choudhury *et al.* (1992) examined the effect of pH and temperature on the formation in model systems of individual theaflavins and the possible implications for tea

processing. Optimum fermenting temperature and pH for each individual theaflavins (TF) were investigated *in vitro* model fermentations and production of each TF at these optimum conditions were three/four times higher from the production at the ambient temperature and physiological pH of tea leaf. With the exception of TFDG, other TFs had the optimum fermenting pH 4.5 and pH 4.0 may be optimum for TFDG. It was observed that optimum fermenting temperatures for theaflavin and theaflavin-3'-monogallate were 15^0C and theaflavin-3-mono gallate and theaflavin-3,3'-digallate were 20^0C. With the increase of fermenting temperature and pH, production of TF fractions and total fermented products were decreased and reverse trend was observed for the production of thearubigins, parameter for black tea quality.

Karim *et al.* (2000) was undertaken an experiment in order to find out the correlation between some quality influencing parameters as crude fiber, theaflavin (TF), thearubigin (TR), total liquor colour and high polymerized substances (HPS) with different shoot length. TF content was found the highest in bud followed by one leaf and a bud, and so on up to seven leaves and a bud. But TR content increased from bud to two leaves and a bud and then declined successively to seven leaves and a bud. The total liquor colour content increased from bud to three leaves and a bud and after that declined, whereas HPS content increased from bud to two leaves and a bud and after that declined. In the corresponding sample, the crude fiber content increased step by step from bud to seven leaves and a bud, whereas the crude fibre contents of bud, one leaf and a bud, and two leaves and a bud were found within the permissible limit. A positive correlation was found among the shoot length, crude fibre and TF & TR content.

1.4. RATIONALE AND OBJECTIVES

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 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.

Although, conventional tea breeding is well established and contributed much for tea improvement over the past several decades, but the process is slow due to some bottlenecks. Specifically in tea, they are:

– perennial nature,
– long gestation periods,
– high inbreeding depression,
– self-incompatibility,
– unavailability of distinct mutant of different biotic and abiotic stress,
– lack of distinct selection criteria,
– low success rate of hand pollination,
– short flowering time (2–3 months),
– long duration for seed maturation (12–18 months),
– clonal difference of flowering time and fruit bearing capability of some clones.

Tea is one of the important crop and export commodities in Bangladesh. About eighteen indigenous tea varieties have been developed by BTRI are being used for tea production. Under the present circumstances of globalization these varieties should be patented as our indigenous property. Moreover, step should be taken for the development and improvement of our existing tea varieties for producing high quality tea forsake of survival of our tea industry in competitive world market. As per the objectives of the present research molecular marker (DNA) will be develop for selected tea varieties. Out coming DNA marker can be used along morphological and agronomical parameters for patenting the indigenous varieties. Moreover, morphological, biochemical and molecular characterization of existing varieties will create opportunity of the development of new tea varieties with high organoleptic quality.

Therefore, twelve tea accessions, which were commercially planted widespread or new promising selections in the tea estate in Bangladesh, were assessed for the genetic diversity based on qualitative and quantitative characteristics.

1.4.1. Objectives

1. Morphological characterization and extent of genetic variability of selected tea genotypes (12 genotypes) grown in Bangladesh with an aim to establish a broad genetic base tea repository.
2. Biochemical characterization of the selected tea genotypes in order to select high quality tea producing genotype (s).
3. Genotypic characterization of the selected tea genotype to assess the genetic diversity using random amplified polymorphic DNA (RAPD).
4. Selection of the best tea genotype (s) with higher yield and better nutritional qualities and to recommend the selected genotype (s) to BTRI for including in future tea breeding programme.

Chapter 2

EXTENT OF GENETIC VARIABILITY ON MORPHOLOGICAL CHARACTERS OF 12 TEA GENOTYPES

2.1. INTRODUCTION

Commercial tea (*Camellia sinensis* (L) O. Kuntze) has been started in Bangladesh since 1854 using introduced planting materials (Ahmed, 1963). Later Bangladesh Tea Research Institute (BTRI) has been so far released a number of outstanding tea variety selected from natural populations and also through breeding methods (Alam *et al.,* 2001). Since 1960, the Institute has initiated a project on collection of tea germplasm from abroad. Efforts have been made since 1982 to intensify local collection (Rashid, 1983) by picking up the potentially valuable bushes from old sections of commercial tea gardens and from old seed baries (orchards). The vegetative propagated plants from these collections are being added to the central germplasm collection of BTRI. Therefore, the genetic resources of tea in BTRI are undoubtedly the most important source of tea germplasm in Bangladesh. A large number of controlled hybridization was attempted and some of the progeny were also recommended for commercial planting (Dutta *et al.,* 1998). The existing diversity will have to be preserved and characterized for future tea improvement programmes that constitute the fundamental support structure for the tea industry.

In order to increase yield potentiality, information about genetic variability is necessary. Qualitative and quantitative improvement of tea is one of the basic requirements for increasing productivity per unit land area. High yielding tea cultivars require information on the nature and magnitude of variation in the available materials, association of characters with yield and its components.

Variation is the basis of improvement and germplasm represents the sum total of variability or heredity materials or genes available in particular genus or species (Dandin, 1989). Germplasm is the basic foundation of crop improvement and its importance was realized as back as 1898 (Boraiah, 1986). The green revolution was

basically due to the high utilization of “Norin-10” genes, which resulted in doubling of the yield in wheat all over the world. Even in case of paddy, all most all the modern varieties have been developed through utilization of dwarf and high fertilizers responsive genotypes “Dee-geowoo” (Shivashankar, 1989). Germplasm is the raw material of crop improvement and considered as the living museum of the sum total of variability. The conservation and utilization of genetic resources are the two vital components of varietal improvement programme. Without systematic evaluation on nature and magnitude of variation, existing strains, the available gene pool in the germplasm collection can not be utilized to the full extent. The scientists are always looking into usefulness of morphological characters, which are easy and cheap to measure, genetically correlated with yield and have a higher heritability (Gallais, 1984). Gross morphological studies helps to classify the material systematically. It also provides the information on specific gene sources and the sum total of variability available in the genus, which fulfill the requirements of scientists, working in various disciplines (Dandin and Jolly, 1986).

Variability in a population is complex matter. Genetic variability can be measured in a given environment (treatment); the phenotypic variation can not be measured so easily, because it is the result of joint effect of genotype and environment. Fisher (1918) studied the genetic variability in relation to the environmental effect and he was the first to provide the statistical methods of partitioning the total variation into genetic and environmental components with the development of first (mean) and second (variance, co-variance) degree of statistics for the assessment of quantitative characters. Two distinct lines are developed for the measurement of continuous variation. Mather (1949) developed biometrical technique based on the mathematical methods of Fisher *et al.* (1932).

Leaf morphology has an important role in identifying taxa in which variation in floral structures is uninformative or in which flower specimens are infrequent owing to a limited flowering season, for example (Meade *et al.,* 2003). The use of morphological characters is cost-effective when compared to that of biochemical and molecular markers for preliminary characterization of many individuals to identify morphologically similar groups and for simple varietal identification of

phenotypically distinguishable cultivars (Martinez *et al.,* 2003). In tea, morphological characters have been used to study genetic diversity (Wickramaratna, 1981, Toyao *et al.,* 1999 and Rajanna *et al.,* 2011), variation (Gunasekara *et al.,* 2011, Piyasundara *et al.,* 2006 and Su *et al.,* 2007), phylogeny and classification (Chen *et al.,* 2005, Vo, 2006, Piyasundara *et al.,* 2008 and Pi *et al.,* 2009). Leaf features have been largely unexploited in taxonomic studies, resulting from a belief that they respond in a plastic manner to environmental factors. However, in *ex situ* gene banks, the plant materials are grown under similar environmental conditions and farming practices, making it possible to compare taxa.

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).

Pomurugan *et al.* (2000) reported that all six elite tea clones which representing three principal taxa such as "Assam", "China" and "Cambod", were followed similar architectural pattern irrespective of the jats. "Assam" jat showed more vigorous

performance in number of branches, branch length and internodal length than other two.

Alam *et al.* (2001) studied on four test clones against one standard clones BT1 in order to assess their field performances specially yield as well as quality and there were significant variation among the test clones. Three test clones showed the best performance in respect of yield and quality against the control.

Sarwar *et al.* (2002) investigated to study several parametric and non-parametric characteristics of leaves and some non-parametric characters of bushes of eleven tea clones released by Bangladesh Tea Research Institute in order to identify and distinguished them.

Vo (2006) had assessed the genetic diversity of thirty-one tea accession with thirty-four morphological characters and results showed the high diversity of Lam Dong tea. He clustered all tested accessions into four groups.

Mong *et al.* (2007) investigated to assess the taxonomic position of the wild tea plant and explored its relationship with two other closely related taxa, *C. sinenis* var. *sinensis* and *C. sinensis* var. *assamica* with 16 vegetative and 11 floral characters. They opined that the Taiwan native wild tea plant might deserve recognition as a distinct species.

In case of tea genetic variability is shown by different characters viz. angle of first branch (AFB), height of the plucking surface (HPS), length of the plucking surface (LPS), breadth of the plucking surface (BPS), pluckable shoot length (PSL), fresh weight of pluckable shoot (FWPS), dry weight of pluckable shoot (DWPS), fresh weight/dry weight ratio of pluckable shoot (FW/DWPS), leaf area (LA), shoot density (SD), pluckable shoot yield/plant (Y) of twelve cultivars of tea can be measured from the component of variation, coefficients of variability, heritability, genetic advance as % of mean correlation coefficient and path analysis. Under the present investigation all of these were calculated, following Johnson *et al.* (1955), Lush (1949) and Burton and De Van (1953).

The degree of relationship or association of the morphological traits of tea can be ascertained by correlation studies. Determination of correlation coefficient among the yield contributing components is therefore, prime importance in selecting suitable plant types and in designing effective research programme.

To get the idea of the genetic variability existing among the cultivars with regard to the quantitative characters of economic importance, it become necessary to study under an array of distinguishable treatments (environment). The concept of quantitative inheritance is now one of the most important principles of genetics.

Statistical methods have been reported (Kirchoff *et al.,* 2004; Plotze *et al.,* 2005 and Kirchoff *et al.,* 2007) and there are two main types of techniques to represent taxonomic structure: cluster analysis and principal component analysis (PCA). It should be useful for both the breeding programme and the germplasm conservation of tea plants to understand the diversity and differentiation of morphology among those taxa. In the present study, cultivated tea clones in BTRI germplasm are morphologically described and assessed for their diversity by applying statistical methods. The aim is to detect intra-specific boundaries and to identify reliable distinguishing characters. This study will provide a basis for further investigations of systematic classification using the data from morphological characters.

2.1.1. Objectives

Variability and the magnitude of diversity of tea cultivars, growing in different areas of Bangladesh has not been well studied. The present part of work is intended to make an in-depth study of variability present in morphological traits of tea genotypes on all possible genetic parameters in order to address their merits in the gene pool configuration and for future needs of workers.

2.2. MATERIALS AND METHODS

2.2.1. Plant Materials

A total of 10 test genotypes coded as MZ/39, E/4, D/13, B2×T1, Br/2/97, SDL/1, Ph/9/4, Ph/9/25, Ph/9/40, BS-67 and two standard genotypes BT2 and BT5 were included in this study (**Table 2.1**).

Table 2.1 General information of the tea genotype used under the study.

Accession Name	Identity	Pedigree	Type	Special attributes
MZ/39	BTRI Germplasm	Estate selection	Assam hybrid	Test clone, high drought tolerant, pruning recovery is very good
E/4	BTRI Germplasm	Estate selection	Assam-Manipuri hybrid	Test clone, , drought susceptible, pruning recovery is fair
D/13	BTRI Germplasm	Estate selection	Assam	Test clone, moderate drought tolerant, pruning recovery is good
B2×T1	Hybrid line	BT2×TV1	Manipuri hybrid	Commercial clone named as BT17, high drought tolerant, pruning recovery is very good, cup quality is above average and coloury with good strength and briskness
Br/2/97	BTRI Germplasm	Estate selection	Assam hybrid	Test clone, moderate drought tolerant, pruning recovery is good
SDL/1	BTRI Germplasm	Estate selection	Assam-Manipuri hybrid	Test clone, poor drought tolerant, pruning recovery is moderate
BT2	BTRI Germplasm	Estate selection	Manipuri hybrid	Commercial clone, very high drought tolerant, pruning recovery is very good, cup quality is above average and coloury liquor with consistent touch of flavour
Ph/9/4	Old seedlings from Phulcherra T. E., Section No. 9, Acc. No. 4	Estate selection	Assam hybrid	Test clone, pruning recovery is very good during drought
Ph/9/25	Old seedlings from Phulcherra T. E., Section No. 9, Acc. No. 25	Estate selection	Assam hybrid	Test clone, moderate drought tolerant, pruning recovery is good
Ph/9/40	Old seedlings from Phulcherra T. E., Section No. 9, Acc. No. 40	Estate selection	Manipuri hybrid	Test clone, high drought tolerant, pruning recovery is very good
BS-67	BTRI Germplasm	Estate selection	Assam hybrid	Test clone, high drought tolerant, pruning recovery is very good
BT5	Hybrid line	BT1×TV1	Assam hybrid	Commercial clone, moderate to high drought tolerant, cup quality above average and taste of liquor is brisk

The morphological description of the test genotypes and the standard genotypes used as control are given below:

MZ/39: Ortho-plagotropic, compact bush, densely branched with heavy girth and floriferous. Patina is quite glossy, leaves are semi-dark green, medium in size, texture is fairly thick and hard, prominent long apex, serration uniform, leaf blade is slightly wavy and leaf pose is semi-erect and moderately embossed (**Plate 1- Figure 1A** and **1B**).

E/4: Plagiotropic, loose frame, not very compact but having effective plucking points and poorly floriferous. Leaves are medium in size, light green, quite glossy, leaf texture thin and soft, semi-erect leaf pose, apex is less prominent, long lamina, slightly boat shape, serration uniform and venation is less prominent (**Plate 1- Figure 1C** and **1D**).

D/13: Plagiotropic, compact plucking table, fairly dense plucking points, heavy girth with good branching behaviour and poorly floriferous. Patina is glossy, leaves are light green, broad in size, texture is thick, soft and leathery, and apex is less prominent, leaf pose semi horizontal (**Plate 1- Figure 1E** and **1F**).

B2×T1: Biclonal progeny of BT2 and TV1. Ortho-plagiotropic, compact bush with dense plucking points, very uniform flushing behaviour. Patina is quite glossy, dark green foliage, texture is thick and hardy, erect leaf pose, apex is prominent, uniformly dentate serration and venation is fairly prominent (**Plate 2- Figure 2A** and **2B**).

Br/2/97: Orthotropic, fairly compact bush, good branching, fair growth and quite hardy. Patina quite glossy, texture is fairly thick, dark green foliage, blades are wavy with prominent apex, serration is uniform but dent bluntish, leaves are long with erect pose (**Plate 2- Figure 2C** and **2D**).

SDL/1: Typically plagiotropic, very compact bush and profuse branch, highly plucking density. Patina is glossy, light green foliage, semi erect leaf pose, leaves are small in size and texture is thin and soft. Apex is less prominent; serration is less uniform and venation is fairly prominent (**Plate 2- Figure 2E** and **2F**).

BT2: Orthotropic, not densely branched, comparatively loose frame, but effective branches, very well and uniform flushing behaviour, moderately floriferous. Leaf

texture is fairly thick and soft, semi-dark green. Apex less pointed, lamina is considerably equal width in mid region, serration uniform and semi-erect and Assam-Manipuri hybrid type (**Plate 3- Figure 3A** and **3B**).

Ph/9/4: Assam hybrid, plagio-orthotropic, heavy girth with fairly compact bush, leaves are elongated and light in colour, blades are boat shaped with prominent apex, serration is irregular and bluntish, venation is deeply embossed (**Plate 3- Figure 3C** and **3D**).

Ph/9/25: Assam type, well develop girth, compact bush, medium to large leaf with dark green colour, prominent long apex, serration is regular but bluntish, horizontal leaf pose (**Plate 3- Figure 3E** and **3F**).

Ph/9/40: Manipuri hybrid, heavy girth, dense plucking points, plucking shoots are pale green in colour, semi erect leaf pose, apex is less prominent, serration is regular acute (**Plate 4- Figure 4A** and **4B**).

BS-67: Assam hybrid type, bush having profuse branch with many plucking points, leaves are light green in colour and elongated in shape with less prominent apex, leaf pose is horizontal, serration is regular blunt (**Plate 4- Figure 4C** and **4D**).

BT5: Assam hybrid, compact bush with excellent spread, leave size is medium to large and light green in colour, leaves having prominent apex, serration is regular acute, venation is prominent and slight embossed between two veins (**Plate 4- Figure 4E** and **4F**).

Plate 1

Figure 1A. A mother bush of MZ/39

Figure 1B. An isolated shoot of MZ/39

Figure 1C. A mother bush of E/4

Figure 1D. An isolated shoot of E/4

Figure 1E. A mother bush of D/13

Figure 1F. An isolated shoot of D/13

Plate 2

Figure 2A. A mother bush of B2×T1

Figure 2B. An isolated shoot of B2×T1

Figure 2C. A mother bush of Br/2/97

Figure 2D. A shoot of Br/2/97

Figure 2E. A mother bush of SDL/1

Figure 2F. An isolated shoot of SDL/1

Plate 3

Figure 3A. A mother bush of BT2

Figure 3B. An isolated shoot of BT2

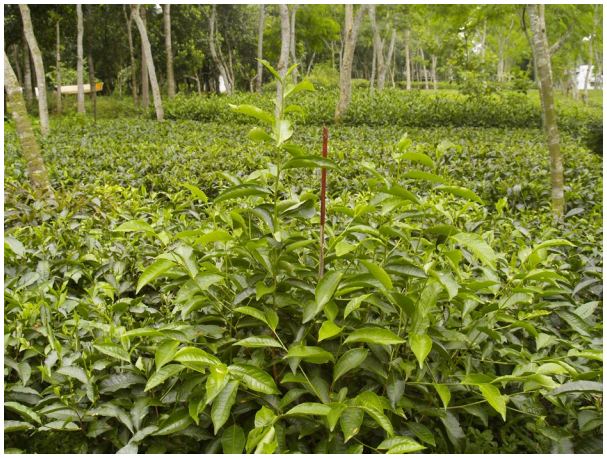


Figure 3C. A mother bush of Ph/9/4

Figure 3D. A shoot of Ph/9/4

Figure 3E. A mother bush of Ph/9/25

Figure 3F. An isolated shoot of Ph/9/25

Plate 4

Figure 4A. A mother bush of Ph/9/40

Figure 4B. An isolated shoot of Ph/9/40

Figure 4C. A mother bush of BS-67

Figure 4D. An isolated shoot of BS-67

Figure 4E. A mother bush of BT5

Figure 4F. An isolated shoot of BT5

2.2.2. Study Area

Location

The Bangladesh Tea Research Institute (BTRI) is situated about 3.2 km away from Srimangal town, located at latitude $24^0$18N and longitude $91^0$44E, occupies the North-Eastern side of Bangladesh. Its altitude is 21.95 m above sea level.

Geology and soil

The tea soil of Bangladesh is red-yellow podzolic and reddish-brown lateritic (Hajra, 2001b). The soil is sandy loam in texture with low to marginal contents of organic carbon (0.96 to 1.09%), nitrogen (0.096 to 0.011%), phosphorus, potassium, calcium, magnesium and other nutrients. The pH value is low (3.9 to 4.4) with highly acidic reaction having low base saturation. Organic matter content is low (1.65 to 1.87%), which affects the physico-chemical properties of soil (Shahiduzzaman, 2004). The reason for low nitrogen content also seems to be due to marginal organic matter content of soil.

Climatic condition

Bangladesh climate is considered to be tropical monsoon with three distinct seasons: Warm season (mid February to mid may), Monsoon season (mid May to mid October) and Cold season (mid October to mid February). The year begins and ends with a dry period. By March rain begins and the south-west monsoon initiates in southern region, progressing northwards until the whole country is covered by the unstable south-west air mass during monsoon period.

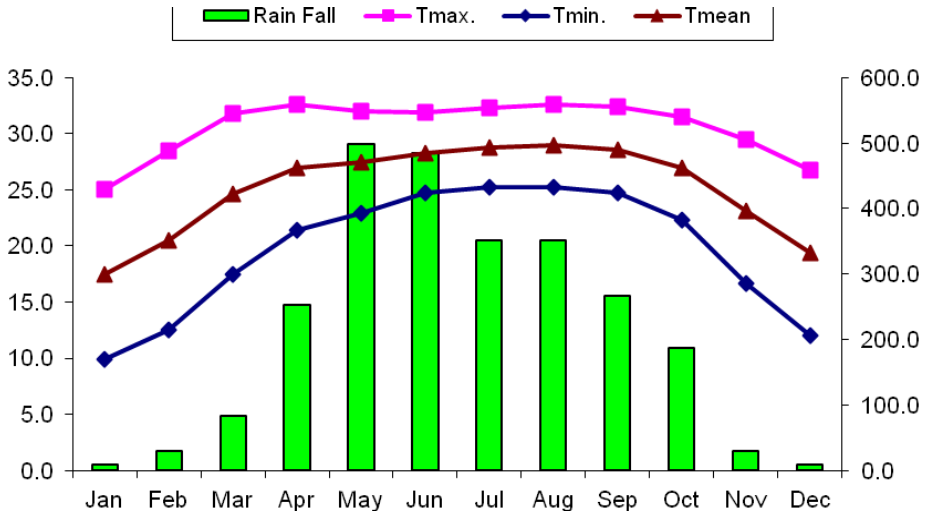


Figure 2.1 Average temperatures and rainfall at BTRI, Srimangal from 2000 to 2010.
(**Source:** Agricultural Metrological Division, Bangladesh Metrological Department)

The wet month, according to the climatic group's standard, is defined as one having 200 mm or more rain while a dry month receives 100 mm of rain or less. To this standard, the wet areas of Bangladesh comprise of Sylhet, Mymensing, and the estuarine state of Patuakhali, Noakhali and Chitagong that receive over 200 mm of rain per month for six months. The eastern part of Sylhet i.e. Srimangal where tea is grown receives over 200 mm of rain for seven months during April/May – September/October (**Figure 2.1**). Tea zones of Bangladesh experience dry season from November to April while the rainy season continues from May to October and above 80 percent of annual rainfall is obtained during June to September.

In tea grown area of Bangladesh, temperature becomes generally highest in April or May, dips slightly during monsoon period and diminishes after September or October. Prolonged drought occasionally occurs after November or December when rain is meager.

2.2.3. Methods

The experiment was laid out in Randomized Block Design with three replications having twelve plots in each. The size of the each plot was about 4.8m ×3.0m having 120cm × 60cm planting spacing (**Figure 2.2**). There were 30 plants per plot.

Characters were chosen with respect to variation among genotypes mentioned in literature and based on personal observations on specimens. Finally, 15 important quantitative characters of tea bush, the 5th leaf, and pluckable shoots were investigated from the currently harvested tea trees following IPGRI's guide line (IPGRI, 1997) with some minor adjustments.

2.2.3.1. The characteristics of bush

Stems were described by the following characteristics:

The branch angle (AFB, degrees): The angle formed between the first branchlets and the main stem of the bush is denoted as AFB and it was measured in degree.

The height of plucking surface (HPS, cm): HPS was measured from ground level to the plucking surface (**Plate 5- Figure 5A**).

The length of plucking surface (LPS, cm): LPS was measured in metric scale.

The breadth of plucking surface (BPS, cm): BPS was measured in metric scale.

2.2.3.2. The characteristics of the 5th leaf

The 5th leaves counted from the bud were exploited by the following characteritics:

The leaf length of the 5th leaf (LLFL, cm): LLFL was measured in metric scale from the leaf base to the tip (**Plate 5- Figure 5B**).

The leaf breadth of the 5th leaf (LBFL, cm): LBFL was measured at the widest position of the lamina in cm (**Plate 5- Figure 5B**).

The leaf length/breadth ratio of the 5th leaf (LL/LBFL): LL/LBFL was measured by calculated from the data of the leaf length and breadth.

The leaf angle of the 5th leaf (LAFL, degrees): The angle formed between the branches and the lamina was denoted as LAFL and it was measured in degree.

2.2.3.3. Characteristics of the pluckable shoots

Pluckable shoots (consist of two leaves and one bud or three leaves and one bud) were picked up on weekly basis during the cropping season and data of the following characteristics were taken:

Pluckable shoot length (PSL, cm): A total of 50 pluckable shoots was taken randomly from each genotypes and PSL was measured in metric scale.

Fresh weight of pluckable shoot (FWPS, g): A total of 50 pluckable shoots (two leaves and a bud) was taken randomly from each genotypes and FWPS were calculated by electronic weighing machine.

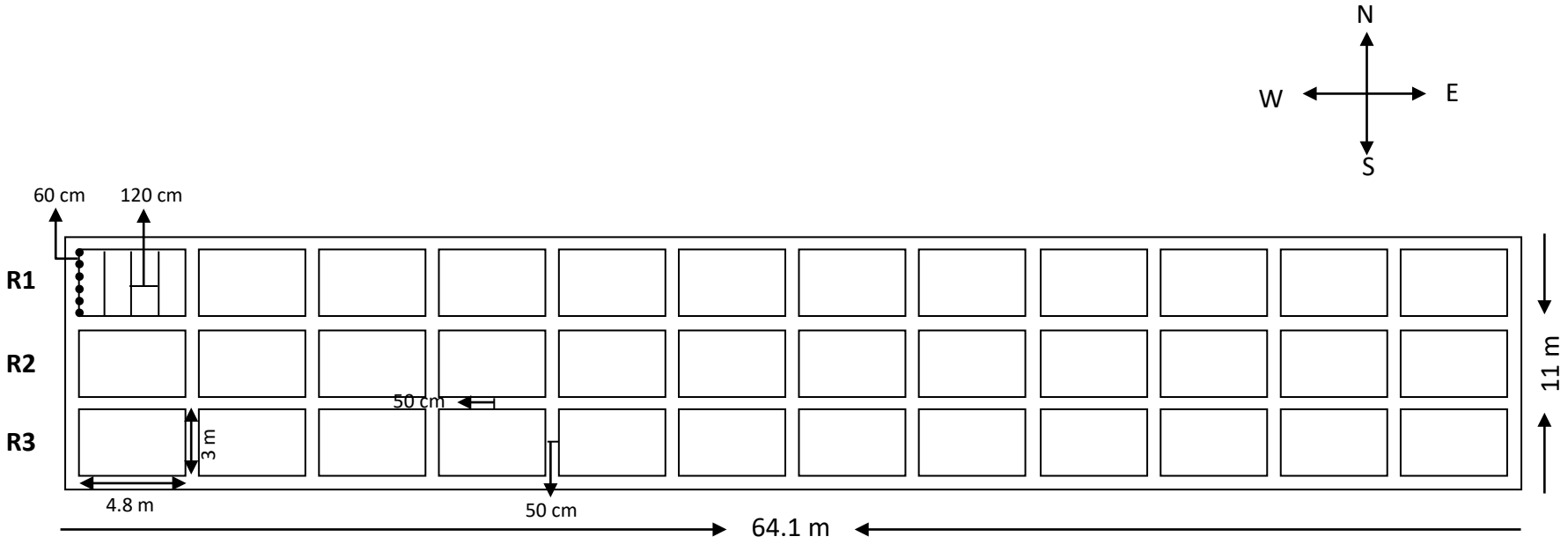
Dry weight of pluckable shoot (DWPS, g): The corresponding 50 pluckable shoots of each genotype had been put in to 75^0C oven for 24 hours in order to dry and DWPS of each genotype were taken by electronic weighing machine.

The fresh/dry weight ratio of pluckable shoot: The fresh/dry weight ratio of pluckable shoots **(FW/DWPS)** was measured by calculated from the corresponding data of the fresh weight and dry weight of pluckable shoots.

Leaf area (LA, cm^2): The corresponding 50 pluckable shoots of each genotype was also taken in order to measure LA by electronic leaf area meter (Systronics Leaf Area Meter 211).

Shoot density (SD): Numbers of pluckable shoots of each bush were counted by 60cm × 60cm Quadrate method. Data was taken two months interval from April to November. Shoot density was denoted as **SD**.

Total pluckable shoot yield/plant (Y, g): Total pluckable shoots from 30 plants/plot was harvested on weekly basis during the cropping season of 2009 (mid-March to mid-December). Yield/plant is presented here as the mean value of the total weight of 30 plants/plot.



Here,

1 cm = 3 cm

Figure 2.2 Design of the experimental field

Plate 5

Figure 5A. Measuring the height of the plucking surface from the ground level to the surface.

Figure 5B. A single leaf shows its length, breadth and size.

Figure 5C. Shoot density on pluckable surface of bushes

2.2.3.4. Statistical analysis

For evaluation and interpreting the results, collected data were analyzed following the biometrical methods developed by Mather (1949) based on the mathematical model of Fisher *et al.* (1932). The techniques used for analysis of data are described under the following subheads:

i) Mean: It is arithmetic mean or average and computed by dividing the total of observations by their number. It was calculated by using the following formula:

$$\overline{X} = \frac{1}{n}\sum_{i=1}^{n} X_i$$

Where, X_i = Individual reading recorded on each plant

$\overline{X}$ = Arithmetic mean

n = No. of observation

$\sum_{i=1}^{n} X_i$ = Summation of variable

i = 1,2,3..... n.

ii) Standard deviation (SD): Standard deviation is the average deviation of the individual observation from the mean. It was calculated as the square root of the variance as follows:

$$SD = \sqrt{\sigma^2}$$

Where, σ^2 = Variance

SD = Standard deviation

iii) Standard error of mean (SE): If several samples are considered instead of taking one, it will be found that the standard deviations of the different samples also vary. This variation is measured by the standard error of mean, which was calculated as follows:

$$SE = \frac{SD}{\sqrt{n}}$$

Where,

SD = Standard deviation

SE = Standard error of mean

n = Total number of individual

iv) Least significant difference (LSD): In order to compare the mean of various entries, it requires to calculate the least significant difference. Least significant differences were carried out, using the following formula:

$$LSD = \sqrt{\frac{2EMS}{r}} \times t$$

Where,

EMS = Error mean square

r = Number of replication

t = Tabulated value of 't' at 5% and 1% level at EMS df.

v) Range: It is the difference between the lowest and highest values present in the observation included in the study. In this study lowest and highest values are shown to represent the range of variation.

vi) Analysis of variance: The analysis of variance was done for sorting out the variance due to difference sources and for testing the significance among the sources. It is expressed as the sum of square of deviations of all observations of a sample from its mean and divided by the degree of freedom (n-1). It was computed by using formula, following Singh and Chaudhary (1977) as follows:

$$\sigma^2 = \frac{\sum_{i=I}^{n} x_i^2 - \left(\sum_{i=I}^{n} \times x_i^2\right)^2 /n}{n-1}$$

Where,

σ^2 = Variance

x = The individual reading recorded on each plant

n = The total number of individuals

$\sum$ = Summation

i = 1,2,3.....n

n-1 = Degree of freedom

In analysis of variance, the mixed model was used and the expected mean sum square (EMS) was determined as follows:

Analysis of Variance (ANOVA)

Item	df	MS	EMS
Variety (V)	V – 1	MS_1	$\sigma^2_{W11} + S\sigma^2_{VR11} + SR\sigma^2_{V11}$
Replication (R)	R – 1	MS_2	$\sigma^2_{W11} + SV\sigma^2_{R11}$
V × R	(V – 1)(R – 1)	MS_3	$\sigma^2_{W11} + S\sigma^2_{VR11}$
Within Error	VR(S – 1)	MS_4	σ^2_{W11}

Where,

G = Genotype
R = Replication
MS_t = Mean square of total
MS_g = Mean square of genotype
MS_r = Mean square of replication
MS_e = Mean square of error
σ^2_g = Genotypic variance
σ^2_e = Environmental variance

viii) Component of variance: The components of variance were of three types viz. genotypic variance (σ^2_g), phenotypic variance (σ^2_p) and environmental variance (σ^2_e). These components were estimated, following the methods as described by Singh and Chaudhary (1977).

$$\text{Genotypic variance } (\sigma^2_g) = \frac{MS_g - MS_e}{r}$$

$$\text{Phenotypic variance } (\sigma^2_p) = \sigma^2_g + MS_e$$

$$\text{Environment variance } (\sigma^2_e) = MS_e$$

Where,

MS_g = Mean square of genotype
MS_e = Mean square of error
r = No. of replication

ix) Co-efficient of variation: Deviation is also expressed by the coefficient of variation. Coefficient of variability at phenotypic, genotypic and environmental levels

were computed following Johnson et al. (1955) and Burton and De Vane (1953) as follows:

a) Phenotypic coefficient of variation (PCV) = $\frac{\sqrt{\sigma^2_p}}{\bar{x}} \times 100$

b) Genotypic coefficient of variation (GCV) = $\frac{\sqrt{\sigma^2_g}}{\bar{x}} \times 100$

c) Environmental coefficient of variation (ECV) = $\frac{\sqrt{\sigma^2_e}}{\bar{x}} \times 100$

Where,

(σ^2_p)	=	Phenotypic variance
(σ^2_g)	=	Genotypic variance
(σ^2_e)	=	Environmental variance
$\bar{x}$	=	Grand mean

x) Heritability (h^2_b): Estimation of heritability (in broad sense) was computed by dividing the genotypic variance with phenotypic variance and then multiplying it by 100 as suggested by Warner (1952).

$$h^2_b = \frac{\sigma^2_g}{\sigma^2_p} \times 100$$

Where,

h^2_b	=	Heritability in broad sense
σ^2_g	=	Genotypic variance
σ^2_p	=	Phenotypic variance

xi) Genetic advance: Genetic advance was calculated as per formula of Lush (1949) as follows:

$$G_A = K(\sigma_p)\ (\sigma^2_g / \sigma^2_p)$$

Where,

G_A	=	Genetic advance
K	=	The selection differential in standard unit, for the present study it was 2.06 at \5% level of selection (Lush 1949).

σ_p = Square root of phenotypic variance e.g. phenotypic standard deviation.

σ^2_g = Genotypic variance

σ^2_p = Phenotypic variance

xii) Genetic advance as % of mean (GA%) : It was measured by using the following formula:

$$GA\% = \frac{GA}{\bar{x}} \times 100$$

Where,

GA% = Genetic advance as % of mean

$\overline{X}$ = Grand mean for a particular character

xiii) Variance ratio test (F- test): F-test was carried out for test of significance among the variance within a source.

$$F\ value = \frac{MS}{EMS}$$

Where,

MS = Mean square value of specific source of variance

EMS = Error mean square

xiv) Selection of yield contribution characters through step wise ridge regression: A number of characters were selected and studied (**Section 2.2.3.1 - 2.2.3.3**) in order to achieve the objectives of the study. Stepwise Ridge regression analysis was carried out with a view to select most important yield contributing characters. Therefore, let the studied variables are considered as pluckable shoot yield/plant (y), angle of the first branch (AFB), height of the plucking surface (HPS), length of the plucking surface (LPS), breadth of the plucking surface (BPS), leaf length of the fifth leaf (LLFL), leaf breadth of the fifth leaf (LBFL), leaf length/breadth ratio of the fifth leaf (LL/LBFL), leaf angle of the fifth leaf (LAFL), pluckable shoot length (PSL), fresh weight of pluckable shoot (FWPS), dry weight of pluckable shoot (DWPS), fresh and dry weight ratio of pluckable shoot (FW/DWPS), leaf area of total pluckable shoot (LA), shoot density of pluckable shoot (SD). Natural log transformation was considered to reduce the variability of the variables and they were defined as *z*

$=\log_e(y)$, $y_1=\log_e(\text{AFB})$, $y_2=\log_e(\text{HPS})$, $y_3=\log_e(\text{LPS})$, $y_4=\log_e(\text{BPS})$, $y_5=\log_e(\text{LLFL})$, $y_6=\log_e(\text{LBFL})$, $y_7=\log_e(\text{LL/LBFL})$, $y_8=\log_e(\text{LAFL})$, $y_9=\log_e(\text{PSL})$, $y_{10}=\log_e(\text{FWPS})$, $y_{11}=\log_e(\text{DWPS})$, $y_{12}=\log_e(\text{FW/DWPS})$, $y_{13}=\log_e(\text{LA})$ and $y_{14}=\log_e(\text{SD})$. Multiple linear regression was considered to estimate the total yield with respect to different phenotype variables. Since the information was collected from each plant of tea genotypes, then the variables might be highly correlated that resulted the multicollinearity problem in the regression model. Therefore, to overcome such problem several researchers proposed different methods. One of them is Ridge estimator, a biased but having minimum variance property of the estimator of the linear regression model. Since the objective of this study was to find out the proper subset of independent variables for explaining maximum variation in dependent variables. This objective was fulfilled by using variable selection method such as stepwise forward selection. This procedure began with the assumption that there was no regressor in the model other than the intercept. An effort was made to find out an optimal subset by inserting regressors into the model once at a time. The first regressor was being selected for entry into the equation was the one that had the largest simple correlation with response variable, second was largest partial correlation (smaller than first variable) with response variables and so on. The variable was entered into the model on the basis of F statistics. So, to fulfill whole objective, stepwise ridge regression model can be used. The mathematical explanation of ridge regression model is given below:

Let us consider multiple linear regression models with 14 regressors. The form of regression model can be written as follows:

$$z_i = \beta_1 y_{1i} + \beta_2 y_{2i} + \beta_3 y_{3i} + \beta_4 y_{4i} + \beta_5 y_{5i} + \beta_6 y_{6i} + \beta_7 y_{7i} + \beta_8 y_{8i} + \beta_9 y_{9i} + \beta_{10} y_{10i} + \beta_{11} y_{11i} + \beta_{12} y_{12i} + \beta_{13} y_{13i} + \beta_{14} y_{14i} + \varepsilon_i$$

; i=1, 2, 3, . . ., 360

$$z_i = \sum_{j=1}^{14} \beta_j y_{ji} + \varepsilon_i;\ i=1, 2, 3, \ldots, 360$$

In matrix notation, the above equation model can be written as follows

$Z = Y\beta + \varepsilon$; where notations are of usual assumption.

Using ordinary least squared methods, the estimator of β is

$$\hat{\beta} = (Y^{/}Y)^{-1}Y^{/}Z$$

The ridge regression model was originally proposed by Hoerl and Kennard (1970a, b). The ridge estimator was found by solving a slightly modified version of the least square normal equations. The ridge estimator is defined as follows:

$$\hat{\beta}_R = (Y^{/}Y + kI)^{-1}Y^{/}Z$$

Where k≥0 is constant selected by the analyst.

Using STATISTICA 7, the summary statistics of stepwise forward ridge regression (method described in the section 2.2.3.4) model with biased parameter k = 0.10 are presented in **Table 2.2**. From **Table 2.2**, the goodness of fit measured by R square and adjusted R square and indicated that the step 5 for the regressor y_2, y_3, y_4, y_1 and y_{14} are sufficient for describing 97.6% variation in z. Therefore regressor y_2, y_3, y_4, y_1 and y_{14} are being choose for response z because after this step the value of R square and adjusted R square has no significant increment.

Table 2.2 Summary of stepwise forward ridge regression model

Step	Model	R square	Adjusted R square
1	y_2	R^2= 0.90555622	adjusted R^2= 0.90529315
2	y_2 y_3	R^2= 0.94870630	adjusted R^2= 0.94841974
3	y_2 y_3 y_4	R^2= 0.96380911	adjusted R^2= 0.96350499
4	y_2 y_3 y_4 y_1	R^2= 0.97153209	adjusted R^2= 0.97121223
5	y_2 y_3 y_4 y_1 y_{14}	R^2= 0.97632239	adjusted R^2= 0.97598891
6	y_2 y_3 y_4 y_1 y_{14} y_{10}	R^2= 0.97943313	adjusted R^2= 0.97908454
7	y_2 y_3 y_4 y_1 y_{14} y_{10} y_7	R^2= 0.98174538	adjusted R^2= 0.98138339
8	y_2 y_3 y_4 y_1 y_{14} y_{10} y_7 y_{11}	R^2= 0.98336750	adjusted R^2= 0.98298949
9	y_2 y_3 y_4 y_1 y_{14} y_{10} y_7 y_{11} y_{12}	R^2= 0.98472196	adjusted R^2= 0.98433022
10	y_2 y_3 y_4 y_1 y_{14} y_{10} y_7 y_{11} y_{12} y_8	R^2= 0.98569557	adjusted R^2= 0.98528687
11	y_2 y_3 y_4 y_1 y_{14} y_{10} y_7 y_{11} y_{12} y_8 y_5	R^2= 0.98643478	adjusted R^2= 0.98600722
12	y_2 y_3 y_4 y_1 y_{14} y_{10} y_7 y_{11} y_{12} y_8 y_5 y_9	R^2= 0.98701641	adjusted R^2= 0.98656870
13	y_2 y_3 y_4 y_1 y_{14} y_{10} y_7 y_{11} y_{12} y_8 y_5 y_9 y_{13}	R^2= 0.98750353	adjusted R^2= 0.98703537
14	y_2 y_3 y_4 y_1 y_{14} y_{10} y_7 y_{11} y_{12} y_8 y_5 y_9 y_{13} y_6	R^2= 0.98776745	adjusted R^2= 0.98727249

Therefore the estimated equation of the selected model is given below

$$\hat{z} = \hat{\beta}_1 y_1 + \hat{\beta}_2 y_2 + \hat{\beta}_3 y_3 + \hat{\beta}_4 y_4 + \hat{\beta}_{14} y_{14}$$

The summary statistics for estimated parameters of the selected model is presented in **Table 2.3**. It reveals from **Table 2.3** that for the one unit increasing of HPS will cause the 0.201490 unit increment in yield considering other variables are kept fixed at a certain level. Similarly, it is found that for the one unit increasing of LPS, BPS, AFB and SD will cause the 0.200051, 0.196599, 0.193712 and 0.187758 unit, respectively increment in yield considering other variables are kept fixed at a certain level.

Table 2.3 Summary statistics for estimated parameters

Variable	$\hat{\beta}_{std.}$ (standardized)	Std. Err. of $\hat{\beta}_{std.}$	$\hat{\beta}_{unstd.}$ (unstandardized)	Std. Err. of $\hat{\beta}_{std.}$	t	p-level
y_2	0.201490	0.023027	0.345969	0.039538	8.750181	0.000000
y_3	0.200051	0.023031	0.338066	0.038921	8.686004	0.000000
y_4	0.196599	0.022974	0.363142	0.042435	8.557544	0.000000
y_1	0.193712	0.022766	0.396801	0.046634	8.508889	0.000000
y_{14}	0.187758	0.022155	0.392349	0.046296	8.474749	0.000000

Thus, among the 14 quantitative characters, five characters e.g., y_2 = HPS, y_3 = LPS, y_4 = BPS, y_1 = AFB and y_{14} = SD were selected for the study of correlation coefficient and path coefficient analysis.

xv) Correlation coefficient: The statistics, which measures the relation between two or more variables, is known as correlation coefficient. Correlation coefficient analysis also measures the mutual relationship between the various plant characters and determines the components character on which selection can be based for improvement of yield.

Correlation coefficient were calculated from the variance and covariance components as follows:

$$r_{x_1x_2} = \frac{Covr_{x_1x_2}}{\sqrt{v(x_1)v(x_2)}}$$

Where,

$r_{x_1x_2}$ = The correlation coefficient between the character x_1 and x_2.

$Covr_{x_1x_2}$ = Covariance between the character x_1 and x_2.

Vx_1 = Variance of the character x_1

Vx_2 = Variance of the character x_2

Phenotypic, genotypic and environmental correlation coefficient for all possible combinations were computed from the components of variance and covariance following Al-Jibouri *et al.* (1958) as follows:

$$r_p = \frac{\sigma^2 p_{12}}{\sqrt{\sigma^2(p_1)\sigma^2(p_2)}}$$

$$r_g = \frac{\sigma^2 g_{12}}{\sqrt{\sigma^2(g_1)\sigma^2(g_2)}}$$

$$r_e = \frac{\sigma^2 e_{12}}{\sqrt{\sigma^2(e_1)\sigma^2(e_2)}}$$

Where,

r_p = Phenotypic correlation

r_g = Genotypic correlation

r_e = Environmental correlation

$\sigma^2 p_{12}$ = Covariance at the phenotypic level for the characters 1 and 2

$\sigma^2 g_{12}$ = Covariance at the genotypic level for the characters 1 and 2

$\sigma^2 e_{12}$ = Covariance at the environmental level for the characters 1 and 2

$\sigma^2 p_1$ = Phenotypic variance for the character 1

$\sigma^2 g_1$ = Genotypic variance for the character 1

$\sigma^2 e_1$ = Environmental variance for the character 1

$\sigma^2 p_2$ = Phenotypic variance for the character 2

$\sigma^2 g_2$ = Genotypic variance for the character 2

$\sigma^2 e_2$ = Environmental variance for the character 2

xvi) Path coefficient analysis: The path analysis is simply standardized partial regression coefficient, which splits the correlation coefficient into the measures of direct and indirect effects of a set of independent variables on the dependant variable. The path coefficient analysis was done by using Wright's (1921 and 1923) formula as

was extended by Dewey and Lu (1959) by solving a set of simultaneous equations as follows:

$$r_{xy} = P_{xy} + r_{x2}P_{2y} + r_{x3}P_{3y} + r_{x4}P_{4y} + ...$$

Where,

r_{xy} = Correlation between one component character (independent variable-x) to the yield (dependent variable-y)

P_{xy} = Path coefficient between the same character and yield.

r_{x2} = Correlation between the same character and one of the remaining yield component in turn.

xvii) Multivariate analysis

Multivariate analysis e.g., principal component analysis (PCA) and cluster analysis (CLU) were done by using STATISTICA 7 software.

2.3. RESULTS

In the present study 15 quantitative characters, angle of the first branch (AFB), height of the plucking surface (HPS), length of the plucking surface (LPS), breadth of the plucking surface (BPS), leaf length of the fifth leaf (LLFL), leaf breadth of fifth leaf (LBFL), leaf length and breadth ratio of the fifth leaf (LL/LBFL), leaf angle of the fifth leaf (LAFL), pluckable shoot length (PSL), fresh weight of the pluckable shoots (FWPS), dry weight of the pluckable shoots (DWPS), fresh and dry weight ratio of pluckable shoots (FW/DWPS), leaf area of the pluckable shoots (LA), shoot density (SD) of pluckable shoots and pluckable shoot yield/plant (Y) studied in 12 different tea genotypes. The results which were obtained for these quantitative characters are described under the following heads.

2.3.1. Ranges, Mean with Standard Error (SE), List Significant Difference (LSD) and Coefficient of Variability Percentage (CV%)

The 15 quantitative characters were analyzed for ranges, mean with standard error (SE), list significant difference (LSD) at 5% and 1% and coefficient of variability percentage (CV%) separately for every characters of tea genotypes. The results are shown in **Table 2.2-2.16.**

Angle of the first branch (AFB): The highest range of variation for the character of AFB was calculated as 30-70^0 in the genotypes of MZ/39 and Ph/9/4 while, the lowest range of variation was found as 30-45^0 in Br/2/97, BT2, Ph/9/25 and BS-67 **(Table 2.4)**.

It appears from the **Table 2.4** that the value of AFB in D/13 was significantly higher than rest of the genotypes. The highest mean with standard error was 61.10 ± 1.14 degree in D/13 and lowest mean with standard error was 36.83 ± 0.88 in the genotype Br/2/97.

Table 2.4 also reflects the highest CV% was recorded in Ph/9/4 with a value of 26.06, while the lowest was noted in E/4 with a value of 9.73 for this character.

Height of the plucking surface (HPS): The highest range of variation in HPS was recorded as 81-105 cm in the genotype D/13 and the lowest range of variation was found as 94-100 cm in the genotypes B2 × T1 and Br/2/97 **(Table 2.5)**.

It reveals from the **Table 2.5** that the genotype SDL/1 was significantly higher values for this attributes in comparison to other genotypes. The highest mean with standard error of 98.77 ± 0.37 cm in SDL/1 while the lowest mean with standard error was 87.83 ± 0.68 cm in Ph/9/40.

For this character the highest CV% was in D/13 with a value of 5.81 and the lowest CV% was observed in Br/2/97 with a value of 1.77 **(Table 2.5)**.

Length of the plucking surface (LPS): The highest range of variation was recorded for LPS as 70-120 cm in BS-67 and the lowest range of variation was found as 90-120 cm in BT2 **(Table 2.6)**.

Table 2.6 shows that the LPS of the genotypes MZ/39 was significantly higher than the other genotypes. The highest mean was recorded for the genotype MZ/39 with a value of 112.90 ± 1.88 cm while the lowest was observed in BS-67 with a value of 94.27 ± 2.21 cm.

It also reveals from the **Table 2.6** that both the highest and the lowest CV% were noted as 12.85 and 7.70 in the genotype BS-67 and BT2, respectively.

Breadth of the plucking surface (BPS): In case of BPS the highest range of variation calculated as 52-100 cm in MZ/39 and BT2. On the other hand, the lowest range of variation was 56-81 cm in BS-67 **(Table 2.7)**.

It appears from the **Table 2.7** that the value of BPS in MZ/39 was significantly higher than the rest of the genotypes. The highest mean was recorded in MZ/39 and the lowest was observed in BT5.

Table 2.7 also shows that the highest CV% was observed in MZ/39 with a value of 16.26, while the lowest was noted in BS-67 with a value of 10.12.

Leaf length of the fifth leaf (LLFL): The highest range of variation in respect of LLFL showed as 7.2-19.0 cm in Ph/9/25 while lowest range of variation was 10.0-11.5 cm in BT2 **(Table 2.8)**.

It reveals from the **Table 2.8** that the genotype Ph/9/4 was significantly higher values for this character in comparison to other genotypes. The highest mean was noted in the genotype Ph/9/4 with a value of 18.33 ± 0.32 cm and lowest was noted in BT2 with a value of 10.68 ± 0.07 cm.

In this case, the highest CV% was 19.61 in the genotype Ph/9/25, while the lowest was 3.40 in the genotype BT2 **(Table 2.8)**.

Leaf breadth of the fifth leaf (LBFL): In case of LBFL, the highest range of variation was shown as 5.5-9.0 cm and 6.0-9.5 cm in Ph/9/4 and BS-67 respectively while the lowest range of variation was calculated as 3.3-4.1 cm in BT2 **(Table 2.9)**.

Table 2.9 indicates that the LBFL for all genotypes were significantly diverged in comparison to each other. For this character the highest mean was recorded in BS-67 with a value of 7.96 ± 0.13 cm, while the lowest mean was obtained in BT2 with a value of 3.72 ± 0.04 cm.

It also appears from the **Table 2.9** that the highest CV% was recorded as 13.31 in the genotype BT5, while the lowest was 4.89 in E/4.

Leaf length and breadth ratio of the fifth leaf (LL/LBFL): The highest range of variation was recorded for LL/LBFL as 0.97-2.69 in Ph/9/25 and the lowest range of variation was found as 2.29-2.74 in the genotype of B2 × T1 **(Table 2.8)**.

It reveals from the **Table 2.10** that the genotype E/4 was significantly higher values for this attributes in comparison to other genotypes. In this character, the highest mean was noted in E/4 and the lowest was observed in Ph/9/40 where the values with standard error were 3.36 ± 0.03 and 2.05 ± 0.03 cm, respectively.

It also exposes from **Table 2.10** the highest CV% was noted as 19.14 in the genotype Ph/9/25, while the lowest was 4.51 in E/4.

Leaf angle of the fifth leaf (LAFL): The highest range of variation in respect of LAFL was recorded as 35-80^0 in the genotype of SDL/1 while the lowest was shown as 70-85^0 in Br/2/97 **(Table 2.11)**.

It appears from the **Table 2.11** that the all genotypes showed significant differences in comparison to each other for the character of LAFL. The genotype BS-67 obtained the highest mean with standard error of 78.00 ± 1.26 cm and BT2 was got the lowest mean with a value of 38.77 ± 0.92 cm.

It also reveals from the **Table 2.11** that both the highest and the lowest CV% were noted as 17.55 and 4.24 in the genotype SDL/1 and Br/2/97, respectively.

Pluckable shoot length (PSL): The highest range of variation in case of the character of PSL was found as 7.0-18.0 cm in Ph/9/4. On the other hand, lowest range was calculated as 5.4-11.6 cm in Ph/9/25 **(Table 2.12)**.

It reflects from the **Table 2.12** that the PSL indicates the significant differences among the all genotypes. The highest mean was noted in Ph/9/4 and the lowest was observed in B2×T1 where the values with standard error were 12.04 ± 0.50 and 7.35 ± 0.32 cm, respectively.
For this character, the highest CV% was observed as 24.80 in the genotype D/13 and the lowest was noted as 13.66 in Ph/9/40 **(Table 2.120)**.

Fresh weight of the pluckable shoot (FWPS): The highest range of variation in respect of FWPS was recorded as 31.31- 77.16 g in B2×T1 while the lowest range of variation was shown as 49.56-52.25 g in BS-67 **(Table 2.13)**.

Table 2.13 shows that the fresh weight of the pluckable shoot of the genotypes BT5 was significantly higher than the other genotypes. The highest mean of this character was recorded in BT5 with a value of 78.44 ± 0.28 g, while the lowest was recorded in SDL/1 with a value of 48.32 ± 0.28 g.

It also exposes from **Table 2.13** that the highest CV% was noted as 25.70 in the genotype B2×T1, while the lowest was 1.74 in BS-67.

Dry weight of the pluckable shoot (DWPS): The highest range of variation in case of DWPS was recorded as 10.56-29.32 g in Ph/9/25 and the lowest was found as 11.65-12.28 g in Br/2/97 **(Table 2.14)**.

It reveals from the **Table 2.14** that there are significant differences between all genotypes. For this character the highest mean with standard error was 19.84 ± 1.05 g in Ph/9/4 and lowest mean with standard error was 9.49 ± 0.04g in the genotype SDL/1.

Table 2.14 also displays that both the highest and the lowest CV% were observed as 28.93 and 1.85 in the genotype Ph/9/4 and Br/2/97, respectively.

Fresh and dry weight ratio of pluckable shoot (FW/DWPS): The highest range of variation was calculated in FW/DWPS as 2.86-10.17 in B2×T1 while the lowest range of variation was computed as 4.13-4.69 in BS-67 **(Table 2.15)**.

Table 2.15 reflects that all genotypes showed significant differences in respect to FW/DWPS. The highest mean was noted in B2×T1 and the lowest was observed in D/13 where the values with standard error were 5.85 ± 0.34 and 3.82 ± 0.03, respectively.

It also reveals from **Table 2.15** that the highest CV% was recorded as 32.18 in the genotype B2×T1, while the lowest was 3.36 in E/4.

Leaf area of pluckable shoot (LA): The highest range of variation in respect of LA was recorded as 20.2-132.5 cm^2 in the genotype of D/13 while the lowest range was calculated as 23.0-59.5 cm^2 in SDL/1 **(Table 2.16)**.

It appears from the **Table 2.16** that the all genotypes showed significant differences in comparison to each other for the character of LA. The highest mean of this character was recorded in Ph/9/25 with a value of 110.63 ± 5.07 cm^2, while the lowest was recorded in BT5 with a value of 33.17 ± 1.64 cm^2.

For this character, the highest CV% was observed as 39.19 in the genotype D/13 and the lowest was recorded as 21.55 in Ph/9/40 **(Table 2.16)**.

Shoot density (SD): The highest range of variation in case of the character of SD was computed as 21-199 numbers in the genotype of MZ/39 while the lowest range of variation was found as 21-53 numbers in D/13 **(Table 2.17)**.

Table 2.17 shows that SD of the genotype MZ/39 was significantly higher than the other genotypes. In this attribute the highest mean was recorded in MZ/39 and the

lowest was observed in E/4 where the values were 82.93 ± 8.18 and 32.43 ± 2.06, respectively.

It also appears from the **Table 2.17** that the highest CV% was noted as 54.02 in the genotype MZ/39, while the lowest was 22.26 in E/4.

Total pluckable shoot yield/plant (Y): The highest range of variation in respect of the character of Y was recorded as 900-7050 g in the genotype of MZ/39 while the lowest range of variation was calculated as 400-2950 g in Ph/9/25 **(Table 2.18)**.

It reveals from the **Table 2.18** that The significant differences with LSD value of 502.82 at 5% level were found between MZ/39 and BS-67, between MZ/39 and Ph/9/40, between MZ/39 and Ph/9/25, between MZ/39 and Ph/9/4, between MZ/39 and Br/2/97, between MZ/39 and D/13 and between MZ/39 and E/4. The highest mean of this character was in MZ/39 with a value of 1419.54 ± 286.53 g, while the lowest was noted in Ph/9/25 with a value of 787.68 ± 101.05 g.

For this character the highest CV% was recorded in D/13 with a value of 116.72, while the lowest was recorded in BT5 with a value of 69.44 **(Table 2.18)**.

Table 2.4 Range, mean ($\overline{X}$) with standard error (SE), coefficient of variability in percentage (CV%), least significant difference (LSD) at 5% and 1% level, and analysis of variance for angle of first branch (AFB) of 12 tea genotype.

Genotypes	Range	Mean with SE	CV%
MZ/39	30-70	48.33 ± 1.68	19.08
E/4	45-70	57.13 ± 1.01	9.73
D/13	45-75	61.10 ± 1.14	10.26
B2×T1	30-50	40.00 ± 0.93	12.71
Br2/97	30-45	36.83 ± 0.88	13.09
SDL/1	35-65	52.67 ± 1.41	14.69
BT2	30-45	37.33 ± 0.92	13.50
Ph/9/4	30-70	45.33 ± 2.16	26.06
Ph/9/25	30-45	39.33 ± 1.01	14.06
Ph/9/40	40-70	50.17 ± 2.08	22.74
BS-67	30-45	37.83 ± 1.12	16.16
BT-5	30-60	47.33 ± 1.51	17.49

LSD at 5% level = 3.94
LSD at 1% level = 5.18

Table 2.5 Range, mean ($\overline{X}$) with standard error (SE), coefficient of variability in percentage (CV%), least significant difference (LSD) at 5% and 1% level, and analysis of variance for height of the plucking surface (HPS) of 12 tea genotype.

Genotypes	Range	Mean with SE	CV%
MZ/39	90-100	95.23 ± 0.57	3.29
E/4	87-100	93.53 ± 0.70	4.11
D/13	81-105	94.03 ± 1.00	5.81
B2×T1	94-100	97.10 ± 0.35	1.98
Br2/97	94-100	97.07 ± 0.31	1.77
SDL/1	95-105	98.77 ± 0.37	2.07
BT2	90-105	97.17 ± 0.84	4.74
Ph/9/4	86-100	93.23 ± 0.62	3.63
Ph/9/25	80-97	90.50 ± 0.74	4.47
Ph/9/40	81-95	87.83 ± 0.68	4.24
BS-67	90-100	95.10 ± 0.51	2.93
BT-5	87-99	93.30 ± 0.61	3.56

LSD at 5% level = 1.62
LSD at 1% level = 2.13

Table 2.6 Range, mean ($\overline{X}$) with standard error (SE), coefficient of variability in percentage (CV%), least significant difference (LSD) at 5% and 1% level, and analysis of variance for length of the plucking surface (LPS) of 12 tea genotype.

Genotypes	Range	Mean with SE	CV%
MZ/39	95-130	112.90 ± 1.88	9.12
E/4	80-125	106.93 ± 2.22	11.37
D/13	95-128	107.97 ± 1.53	7.76
B2×T1	90-130	105.90 ± 2.01	10.40
Br2/97	74-120	102.70 ± 1.98	10.57
SDL/1	75-120	97.50 ± 1.76	9.87
BT2	90-120	103.73 ± 1.46	7.70
Ph/9/4	76-120	97.20 ± 2.04	11.48
Ph/9/25	80-120	96.73 ± 1.89	10.68
Ph/9/40	82-120	100.23 ± 1.60	8.77
BS-67	70-120	94.27 ± 2.21	12.85
BT-5	73-120	97.73 ± 1.95	10.90

LSD at 5% level = 5.16
LSD at 1% level = 6.78

Table 2.7 Range, mean ($\overline{X}$) with standard error (SE), coefficient of variability in percentage (CV%), least significant difference (LSD) at 5% and 1% level, and analysis of variance for breadth of the plucking surface (BPS) of 12 tea genotype.

Genotypes	Range	Mean with SE	CV%
MZ/39	52-100	75.93 ± 2.25	16.26
E/4	55-100	75.83 ± 2.22	16.05
D/13	55-100	71.90 ± 2.04	15.51
B2×T1	50-84	63.80 ± 1.53	13.17
Br2/97	54-93	71.13 ± 1.77	13.59
SDL/1	56-100	69.20 ± 2.00	15.80
BT2	52-100	69.43 ± 2.01	15.87
Ph/9/4	53-84	63.87 ± 1.58	13.51
Ph/9/25	53-100	69.00 ± 2.04	16.17
Ph/9/40	52-95	68.97 ± 2.02	16.07
BS-67	56-81	66.80 ± 1.23	10.12
BT-5	50-80	63.33 ± 1.36	11.80

LSD at 5% level = 5.15
LSD at 1% level = 6.77

Table 2.8 Range, mean ($\overline{X}$) with standard error (SE), coefficient of variability in percentage (CV%), least significant difference (LSD) at 5% and 1% level, and analysis of variance for leaf length of the fifth leaf (LLFL) of 12 tea genotype.

Genotypes	Range	Mean with SE	CV%
MZ/39	11.6-16.6	14.41 ± 0.21	8.10
E/4	11.9-15.3	13.70 ± 0.14	5.78
D/13	11.5-18	14.60 ± 0.27	10.05
B2×T1	10-13.5	11.82 ± 0.15	7.13
Br2/97	13-21.3	16.53 ± 0.35	11.43
SDL/1	10.5-15	12.54 ± 0.18	7.81
BT2	10-11.5	10.68 ± 0.07	3.40
Ph/9/4	15.9-23.5	18.33 ± 0.32	9.64
Ph/9/25	7.2-19	14.93 ± 0.53	19.61
Ph/9/40	9.5-14.5	11.83 ± 0.19	8.71
BS-67	15-20.5	17.42 ± 0.27	8.39
BT-5	11-15.5	13.08 ± 0.26	11.04

LSD at 5% level = 0.75
LSD at 1% level = 0.99

Table 2.9 Range, mean ($\overline{X}$) with standard error (SE), coefficient of variability in percentage (CV%), least significant difference (LSD) at 5% and 1% level, and analysis of variance for leaf breadth of the fifth leaf (LBFL) of 12 tea genotype.

Genotypes	Range	Mean with SE	CV%
MZ/39	4.2-5.9	4.92 ± 0.067	7.37
E/4	3.6-4.5	4.08 ± 0.04	4.89
D/13	4.5-7.7	6.02 ± 0.14	12.35
B2×T1	3.9-5.5	4.75 ± 0.07	8.20
Br2/97	4.2-6.2	5.12 ± 0.08	8.79
SDL/1	3.4-5.2	4.26 ± 0.07	9.07
BT2	3.3-4.1	3.72 ± 0.04	5.75
Ph/9/4	5.5-9.0	7.65 ± 0.13	9.32
Ph/9/25	6.0-8.2	7.24 ± 0.10	7.89
Ph/9/40	5.0-6.4	5.78 ± 0.07	6.72
BS-67	6.0-9.5	7.96 ± 0.13	8.79
BT-5	4.5-7.4	5.55 ± 0.13	13.31

LSD at 5% level = 0.26
LSD at 1% level = 0.35

Table 2.10 Range, mean ($\overline{X}$) with standard error (SE), coefficient of variability in percentage (CV%), least significant difference (LSD) at 5% and 1% level, and analysis of variance for leaf length and breadth ration of the fifth leaf (LL/LBFL) of 12 tea genotype.

Genotypes	Range	Mean with SE	CV%
MZ/39	2.54-3.46	2.94 ± 0.05	8.42
E/4	3.07-3.66	3.36 ± 0.028	4.51
D/13	2.22-3.11	2.43 ± 0.03	7.02
B2×T1	2.29-2.74	2.50 ± 0.02	4.82
Br2/97	2.80-4.04	3.24 ± 0.06	10.58
SDL/1	2.63-3.34	2.95 ± 0.03	6.43
BT2	2.53-3.17	2.88 ± 0.03	5.37
Ph/9/4	1.98-3.13	2.41 ± 0.05	10.77
Ph/9/25	0.97-2.69	2.07 ± 0.07	19.14
Ph/9/40	1.76-2.42	2.05 ± 0.03	7.03
BS-67	1.63-2.63	2.20 ± 0.04	10.35
BT-5	2.00-2.83	2.37 ± 0.04	8.52

LSD at 5% level = 0.12
LSD at 1% level = 0.15

Table 2.11 Range, mean ($\overline{X}$) with standard error (SE), coefficient of variability in percentage (CV%), least significant difference (LSD) at 5% and 1% level, and analysis of variance for leaf angle of the fifth leaf (LAFL) of 12 tea genotype.

Genotypes	Range	Mean with SE	CV%
MZ/39	45-80	60.50 ± 1.45	13.09
E/4	50-80	70.17 ± 1.14	8.87
D/13	65-90	76.23 ± 0.99	7.10
B2×T1	30-60	43.63 ± 1.30	16.30
Br2/97	70-85	76.90 ± 0.60	4.24
SDL/1	35-80	55.43 ± 1.78	17.55
BT2	30-55	38.77 ± 0.92	12.99
Ph/9/4	70-90	77.23 ± 1.01	7.13
Ph/9/25	65-90	76.83 ± 1.30	9.28
Ph/9/40	40-70	54.50 ± 1.52	15.30
BS-67	70-90	78.00 ± 1.26	8.84
BT-5	30-65	46.10 ± 2.00	23.70

LSD at 5% level = 3.67
LSD at 1% level = 4.82

Table 2.12 Range, mean ($\overline{X}$) with standard error (SE), coefficient of variability in percentage (CV%), least significant difference (LSD) at 5% and 1% level, and analysis of variance for pluckable shoot length (PSL) of 12 tea genotype.

Genotypes	Range	Mean with SE	CV%
MZ/39	5.5-13.8	9.38 ± 0.34	19.67
E/4	4.0-12.2	8.81 ± 0.34	21.29
D/13	3.0-12.8	9.06 ± 0.41	24.80
B2×T1	4.3-11.0	7.35 ± 0.32	24.11
Br2/97	5.0-13.0	9.98 ± 0.30	16.64
SDL/1	5.0-11.5	9.17 ± 0.25	15.21
BT2	6.5-13.0	9.82 ± 0.30	16.71
Ph/9/4	7.0-18.0	12.04 ± 0.50	22.51
Ph/9/25	5.4-11.6	8.49 ± 0.25	16.13
Ph/9/40	6.5-13.0	10.53 ± 0.26	13.66
BS-67	5.5-14.0	9.52 ± 0.35	20.22
BT-5	6.0-13.0	9.99 ± 0.27	14.70

LSD at 5% level = 0.89
LSD at 1% level = 1.17

Table 2.13 Range, mean ($\overline{X}$) with standard error (SE), coefficient of variability in percentage (CV%), least significant difference (LSD) at 5% and 1% level, and analysis of variance for fresh weight of pluckable shoot (FWPS) of 12 tea genotype.

Genotypes	Range	Mean with SE	CV%
MZ/39	42.14-75.16	58.78 ± 1.84	17.13
E/4	56.57-60.70	58.70 ± 0.23	2.18
D/13	54.48-61.08	57.96 ± 0.41	3.85
B2×T1	31.31-77.16	54.40 ± 2.55	25.70
Br2/97	50.50-73.60	62.12 ± 1.29	11.33
SDL/1	45.75-50.80	48.32 ± 0.28	3.20
BT2	52.36-61.40	56.89 ± 0.50	4.84
Ph/9/4	56.60-91.43	72.65 ± 1.86	14.00
Ph/9/25	54.14-85.27	69.56 ± 1.72	13.54
Ph/9/40	55.59-60.83	58.21 ± 0.29	2.73
BS-67	49.56-52.25	50.91 ± 0.16	1.74
BT-5	75.88-80.84	78.44 ± 0.28	1.97

LSD at 5% level = 3.11
LSD at 1% level = 4.09

Table 2.14 Range, mean ($\overline{X}$) with standard error (SE), coefficient of variability in percentage (CV%), least significant difference (LSD) at 5% and 1% level, and analysis of variance for dry weight of pluckable shoot (DWPS) of 12 tea genotype.

Genotypes	Range	Mean with SE	CV%
MZ/39	9.28-11.95	11.02 ± 0.15	7.48
E/4	10.96-12.05	11.46 ± 0.06	3.00
D/13	14.66-15.65	15.19 ± 0.07	2.40
B2×T1	7.06-11.05	9.55 ± 0.25	14.45
Br2/97	11.65-12.28	11.90 ± 0.04	1.85
SDL/1	8.70-9.65	9.49 ± 0.04	2.32
BT2	11.52-12.85	12.58 ± 0.06	2.62
Ph/9/4	10.56-29.32	19.84 ± 1.05	28.93
Ph/9/25	14.66-15.84	15.21 ± 0.07	2.34
Ph/9/40	10.55-11.52	11.06 ± 0.05	2.70
BS-67	10.97-12.33	11.63 ± 0.07	3.47
BT-5	11.74-21.41	16.52 ± 0.54	17.90

LSD at 5% level = 0.86
LSD at 1% level = 1.13

Table 2.15 Range, mean ($\overline{X}$) with standard error (SE), coefficient of variability in percentage (CV%), least significant difference (LSD) at 5% and 1% level, and analysis of variance for fresh and dry weight ratio of pluckable shoot (FW/DWPS) of 12 tea genotype.

Genotypes	Range	Mean with SE	CV%
MZ/39	3.59-6.51	5.35 ± 0.16	16.24
E/4	4.77-5.49	5.12 ± 0.03	3.36
D/13	3.50-4.11	3.82 ± 0.03	4.06
B2×T1	2.86-10.17	5.85 ± 0.34	32.18
Br2/97	4.12-6.11	5.22 ± 0.11	11.62
SDL/1	4.77-5.44	5.09 ± 0.03	3.55
BT2	4.12-5.21	4.52 ± 0.04	5.14
Ph/9/4	2.75-6.41	3.88 ± 0.17	24.50
Ph/9/25	3.61-5.71	4.58 ± 0.12	14.57
Ph/9/40	4.97-5.61	5.27 ± 0.04	4.64
BS-67	4.13-4.69	4.39 ± 0.04	4.55
BT-5	3.58-6.55	4.90 ± 0.16	18.08

LSD at 5% level = 0.35
LSD at 1% level = 0.45

Table 2.16 Range, mean ($\overline{X}$) with standard error (SE), coefficient of variability in percentage (CV%), least significant difference (LSD) at 5% and 1% level, and analysis of variance for leaf area of pluckable shoot (LA) of 12 tea genotype.

Genotypes	Range	Mean with SE	CV%
MZ/39	23.2-68.5	43.72 ± 2.42	30.30
E/4	9.9-62.4	42.13 ± 2.21	28.67
D/13	20.2-132.5	73.54 ± 5.26	39.19
B2×T1	10.3-79.6	43.12 ± 2.96	37.61
Br2/97	26.0-110.3	67.49 ± 3.76	30.49
SDL/1	23.0-59.5	38.08 ± 1.82	26.14
BT2	29.9-76.7	44.31 ± 2.17	26.82
Ph/9/4	27.5-127.8	83.14 ± 4.05	26.71
Ph/9/25	74.1-183.0	110.63 ± 5.07	25.12
Ph/9/40	31.2-77.6	49.97 ± 1.97	21.55
BS-67	22.9-81.8	47.97 ± 2.64	30.16
BT-5	18.3-56.5	33.17 ± 1.64	27.03

LSD at 5% level = 9.23
LSD at 1% level = 12.13

Table 2.17 Range, mean ($\overline{X}$) with standard error (SE), coefficient of variability in percentage (CV%), least significant difference (LSD) at 5% and 1% level, and analysis of variance for pluckable shoot density (SD) of 12 tea genotype.

Genotypes	Range	Mean with SE	CV%
MZ/39	21-199	82.93 ± 8.18	54.02
E/4	18-65	32.43 ± 2.06	34.72
D/13	21-53	35.70 ± 1.65	25.27
B2×T1	19-79	40.03 ± 2.34	32.01
Br2/97	18-67	36.67 ± 2.32	34.67
SDL/1	15-78	45.83 ± 2.44	29.19
BT2	18-64	39.07 ± 1.70	23.78
Ph/9/4	13-60	35.67 ± 2.19	33.56
Ph/9/25	26-59	38.33 ± 1.69	24.20
Ph/9/40	23-82	55.17 ± 2.24	22.26
BS-67	17-55	36.73 ± 1.66	24.80
BT-5	20-91	58.20 ± 2.37	22.29

LSD at 5% level = 6.50
LSD at 1% level = 8.54

Table 2.18 Range, mean ($\overline{X}$) with standard error (SE), coefficient of variability in percentage (CV%), least significant difference (LSD) at 5% and 1% level, and analysis of variance for total pluckable shoot yield/plant (Y) of 12 tea genotype.

Genotypes	Range	Mean with SE	CV%
MZ/39	900-7050	1419.54 ± 286.53	110.56
E/4	400-4900	879.38 ± 176.74	110.08
D/13	200-4100	898.84 ± 191.54	116.72
B2×T1	1000-6200	1137.22 ± 228.26	109.94
Br2/97	900-4300	900.00 ± 158.76	96.62
SDL/1	300-5000	1237.82 ± 202.86	89.76
BT2	900-4850	1115.91 ± 189.22	92.87
Ph/9/4	750-4100	897.14 ± 127.57	77.88
Ph/9/25	400-2950	787.68 ± 101.05	70.27
Ph/9/40	350-4400	891.67 ± 169.32	104.01
BS-67	700-3450	859.72 ± 118.83	75.70
BT-5	1000-4300	1160.87 ± 147.17	69.44

LSD at 5% level = 502.82
LSD at 1% level = 660.85

2.3.2. Analysis of Variance

The results of the analysis of variance for all the 15 quantitative characters of 12 different genotypes were done separately which are presented in **Appendix I-XV**. For significant test the main items and their interaction effects, a mixed model was followed.

The analysis of variance showed that the item genotype (G) was highly significant for all the characters when tested against the within and pooled error. These indicate that the genotypes were significantly different from each other in respect of those characters. Replication (R) item was non-significant for most of the characters except HPS, BPS, FWPS, DWPS and SD. The interaction G × R was non-significant for most of the characters except HPS, PSL, FWPS, DWPS, FW/DWPS and SD.

2.3.3. Genetic Parameters

Genotypic variation (σ^2_g), phenotypic variation (σ^2_p), interaction (σ^2_{GR}), error (σ^2_w), genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), interaction coefficient of variation (G × R CV), within error coefficient of variability (ECV), heritability (h^2_b), genetic advance (GA) and genetic advance as percent of mean (GA%) for different characters were estimated and results are presented in **Table 2.19-2.21**. Results obtained on different genetic parameters were described separately.

2.3.3.1. Genotypic variance (σ^2_g)

The estimation of genotypic variance among all the studied characters of the genotypes reveals highest genotypic variance for Y (435861.62) and it was followed by LA (521.35), LAFL (218.81) and SD (143.70). The lowest genotypic variance was 0.20 estimated for LL/LBFL **(Table 2.19)**.

2.3.3.2. Phenotypic variance (σ^2_p)

For all characters, phenotypic variation (σ^2_p) was greater than those of genotypic (σ^2_g), interaction (σ^2_{GR}) and error (σ^2_w) component of variation as expected, except error (σ^2_w) component of variation of Y. The phenotype is the joint product of genotypic (σ^2_g), interaction (σ^2_{GR}) and error (σ^2_w). **Table 2.19** showed the greater portion of the total phenotypic variation was appeared mostly due to error variation for all the characters. The

maximum variation was observed for Y with a value of 1417880.56 and the lowest phenotypic variation was 0.25 shown by LL/LBFL **(Table 2.19)**.

2.3.3.3. Interaction variance (σ^2_{GR})

Regarding variation of the interaction between genotype and replication, the highest value was found for SD, while the lowest value was observed for Y **(Table 2.19)**.

2.3.3.4. Error variance (σ^2_w)

The highest and the lowest values of error variation (σ^2_w) was recorded for Y and LL/LBFL **(Table 2.19)**.

2.3.3.5. Genotypic coefficient of variation (GCV)

For this item pluckable shoot yield/plant showed the highest GCV, while lowest GCV was found for FW/DWPS with the values of 16162.98 and 6.06, respectively **(Table 2.20)**.

2.3.3.6. Phenotypic coefficient of variation (PCV)

In general, the phenotypic coefficient of variability (PCV), was greater than genotypic (GCV), interaction (G × R CV) and error coefficient of variability (ECV) for all the characters. PCV is the joint product of GCV, G × R CV and ECV. Estimates of the PCV was the highest for Y with a value of 52529.56 and the lowest PCV was estimated for the LL/LBFL with a value of 9.53 **(Table 2.20)**.

2.3.3.7. G × R interaction coefficient of variation (G × R CV)

The highest and the lowest values of this interaction coefficient of variability were noted for SD and Y (**Table 2.20)**.

2.3.3.8. Error coefficient of variability (ECV)

The character, Y showed the highest ECV (36608.64) followed by LA (588.96), SD (368.56), BPS (149.93), AFB (131.57) and LPS (101.82). The lowest ECV (2.05) was observed for the LL/LBFL **(Table 2.20)**.

2.3.3.9. Heritability (h^2_b)

Broad sense heritability (h^2_b) for 15 quantitative characters of tea were estimated and the results are presented in **Table 2.21**. In the present investigation the highest heritability was estimated for LBFL (87.65) and the lowest was recorded for BPS

(13.85). Heritability were also higher for LAFL (80.96), LL/LBFL (78.82), LLFL (71.28), DWPS (70.13), LA (63.28), FWPS (61.21) and AFB (53.75). The lowest heritability was recorded for BPS (13.85).

2.3.3.10. Genetic advance (GA)

The estimation of genetic advance shows the highest value for Y (754.40). In comparison to Y, GAs for rest of the LA (37.42), LAFL (27.42), FWPS (13.88), SD (13.43) and AFB (12.23) were very low. The lowest GA was 0.62 estimated for FW/DWFL **(Table 2.21)**.

2.3.3.11. Genetic advance as % of mean (GA%)

The estimation of GA% reveals **(Table 2.21)** the highest value for LA (66.30). In comparison to LA, GA% for rest of the characters such as LBFL (48.41), LAFL (43.62), DWPS (40.45), LL/LBFL (30.99), SD (30.03), LLFL (28.85), Y (27.98), AFB (26.51), FWPS (22.91), FW/DWPS (12.80), PSL (11.30), LPS (4.70) and BPS (4.44) were low. The lowest value of GA% was 4.09 found for HPS.

Table 2.19 Genotypic, phenotypic, environmental and error component of variation of different quantitative characters in tea.

Characters	Genetic parameter			
	Genotypic variance (σ^2_g)	Phenotypic variance (σ^2_p)	Interaction variance (σ^2_{GR})	Error variance (σ^2_w)
AFB	65.55	121.94	-4.28	60.68
HPS	8.52	20.62	1.81	10.30
LPS	27.00	134.81	3.97	103.84
BPS	15.98	115.33	-4.25	103.60
LLFL	5.51	7.73	0.02	2.20
LBFL	1.97	2.24	0.005	0.27
LL/LBFL	0.20	0.25	-0.001	0.05
LAFL	218.81	270.28	-1.13	52.60
PSL	1.11	4.55	0.32	3.12
FWPS	74.13	121.09	9.11	37.86
DWPS	9.22	13.15	1.05	2.88
FW/DWPS	0.29	0.95	0.19	0.47
LA	521.35	823.91	-29.85	332.40
SD	143.70	485.51	176.96	164.86
Y	435861.62	1417880.56	-6575.51	9888694.44

AFB = Angle of the first branch, HPS = Height of the plucking surface, LPS = Length of the plucking surface, BPS = Breadth of the plucking surface, LLFL = Leaf length of the fifth leaf, LBFL = Leaf breadth of the fifth leaf, LL/LBFL = Leaf length and breadth ration of the fifth leaf, LAFL = Leaf angle of the fifth leaf, PSL = Pluckable shoot length, FWPS = Fresh weight of the pluckable shoot, DWPS = Dry weight of the pluckable shoot, FW/DWPS = Fresh weight and dry weight ratio of the pluckable shoot, LA = Leaf area of pluckable shoot, SD = Shoot density of pluckable shoot and Y = Total pluckable shoot yield/plant.

Table 2.20 Genotypic, phenotypic, environmental and error coefficient of variability of different quantitative characters in tea.

Characters	Genetic parameter			
	Genotypic coefficient of variation (GCV)	**Phenotypic coefficient of variation (PCV)**	**Interaction coefficient of variation (G×RCV)**	**Error coefficient of variation (ECV)**
AFB	142.13	264.42	-9.28	131.57
HPS	9.02	21.85	1.91	10.91
LPS	26.47	132.19	3.89	101.82
BPS	23.12	166.91	-6.14	149.93
LLFL	38.94	54.63	0.15	15.54
LBFL	35.20	40.16	0.08	4.88
LL/LBFL	7.51	9.53	-0.03	2.05
LAFL	348.10	429.99	-1.79	83.68
PSL	11.70	47.79	3.32	32.77
FWPS	122.37	199.90	15.03	62.50
DWPS	71.22	101.55	8.13	22.20
FW/DWPS	6.06	19.66	3.96	9.65
LA	923.75	1459.82	-52.89	588.96
SD	321.26	1085.42	395.60	368.56
Y	16162.98	52529.56	-242.05	36608.64

AFB = Angle of the first branch, HPS = Height of the plucking surface, LPS = Length of the plucking surface, BPS = Breadth of the plucking surface, LLFL = Leaf length of the fifth leaf, LBFL = Leaf breadth of the fifth leaf, LL/LBFL = Leaf length and breadth ration of the fifth leaf, LAFL = Leaf angle of the fifth leaf, PSL = Pluckable shoot length, FWPS = Fresh weight of the pluckable shoot, DWPS = Dry weight of the pluckable shoot, FW/DWPS = Fresh weight and dry weight ratio of the pluckable shoot, LA = Leaf area of pluckable shoot, SD = Shoot density of pluckable shoot and Y = Total pluckable shoot yield/plant.

Table 2.21 Heritability, Genetic advance (GA) and Genetic advance as percentage of mean (GA %) different characters in tea.

Characters	Genetic parameter		
	Heritability (h^2_b)	Genetic Advance (GA)	GA% of mean
AFB	53.75	12.23	26.51
HPS	41.30	3.86	4.09
LPS	20.03	4.79	4.70
BPS	13.85	3.06	4.44
LLFL	71.28	4.08	28.85
LBFL	87.65	2.71	48.41
LL/LBFL	78.82	0.81	30.99
LAFL	80.96	27.42	43.62
PSL	24.48	1.08	11.30
FWPS	61.21	13.88	22.91
DWPS	70.13	5.24	40.45
FW/DWPS	30.82	0.62	12.80
LA	63.28	37.42	66.304
SD	29.60	13.43	30.03
Y	30.77	754.40	27.98

AFB = Angle of the first branch, HPS = Height of the plucking surface, LPS = Length of the plucking surface, BPS = Breadth of the plucking surface, LLFL = Leaf length of the fifth leaf, LBFL = Leaf breadth of the fifth leaf, LL/LBFL = Leaf length and breadth ration of the fifth leaf, LAFL = Leaf angle of the fifth leaf, PSL = Pluckable shoot length, FWPS = Fresh weight of the pluckable shoot, DWPS = Dry weight of the pluckable shoot, FW/DWPS = Fresh weight and dry weight ratio of the pluckable shoot, LA = Leaf area of pluckable shoot, SD = Shoot density of pluckable shoot and Y = Total pluckable shoot yield/plant.

2.3.4. Correlation Coefficient

Stepwise forward Ridge regression (Hoerl and Kennard, 1970) was carried out on 14 quantitative characters of tea in order to select the important yield contributing characters to be used for correlation analysis. The results of Ridge regression analysis are shown in **Table 2.2.** Depending upon the Ridge regression analysis five characters e.g. HPS, LPS, BPS, AFB and SD were selected for the study of correlation coefficient and path coefficient analysis.

Correlation co-efficient between Y and yield attributing characters and correlation co-efficient among yield attributing characters of the studied tea genotypes at genotypic and phenotypic levels were calculated. The results on correlation coefficient between Y and yield components and among yield components are presented in **Table 2.22-2.23**. Item wise results were described under following heads.

2.3.4.1. Genotypic correlation co-efficient among different pairs of characters

Genotypic correlation co-efficient between Y and yield attributing characters is presented in **Table 2.22**. Y was highly significant and positively correlated with SD followed by LPS, HPS surface and BPS surface was recorded.

Genotypic correlation co-efficient among the different pairs of characters were estimated and the results are presented in **Table 2.22**. HPS showed highly significant positive correlation with LPS and Y while correlation co-efficient of HPS with AFB exhibited highly significant but negative correlation. Negative significant association with SD with HPS was also observed. LPS was showed highly significant and positive correlation with BPS, AFB, SD and Y. Highly positive significant association of BPS with AFB, SD and Y also observed. Highly significant and positive correlation of AFB with SD was observed. Highly significant and positive correlation of SD with Y was found.

2.3.4.2. Phenotypic correlation co-efficient among different pairs of characters

At phenotypic level, Y was showed highly significant and positive correlation with HPS followed by LPS and SD (**Table 2.23)**.

Phenotypic correlation co-efficient among different pairs of characters are presented in **Table 2.23**. Among the different inter-character associations HPS showed positive significant association with the LPS and Y. Significant but negative association of HPS with AFB was observed, while the correlation coefficient with rest of the characters was non-significant. Relationship of LPS was highly significant and positive with BPS, SD and Y, while the association with AFB was positively significant. Highly significant and positive correlation of BPS with AFB was also observed. The association of the AFB with the rest of the character viz. SD and Y was non-significant. Highly significant and positive correlation of SD with Y was also observed.

Table 2.22 Genotypic correlation co-efficient among different pairs of characters in tea.

Character	HPS	LPS	BPS	AFB	SD	Y
HPS	1.0000	0.1767**	0.0263	-0.2161**	-0.1150*	0.6613**
LPS		1.0000	0.7442**	0.4039**	0.3945**	0.7093**
BPS			1.0000	0.4366**	0.2155**	0.2952**
AFB				1.0000	0.1712**	0.0231
SD					1.0000	0.8199**

* & ** indicates significant at 5% and 1% levels of probability, respectively.

HPS = Height of the plucking surface, LPS = Length of the plucking surface, BPS = Breadth of the plucking surface, AFB = Angle of the first branch, SD = Shoot density of pluckable shoot and Y= pluckable shoot yield/plant.

Table 2.23 Phenotypic correlation co-efficient among different pairs of characters in tea.

Character	HPS	LPS	BPS	AFB	SD	Y
HPS	1.0000	0.1047*	0.0032	-0.1342*	-0.0783	0.2921**
LPS		1.0000	0.3166**	0.1157*	0.1480**	0.2561**
BPS			1.0000	0.1416**	0.0922	0.0682
AFB				1.0000	0.0324	0.0807
SD					1.0000	0.1777**

* & ** indicates significant at 5% and 1% levels of probability, respectively.

HPS = Height of the plucking surface, LPS = Length of the plucking surface, BPS = Breadth of the plucking surface, AFB = Angle of the first branch, SD = Shoot density of pluckable shoot and Y= pluckable shoot yield/plant.

2.3.5. Path Coefficient Analysis

The correlation coefficients among the Y and other Y components were partitioned in to direct and indirect effects through path coefficient analysis in order to find out more realistic picture of relationship. Path coefficient analysis was performed using the values of genotypic and phenotypic correlation and are presented in **Table 2.24** and **2.25**. The results of path coefficient analysis at genotypic and phenotypic levels are described below.

2.3.5.1. Path coefficient at genotypic level

The direct and indirect effect of yield component characters towards Y at genotypic level was calculated and the results presented in **Table 2.24**. Results in **Table 2.24** reveals SD had the highest (0.7644) direct positive effect on pluckable shoot yield/plant followed by HPS (0.6564).The lowest direct positive effect 0.4768 towards Y was found for LPS. The highest direct negative effect towards Y was found for BPS (-0.2131) and the lowest direct negative effect -0.0655 was found for AFB towards Y.

2.3.5.2. Path coefficient at phenotypic level

Path coefficient analysis at phenotypic level was also performed and the results are presented in **Table 2.25**. Path coefficients at phenotypic level reveals that HPS had highest direct positive effect (0.2970) towards Y and which was followed by LPS (0.1961) and SD (0.1709). The lowest direct positive effect 0.0959 was found for AFB. The highest only one direct negative effect towards Y was found for BPS (-0.0242).

Table 2.24 Genotypic path co-efficient analysis showing direct and in direct effect of various characters on total pluckable shoot yield/plant in tea.

Character	HPS	LPS	BPS	AFB	SD	r_g with Y
HPS	**0.6564**	0.0842	-0.0056	0.0142	-0.0879	0.6613**
LPS	0.1160	**0.4768**	-0.1586	-0.0265	0.3016	0.7093**
BPS	0.0173	0.3548	**-0.2131**	-0.0286	0.1647	0.2952**
AFB	-0.1418	0.1926	-0.0930	**-0.0655**	0.1309	0.0231
SD	-0.0755	0.1881	-0.0459	-0.0112	**0.7644**	0.8199**

Residual = 0.4520

Bold figure indicates direct effect.

* & ** indicates significant at 5% and 1% levels of probability, respectively.

HPS = Height of the plucking surface, LPS = Length of the plucking surface, BPS = Breadth of the plucking surface, AFB = Angle of the first branch, SD = Shoot density of pluckable shoot and Y= pluckable shoot yield/plant.

Table 2.25 Phenotypic path co-efficient analysis showing direct and indirect effect of various characters on total pluckable shoot yield/plant in tea.

Character	HPS	LPS	BPS	AFB	SD	r_p with Y
HPS	**0.2979**	0.0205	-0.0001	-0.0129	-0.0134	0.2921**
LPS	0.0312	**0.1961**	-0.0077	0.0111	0.0253	0.2561**
BPS	0.0010	0.0621	**-0.0242**	0.0136	0.0158	0.0682
AFB	-0.0400	0.0227	-0.0034	**0.0959**	0.0055	0.0807
SD	-0.0233	0.0290	-0.0022	0.0031	**0.1709**	0.1777**

Residual = 0.9090

Bold figure indicates direct effect.

* & ** indicates significant at 5% and 1% levels of probability, respectively.

HPS = Height of the plucking surface, LPS = Length of the plucking surface, BPS = Breadth of the plucking surface, AFB = Angle of the first branch, SD = Shoot density of pluckable shoot and Y= pluckable shoot yield/plant.

2.3.6. Genetic Diversity of 12 Tea Genotypes

In order to find out the extent of genetic diversity principal component analysis (PCA) and cluster analyses were performed on different characters of genotypes of tea. The results of these analyses are described below.

2.3.6.1. Principal component analysis (PCA)

Contribution of characters towards divergence of the genotypes is given in **Table 2.26**. The first three components accounted for 46.92% of the total variance in the data set. The first component alone accounted for 26.02% of the total variance and was far more important than other components. The first component with a Cronbach's alpha value of 0.80 was the only one considered to be reliable. The component loadings correspond to the correlation coefficient between characters and the derived components. Characters with high loadings were LBFL (0.87) followed by DWPS (0.70), LLFL, LAFL and LA (over 0.60).

2.3.6.2. Cluster analysis

A dissimilarity matrix based on Euclidian distances for the 12 tea genotypes is presented in **Table 2.27**. The clones exhibited large variation between and within the genotypes. The estimated dissimilarity of 11.9 was the highest between the genotypes Ph/9/40 and Br2/97 as well as 11.1% was between the genotypes MZ/39 and Ph/9/40, while the least dissimilarity (4.5) was between two genotypes, namely B2×T1 and E/4.

A phenogram based on the Euclidian distances from the morphological data divided the tea genotypes in to two major clusters, viz. C1 and C2 (**Figure 2.3**). C1 is consisted of maximum seven genotypes, viz., MZ/39, E/4, B2×T1, SDL/1, BT2, D/13 and BR2/97. On the other hand, C2 has five genotypes which are Ph/9/4, BT5, Ph/9/40, BS-67 and Ph/9/25. Thus, C1 has two sub-cluster S_1C_1 and S_1C_2, which are consisted of five and two genotypes, respectively. Sub-cluster S_1C_1 is consisted MZ/39, E/4, B2×T1, SDL/1 and BT2 while S_1C_2 is comprised of D/13 and BR2/97 genotypes. Similarly, C2 has two sub-cluster S_2C_1 and S_2C_2, which are involved four and single genotypes, respectively. Sub-cluster S_2C_1 is contained Ph/9/4, BT5, Ph/9/40 and BS-67 genotypes, while S_2C_2 has single genotype Ph/9/25 only.

Table 2.26 Variation in morphological characteristics of investigated tea samples. Loadings of the 15 morphological characters on the first three components are from PCA. Eigenvalues, percentages of variance explained and cumulated, and Cronbach's alpha are given for each component.

Characters	Ranges of variation (Standard deviation)	Data type	Component		
			1	2	3
AFB	36.83 (4.82) – 61.10 (6.27)	Quantitative	-0.09	-0.20	-0.37
HPS	87.83 (3.72) – 98.77 (2.05)	Quantitative	-0.39	-0.38	0.30
LPS	94.27 (12.11) – 112.90 (10.30)	Quantitative	-0.33	-0.36	-0.45
BPS	63.33 (7.47) – 75.93 (12.35)	Quantitative	-0.18	-0.48	-0.44
LLFL	10.68 (0.36) – 18.33 (1.77)	Quantitative	0.68	-0.45	0.10
LBFL	3.72 (0.21) – 7.96 (0.70)	Quantitative	0.87	0.09	0.12
LL/LBFL	2.05 (0.14) – 3.36 (0.15)	Quantitative	-0.50	-0.62	-0.04
LAFL	38.77 (5.04) – 78.00 (6.90)	Quantitative	0.65	-0.49	0.11
PSL	7.35 (1.77) – 12.04 (2.71)	Quantitative	0.27	-0.01	-0.27
FWPS	48.32 (1.55) – 78.44 (1.55)	Quantitative	0.40	0.25	-0.63
DWPS	9.49 (0.22) – 19.84 (5.74)	Quantitative	0.70	-0.03	-0.40
FW/DWPS	3.82 (0.16) – 5.85 (1.88)	Quantitative	-0.51	0.27	-0.11
LA	33.17 (8.97) – 110.63 (27.79)	Quantitative	0.64	-0.16	-0.001
SD	32.43 (11.26) – 82.93 (44.80)	Quantitative	-0.23	0.25	-0.44
Y	787.68 (553.47) – 1419.54 (1569.39)	Quantitative	-0.50	-0.17	-0.07
		Eigenvalue	3.90	1.63	1.50
		Variance explained (%)	26.02	10.89	10.01
		Variance cumulative (%)	26.02	36.91	46.92
		Cronbach's alpha	0.80	0.43	0.37

AFB = Angle of the first branch (degree), HPS = Height of the plucking surface (cm), LPS = Length of the plucking surface (cm), BPS = Breadth of the plucking surface (cm), LLFL = Leaf length of the fifth leaf (cm), LBFL = Leaf breadth of the fifth leaf (cm), LL/LBFL = Leaf length and breadth ration of the fifth leaf, LAFL = Leaf angle of the fifth leaf (degree), PSL = Pluckable shoot length (cm), FWPS = Fresh weight of the pluckable shoot (g), DWPS = Dry weight of the pluckable shoot (g), FW/DWPS = Fresh weight and dry weight ratio of the pluckable shoot, LA = Leaf area of pluckable shoot (cm^2), SD = Shoot density of pluckable shoot (no.) and Y= Total pluckable shoot yield/plant (g).

Table 2.27 Dissimilarity matrix of 12 treatments based on morphological characters.

Genotypes	MZ/39	E/4	D/13	B2×T1	Br2/97	SDL/1	BT2	Ph/9/4	Ph/9/25	Ph/9/40	BS-67	BT-5
MZ/39	0.00											
E/4	5.70	0.00										
D/13	7.50	6.80	0.00									
B2×T1	4.80	4.50	6.90	0.00								
Br2/97	6.10	7.70	5.60	6.40	0.00							
SDL/1	6.30	7.20	6.20	6.20	6.90	0.00						
BT2	6.10	6.50	6.60	5.50	6.10	5.33	0.00					
Ph/9/4	8.60	9.10	10.60	8.60	10.40	8.28	9.40	0.00				
Ph/9/25	9.00	8.70	9.10	9.00	9.40	8.36	9.80	7.60	0.00			
Ph/9/40	11.10	10.30	10.50	10.70	11.90	8.84	10.50	6.70	8.66	0.00		
BS-67	10.5	9.70	9.10	10.30	10.20	7.68	8.30	7.70	7.48	7.20	0.00	
BT-5	9.8	10.00	10.70	10.20	10.80	8.25	9.50	4.90	7.63	6.10	5.90	0.00

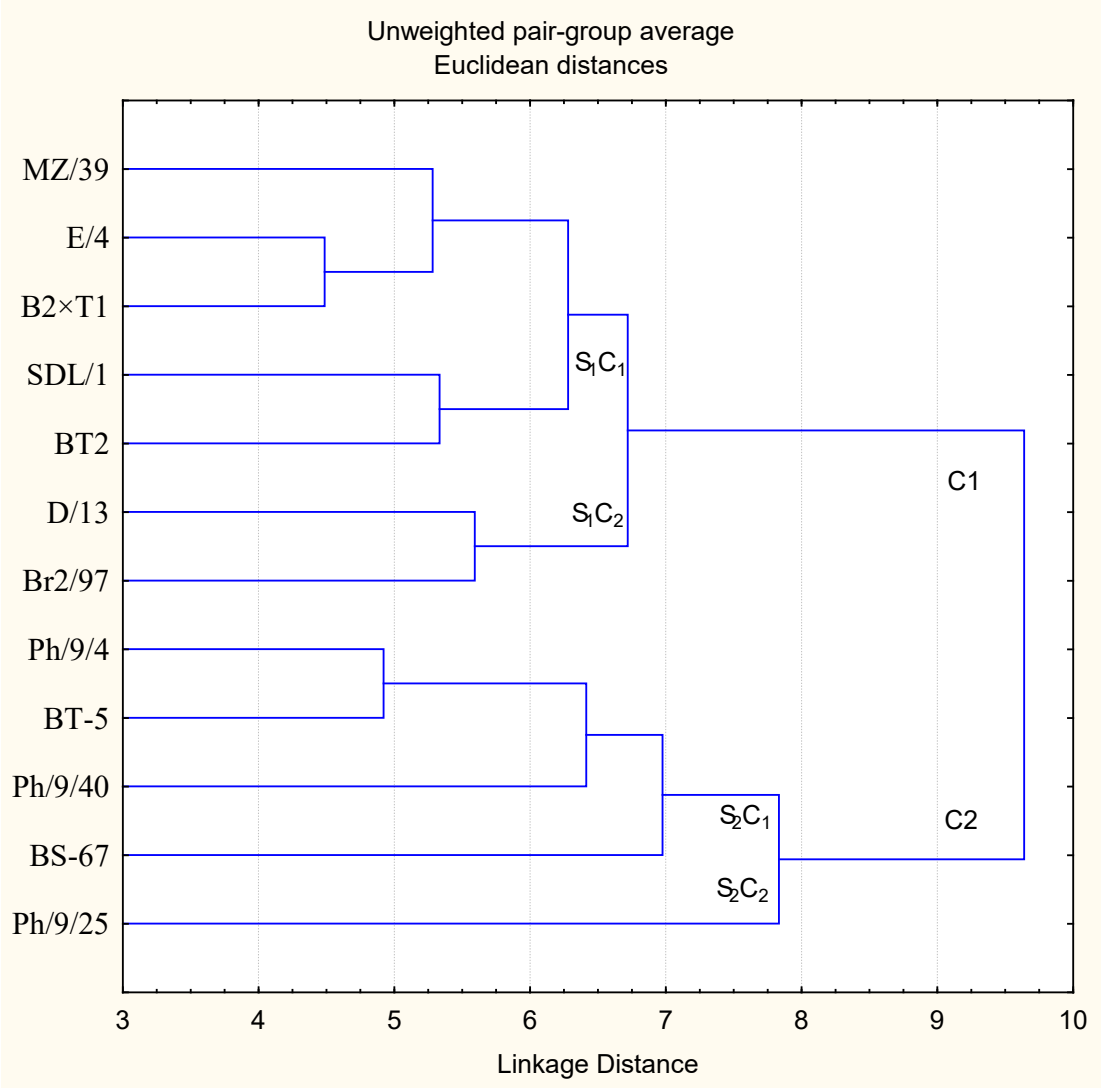


Figure 2.3 Dendrogram of the 12 tea genotypes based on Euclidean distances of morphological characters

2.4. DISCUSSIONS

Tea is an important perennial crop. The vegetative parts i.e. two leaf and a bud is economically important parts of tea plants. The aim of tea breeding is to develop high yielding tea per unit area of bush surface with acceptable quality under different agro climatic conditions.

Clones are selected from tea fields in production farms, seedling nurseries and from special plantings of selected seeds. Studies of morphological and physiological characteristics of clones in relation to the components of yield should aid selection efficiency (Squire, 1985) and hence reduce the proportion of poor clones involved in the clonal trials. Productivity in tea is mainly related to the production of dry matter and its partitioning to the part of plant which makes up the commercial yield.

In genetics and breeding research quantitative characters are no doubt important and most of the quantitative characters are economically important. The present investigation was carried out to study the 15 economically important characters viz. angle of the first branch (AFB), height of the plucking surface (HPS), length of the plucking surface (LPS), breadth of the plucking surface (BPS), leaf length of the fifth leaf (LLFL), leaf breadth of fifth leaf (LBFL), leaf length and breadth ratio of the fifth leaf (LL/LBFL), leaf angle of the fifth leaf (LAFL), pluckable shoot length (PSL), fresh weight of the pluckable shoots (FWPS), dry weight of the pluckable shoots (DWPS), fresh and dry weight ratio of pluckable shoots (FW/DWPS), leaf area of the pluckable shoots (LA), shoot density (SD) of pluckable shoots and total pluckable shoot/plant (Y) in 12 different tea cultivars. Analysis were done for variability, heritability, genetic advance, correlation coefficient, path analysis and genetic diversity.

2.4.1. Ranges, Mean with Standard Error (SE), List Significant Difference (LSD) and Coefficient of Variability Percentage (CV%)

In the analysis all the characters showed a wide and pronounced range of variation indicating that they are under polygenic control and hence quantitative in nature. The wide range of variation showed that these tea genotypes are good breeding materials. Similar results were obtained in tea by Rajanna *et al.* (2011), Rajkumar *et al.* (2010), Kamunya *et al.* (2010) and Rahman *et al.* (2010), in lentil by Malhotra *et al.* (1974)

and Azad (1991), in cheakpea by Haque (1989), Begum (1995), Hasan (2001) and Deb (2002), in green gram by Bhargava *et al.* (1966), in mustard by Paul *et al.* (1976), Chaudhari Prasad (1968), Joarder and Eunus (1968), in sugarcane by Nahar and Khaleque (1996), Nahar (1997) and in Chilli by Husain (1997).

Mean of the 15 tea genotypes of these characters as compared with their respective standard error were found to be highly significant. This indicate that the genotypes were different regarding these characters. This result is in agreement with the analysis of variance in which the genotype item was found to be highly significant for all the 15 characters. It shows that the genotypes are genetically different from each other, which justifies their inclusion as materials in the present work. Similar results were obtained in tea by Rajanna *et al.* (2011), Rajkumar *et al.* (2010), Kamunya *et al.* (2010), Rahman *et al.* (2010) and Ariyarathna *et al.* (2010). Aziz *et al.* (2011) studied ten morphological characters and reported a significant differences among seven cultivars in *Camellia sinensis*. Sarwar *et al.* (2002) investigated several parametric and non-parametric characteristics of leaves and some non-parametric characters of the bushes of 11 BTRI released clones and got the significant differences among the clones. Dutta *et al.* (1999) studied on the cross compatibility of some clones and the growth characteristics of their progenies and reported the similar results. Shiblee *et al.* (1994) studied to examine the comparative performance of different seedlings agrotypes of tea and got the akin outcomes. Similar results were also obtained in respect of yield and quality of different tea clones by Alam *et al*. (2001), Dutta *et al.* (2000), Alam *et al.* (1992), Alam *et al.* (1990) and Chakraborty *et al.* (1975). Alam *et al.* (1978) reported a significant differences among 41 strain in *Brassica campestris* L. Similar results were also obtained in lentil by Azad (1991), in chickpea by Deb (2002) and in rape seed and mustard by Mandal *et al.* (1978).

For each of the characters the mean differences between the genotypes were tested with LSD values. The significant differences were found in all genotypes. However, for all the genotypes the significant differences of a particular character varied. This result is akin to the findings of Aziz *et al.* (2011), Rajkumar *et al.* (2010), Kamunya *et al.* (2010), Rahman *et al.* (2010), Ariyarathna *et al.* (2010), Sarwar *et al.* (2002), Alam *et al*. (2001), Dutta *et al.* (2000), Dutta *et al.* (1999), Shiblee *et al.* (1994), Alam *et al.*

(1992), Alam *et al.* (1990), and Chakraborty *et al.* (1975) in tea. Similar results were also obtained in lentil by Azad (1991), in chickpea by Deb (2002).

In the present materials the degree of coefficient of variability in percentage (CV%) was indicated by the magnitude of range of variation. For all the genotypes the CV% of a particular character varied from genotype to genotype. Similar results were obtained in tea by Rajanna *et al.* (2011), Rajkumar *et al.* (2010), Rahman *et al.* (2010), Ariyarathna *et al.* (2010), in lentil by Azad (1991) and in chickpea by Deb (2002).

2.4.2. Analysis of Variance

In the analysis of variance the genotype item was highly significant for all the characters when it was tested against within error. Again it was highly significant for all the characters when it was tested against pooled error also. These results indicate that genotypes were significantly and genotypically different from each other and it justifies their inclusion in the present investigation as material. Babar Ali (1998) found similar results for all the eight quantitative characters in ten lentil cultivars. Similar results were also obtained in tea by Ariyarathna *et al.* (2010), Rajkumar *et al.* (2010), Rahman *et al.* (2010), in lentil by Azad (1991), in sugarcane by Nahar (1997) and in chickpea by Deb (2002).

2.4.3. Genetic Parameters

The different component of variation varied differently in different characters. Phenotypic component of variation (σ^2_p) was higher than genotypic (σ^2_g), interactions (σ^2_{GR}) and error (σ^2_w) component of variation. This results are in conformity with the findings of Samad (1991), Nahar (1997) and Deb (2002). The difference between phenotypic and genotypic variation were greater in magnitude for angle of the first branch, length of the plucking surface, breadth of the plucking surface, fresh weight of the pluckable shoots, leaf area of the pluckable shoots, shoot density and total pluckable shoot yield/plant which indicated that the environment had considerable effect on these characters. These results are in agreement with the findings of Podder (1993), Mohamed *et al.* (1991), Nahar and Khaleque (1996), Nahar (1997) and Dev (2002). In the present study, the highest phenotypic and genotypic variations were observed for yield followed by leaf area of the pluckable shoot, leaf angle of the fifth

leaf, shoot density, fresh weight of the pluckable shoots, angle of the first branch, length of the plucking surface and breadth of the plucking surface. In the present materials, high genotypic value causes the high phenotypic value. Larger genotypic value for any character is always helpful for effective selection. These results are in agreement with the findings of Mian and Awal (1979). The pronounced environmental variation indicated that greater portion of the phenotypic variation was environmental in nature. Chandra (1968) reported in gram that variability was affected by environment. Similar results were also obtained in chickpea by Deb (2002). The characters total pluckable shoot yield/plant, leaf area of the pluckable shoot, shoot density, length of the plucking surface, breadth of the plucking surface, angle of the first branch and leaf angle of the fifth leaf showed the highest value for σ^2_w component and shoot density showed highest value for σ^2_{GR} component which indicated better scope for improvement of those character through selection. On the other hand, rest of the characters for σ^2_w and σ^2_{GR} showed the lowest values in the present materials indicating difficulties in improvement of these traits through selection.

In this study phenotypic coefficient of variability (PCV) was greater than all other genotypic coefficient of variability (GCV). The results are in agreement with the findings of Samad (1991), Nahar (1997) and Deb (2002). The difference between PCV and GCV were greater in magnitude for length of the plucking surface, breadth of plucking surface, leaf area of the pluckable shoot, shoot density, angle of the first branch and total pluckable shoot yield/plant which indicated that environment had considerable effect on these characters. The highest amount of PCV, GCV, G × RCV and ECV were observed for shoot density indicating wide scope of selection for this trait. The highest values of PCV, GCV and ECV were also recorded for total pluckable shoot yield/plant, leaf area of the fifth leaf, leaf angle of the fifth leaf, angle of the first branch and breadth of the plucking surface, but the G × RCV was negative value for those traits. These results suggest that the greater variability for those characters among the genotypes was due to genetic cause which are less affected by environment and hence could be improved through selection. Akanda *et al.* (1995), Patil and Bhapker (1987), Pathak *et al.* (1993), Malhotra and Khehra (1986), Parh *et al.* (1990) and Ali *et al.* (1994) observed high GCV and PCV percent for yield and yield related character in different crops.

Heritability estimates in broad sense were relatively high for almost all the characters studied except height of the pluking surface, length of the plucking surface, breadth of the plucking surface, pluckable shoot length, fresh and dry weight ratio, shoot density and total pluckable shoot yield/plant. Although high heritability estimates have found to be helpful in making selection of superior genotypes on the basis of phenotypic performance. Johonson (1955) suggested that heritability estimates along with genetic gain were more useful in predicting the effect for selecting the best individual. The present findings, therefore, are in agreement with the earlier findings of Chaudhury and Sharma (1984), Dayal *et al.* (1972), Metin (1985), Pandita and Sidhu (1981), and Desai and Jaimini (1997). They have observed high heritability for tuber yield, average tuber weight, tuber number, plant height and tuber dry matter content of potato.

Though the evaluation may be reliable through studies of GCV (%) and heritability, still more concrete basis may be formed by studying the performance through genetic advance. It is therefore, necessary to estimate the broad-sense heritability in conjunction with the genetic advance. In the present study high heritability estimates for leaf breadth of the fifth leaf, leaf angle of the fifth leaf, leaf length and breadth ratio of the fifth leaf, leaf length of the fifth leaf, dry weight of the pluckable shoots, leaf area of the pluckable shoots, fresh weight of the pluckable shoot and angle of the first branch were associated with high to moderate genetic advance as percentage of mean, suggested that these characters are more influenced by the environment and improvement of these genotypes could be practiced following simple selection methods in future breeding strategy. The findings reported by Desai and Jaimini (1997), Chaudhary (1985), Pandita and Sidhu (1981), Pandita *et al.* (1980), Sidhu and Pandita (1979) and Metin (1985) were also in agreement with the present results. High heritability does not necessarily mean that the character will show high genetic advance. However, whenever this association exists, it is important from the breeding point of view. High heritability but high genetic advance (% of mean) for length breadth of the fifth leaf in the present study suggested that there is less scope for further improvement by selection for this trait. Similar results have also been reported by Desai and Jaimini (1997), Swaminathan and Pushkarnath (1962) and Gaur *et al.* (1978) in potato. They also reported low genetic advance for tuber yield/plant and number of stems/hill which is in agreement with the result of present study.

Chaudhary (1985) in another study found low heritability and low amount of genetic advance for tuber dry matter content, which is in contrast with the results of the present study.

Thus the results of the present study indicated that leaf area of the fifth leaf, leaf angle of the fifth leaf and angle of the first branch exhibited high GCV (%), high heritability as well as high GA (% of mean).

2.4.4. Correlation Coefficient

All quantitative characters included in this research are not yield contributing characters. Therefore, it is important to know that which quantitative character is treated as yield contributing character. Ridge estimator is a biased estimator for linear regression model can be used to overcome such problem. For correlation studies, five yield contributing characters among 14 quantitative characters used in this study, were selected through forward Ridge regression analysis using the model proposed by Hoerl and Kennard (1970a, b). The correlation coefficient between pluckable shoot yield/plant and its component characters and among various components themselves were estimated at genotypic and phenotypic levels. It was revealed that in most of the cases, the values of genotypic correlation coefficient were higher than the corresponding phenotypic correlation coefficients indicating less pronounced environmental effect. Lower phenotypic correlation coefficients than genotypic correlation coefficients indicate that both environmental and genotypic correlations in those cases act in same direction and finally maximize their expression at phenotypic level. Among different characters studied total pluckable shoot yield/plant is found to be positively and significantly associated at genotypic level with height of the plucking surface, length of the plucking surface, breadth of the plucking surface and shoot density and also strong positive significant association among themselves. As yield is the ultimate goal, the positive association of these characters will help for selecting best individual. Similar results were also been reported by Desai and Jaimini (1998), Patel *et al.* (1973), Gaur *et al.* (1978), Pandita and Sidhu (1980) and Garg and Bhutani (1991) in potato. Significant negative association of height of the plucking surface with angle of the first branch was observed at both genotypic and phenotypic level.

Significant positive association of height of the plucking surface with length of the plucking surface; length of the plucking surface with breadth of the plucking surface, angle of the first branch and shoot density as well as breadth of the plucking surface with angle of the first branch at both genotypic and phenotypic were observed in the study that was in agreement with previous findings of Gaur *et al.* (1978), Pandita and Sidhu (1980), Patel *et al.* (1973), Singh *et al.* (1979) and Verma *et al.* (1975) in potato.

2.4.5. Path Coefficient

Path coefficient analysis at genotypic and phenotypic level were estimated. At genotypic level the direct effect revealed that the characters, viz. height of the plucking surface, length of the plucking surface and shoot density having positive correlation with total pluckable shoot yield/plant also had direct positive influence on yield, suggesting thereby good scope for improvement of yield/plant by selecting high shoot density tea bush with wide branching pattern. These findings are in agreement with previous report of Dogra and Paul (2002). They also noticed that high density plucking points can be obtained by increasing secondary branches, surface area of the bush and length of growing shoots, thereby signifying the importance of these characters for indirect selection.

Path coefficient values based on phenotypic correlation revealed that height of the plucking surface, length of the plucking surface, angle of the first branch and shoot density had direct positive effect towards total pluckable shoot yield/plant also having positive correlation with total pluckable shoot yield/plant. Therefore, proper attention should be taken on above characters for the improvement of the total pluckable shoot yield/plant. The present investigation on correlation and path analysis suggested that during selection more emphasis should be given on angle of the first branch, height of the plucking surface, length of the plucking surface, breadth of the plucking surface and shoot density, since these characters, have high correlation and high direct effect on total pluckable shoot yield/plant.

2.4.6. Genetic Diversity Analysis

2.4.6.1. Morphological diversity and principal component analysis (PCA)

The variation in morphological characters of 12 tea genotypes is summarized in **Table 2** by applying statistical methods. Multivariate statistical techniques such as PCA and cluster analysis are commonly used methods for characterisation and genetic diversity analysis of germplasm and can increase the accuracy of interpretation of information generated in characterization studies (Piyasundara *et al.*, 2008). Characters were chosen with respect to variations among taxa mentioned in the literature and also based on careful observation of specimens. The PCA results on morphological characters showed that the first three components accounted for 46.92% of the total variance in the dataset (**Table 2**). The principal component of single trait accounted for 26.02% of the total variance and was highly interpretable (Cronbach's alpha = 0.80). If the alpha value of a specific component is high, it is interpreted as indicating that the component has a strong one dimensional structure, or the dimension can reliably account for the total variance. Generally, an alpha value of 0.70 or greater is considered to be reliable (Bland *et al*., 1997). Phenotypic data that had high contributing component loadings were from such characters as leaf breadth of the fifth leaf (0.87) followed by dry weight of the pluckable shoots (over 0.70), leaf length of the fifth leaf, leaf angle of the fifth leaf and leaf area of the pluckable shoots (over 0.6).

A morphometrical analysis of leaf morphology is a useful and rapid method for identification of species (Pi *et al.*, 2009). Morphometric studies on *Taxus* (Taxaceae), a taxonomically complex genus with many sterile specimens like *Camellia*, showed that leaf characters are a powerful tool in separating and identifying species in this morphologically labile plant group (Moller *et al.*, 2007 and Shah *et al.*, 2008). Pi *et al.* (2009) investigated 54 species of *Camellia*. In their study, PCA results accounted for 63.2 and 20.6% of the total variance for component 1 and component 2 respectively. The sum of the two components accounted for most of the total variance, whereas in this study, 46.92% of the total variance was observed in the data set with a sample size of 12 clones. In their report, the average values of lamina vertical length, horizontal width, width-length ratio, leaf area and leaf veins were transformed before they were used for PCA. The results of this study showed that the quantitative characters with high component loadings are in conformity. Su *et al.* (2007) compared

morphological characters of *Camellia sinensis* (*formosensis*) and two closely related taxa of Taiwan native wild tea plants using numerical methods. In their studies, characters with high loadings were bud pubescence, young branchlet pubescence, abaxial midrib pubescence and petiole pubescence (over 0.7). Thus, significant values of the Cronbach's apha and character component loadings in this investigation are on a par with their findings. Similarly, Hu (2004) used 15 leaf characters measured on a tea germplasm collection of Taiwan to evaluate inter-taxa variations among *C. sinensis* var. *sinensis*, *C. sinensis* var. *assamica* and *C. sinensis* var. *formosensisi*.

2.4.6.2. Cluster analysis

A dissimilarity matrix based on Euclidian distances for the 12 tea genotypes is presented in **Table 2.27**. The clones exhibited large variation between and within the genotypes. The estimated dissimilarity of 11.1% was the highest between the clones MZ/39 and Ph/9/40 while the least dissimilarity (4.5) was between two clones, namely B2×T1 and E/4. The clustering of tea clones is also in congruence with a recent report of Rajanna *et al.*, (2011) based on morphological markers.

A phenogram based on the Euclidian distances from the morphological data divides the tea genotypes in to two major clusters, viz. C1 and C2 (**Figure 2.2**). C1 is consisted of maximum seven genotypes, viz., MZ/39, E/4, B2×T1, SDL/1, BT2, D/13 and BR2/97. On the other hand, C2 has five genotypes which are Ph/9/4, BT5, Ph/9/40, BS-67 and Ph/9/25. Thus, C1 has two sub-cluster S_1C_1 and S_1C_2, which are consisted five and two genotypes, respectively. Sub-cluster S_1C_1 is consisted of MZ/39, E/4, B2×T1, SDL/1 and BT2 while S_1C_2 is comprised of D/13 and BR2/97 genotypes. Similarly, C2 has two sub-cluster S_2C_1 and S_2C_2, which are involved four and single genotypes, respectively. Sub-cluster S_2C_1 is contained of Ph/9/4, BT5, Ph/9/40 and BS-67 genotypes, while S_2C_2 has single genotype Ph/9/25 only. Vo *et al.*, (2006), Su *et al.*, (2007), Rajkumar *et al.*, (2010) and Rajanna *et al.*, (2011) studied the morphological diversity of tea and produced similar results in the grouping of some clones.

2.5. SUMMARY

In the present study 15 quantitative characters viz., angle of the first branch (AFB), height of the plucking surface (HPS), length of the plucking surface (LPS), breadth of the plucking surface (BPS), leaf length of the fifth leaf (LLFL), leaf breadth of fifth leaf (LBFL), leaf length and breadth ratio of the fifth leaf (LL/LBFL), leaf angle of the fifth leaf (LAFL), pluckable shoot length (PSL), fresh weight of the pluckable shoots (FWPS), dry weight of the pluckable shoots (DWPS), fresh and dry weight ratio of pluckable shoots (FW/DWPS), leaf area of the pluckable shoots (LA), shoot density (SD) of pluckable shoots and total pluckable shoot yield/plant (Y) were studied in 12 different tea genotypes (e.g., MZ/39, E/4, D/13, B2×T1, Br/2/97, SDL/1, BT2, Ph/9/4, Ph/9/25, Ph/9/40, BS-67 and BT5) in order to find out genetic variability, coefficients of variability, heritability, genetic advance as % of mean, correlation coefficient, path analysis and genetic diversity among the tea genotypes.

The experimental results reveals that the range of variation was very pronounced for all the characters. The presence of wide range of variation of these characters indicated that they are quantitative in nature and are under polygenic control.

Mean values were highly significant with their respective standard error suggesting that the genotypes were genetically different from each other for all characters, which justified their inclusion as materials in the present work. The significant mean differences between the genotypes of particular character varied from genotype to genotype.

The analysis of variance shows that the genotype item was highly significant for all the characters when it was tested against within error. Again it was highly significant for all the characters when it was tested against pooled error also. These results indicate that genotypes were significantly and genotypically different from each other and it justifies their inclusion in the present investigation as material.

The different component of variation varied differently in different characters. Phenotypic component of variation (σ^2_p) was higher than genotypic (σ^2_g), interactions (σ^2_{GR}) and error (σ^2_w) component of variation. The difference between phenotypic and genotypic variation were greater in magnitude for angle of the first branch, length of

the plucking surface, breadth of the plucking surface, fresh weight of the pluckable shoots, leaf area of the pluckable shoots, shoot density and pluckable shoot yield/plant which indicated that the environment had considerable effect on these characters. In the present study, the highest phenotypic and genotypic variations were observed for total pluckable shoot yield/plant followed by leaf area of the pluckable shoot, leaf angle of the fifth leaf, shoot density, fresh weight of the pluckable shoots, angle of the first branch, length of the plucking surface and breadth of the plucking surface. The pronounced environmental variation indicated that greater portion of the phenotypic variation was environmental in nature. The characters total pluckable shoot yield/plant, leaf area of the pluckable shoot, shoot density, length of the plucking surface, breadth of the plucking surface, angle of the first branch and leaf angle of the fifth leaf showed the highest value for σ^2_w component and shoot density showed highest value for σ^2_{GR} component which indicated better scope for improvement of those character through selection. On the other hand, rest of the characters for σ^2_w and σ^2_{GR} showed the lowest values in the present materials indicating difficulties in improvement of these traits through selection.

In this study phenotypic coefficient of variability (PCV) was greater than all other genotypic coefficient of variability (GCV). The difference between PCV and GCV were greater in magnitude for length of the plucking surface, breadth of plucking surface, leaf area of the pluckable shoot, shoot density, angle of the first branch and total pluckable shoot yield/plant which indicated that environment had considerable effect on these characters. The highest amount of PCV, GCV, G × RCV and ECV were observed for shoot density indicating wide scope of selection for this trait. The highest values of PCV, GCV and ECV were also recorded for total pluckable shoot yield/plant, leaf area of the fifth leaf, leaf angle of the fifth leaf, angle of the first branch and breadth of the plucking surface, but the G × RCV was negative value for those traits. These results suggest that the greater variability for those characters among the genotypes was due to genetic cause which are less affected by environment and hence could be improved through selection.

Heritability estimates in broad sense were relatively high for almost all the characters studied except height of the pluking surface, length of the plucking surface, breadth of

the plucking surface, pluckable shoot length, fresh and dry weight ratio, shoot density and total pluckable shoot yield/plant.

In the present study high heritability estimates for leaf breadth of the fifth leaf, leaf angle of the fifth leaf, leaf length and breadth ratio of the fifth leaf, leaf length of the fifth leaf, dry weight of the pluckable shoots, leaf area of the pluckable shoots, fresh weight of the pluckable shoot and angle of the first branch were associated with high to moderate genetic advance as percentage of mean, suggested that these characters are more influenced by the environment and improvement of these genotypes could be practiced following simple selection methods in future breeding strategy.

Forward Ridge regression analysis was done on 14 quantitative characters in order to select most important yield contributing characters. As per Ridge regression analysis 5 characters viz., height of the plucking surface, length of the plucking surface, breadth of the plucking surface, angle of the first branch and shoot density were selected for correlation and path coefficient analysis.

Total pluckable shoot yield/plant was positively and significantly associated at genotypic as well as phenotypic level with height of the plucking surface, length of the plucking surface, breadth of the plucking surface and shoot density. So, selection on the basis of these characters should get preference for future breeding programme.

Path coefficient analysis using genotypic correlation revealed that height of the plucking surface, length of the plucking surface and shoot density had direct positive influence on total pluckable shoot yield/plant. These characters also show positive correlation with total pluckable shoot yield/plant. At phenotypic level height of the plucking surface, length of the plucking surface, angle of the first branch and shoot density showed direct positive effect on total pluckable shoot yield/plant and also positively correlated with total pluckable shoot yield/plant. Therefore, the present investigation on correlation and path coefficient analysis suggested that during selection more emphasis should be given on height of the plucking surface, length of the plucking surface, shoot density and angle of the first branch.

The results of principal component analysis (PCA) on morphological characters in the present study reveals that the first two principal components accounted for 46.92% of the total variance.

Cluster analysis shows that the 12 tea genotypes used in the study could be grouped in two clusters (C1 and C2). Cluster 1 was consisted of seven genotypes, viz., MZ/39, E/4, B2×T1, SDL/1, BT2, D/13 and BR2/97. On the other hand, Cluster 2 has five genotypes viz., Ph/9/4, BT5, Ph/9/40, BS-67 and Ph/9/25. The Cluster 1 had two sub-cluster S_1C_1 and S_1C_2, which were consisted of five and two genotypes, respectively. Similarly, Cluster 2 had two sub-cluster S_2C_1 and S_2C_2, which were consisted of four and single genotypes, respectively.

In conclusion the present study suggested that during the selection more emphasis should be given on the characters of height of the plucking surface, length of the plucking surface, breadth of the plucking surface, shoot density and angle of the first branch. On the other hand, MZ/39 was found to be more promising clone for the tea industry, although BT2, BT5 and B2×T1 (recently released as BT17) are existing as commercial tea clone in the industry.

Chapter 3

ANALYSIS OF BIOCHEMICAL PROPERTIES OF TWELVE TEA GENOTYPES

3.1. INTRODUCTION

The key objective of the current chapter is to perform a comprehensive biochemical profiling of the tea varieties of Bangladesh to make it as an authenticated product to the world tea market. Tea polyphenols (catechins), caffeine, amino acids, and water extract (including inorganic elements, carbohydrates, lipids, etc) are the most important components for tea quality (Friedman, 2009). Owuor (1986) and Magoma (2000) reported the differences in caffeine and tannin contents between tea cultivars preserved in Japan national tea germplasm gardens. However, there is no systematic investigation of the main quality components of BTRI tea genetic resources, although there are some reports of improved clones. Till now the quality of tea is judged only on the basis of tea tasting, yield attributes and good appearance of the prepared tea. Therefore, there is an utmost importance of the biochemical characterization of the tea cultivars in Bangladesh. Study described in the current chapter will not only enrich our tea resource but also will give authenticity of our tea to the foreign importers.

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).

3.1.1. Chemical Composition of Tea Leaves

The chemical composition of tea is complex: polyphenols, alkaloids (caffeine, theophylline, and theobromine), amino acids, carbohydrates, proteins, chlorophyll, volatile compounds, fluoride, minerals and trace elements, and other undefined compounds. Among these, the polyphenols constitute the most interesting group of tea leaf components and exhibit potent antioxidant activity in vitro and in vivo (Wu *et al.*, 2002). Tea is reported to contain nearly 4000 bioactive compounds of which one third is contributed by polyphenols (Mahmood *et al.*, 2010). Polyphenols are bonded benzene rings with multiple hydroxyl groups. Polyphenols are either flavonoids or non-flavonoids but chemicals found in tea are mostly flavonoids (Sumpio *et al.*, 2006). They are secondary metabolites derived from the condensation reaction of cinnamic acid with three malonyl-CoA groups. A number of flavonoids are present but dietary flavonoids are usually categorized into six major groups (Yilmaz, 2006) (**Table 3.1**).

Table 3.1 Classes of flavonoids.

Flavonoids	**Example**
Flavanols	EGCG, EG, ECG and Catechin
Flavonols	Kaempferol and Quercetin
Anthocyanidins	Malvidin, Cyanidin and Delphinidin
Flavones	Apigenin and Rutin
Flavonones	Myricetin
Isoflavonoids	Genistein and Biochanin A

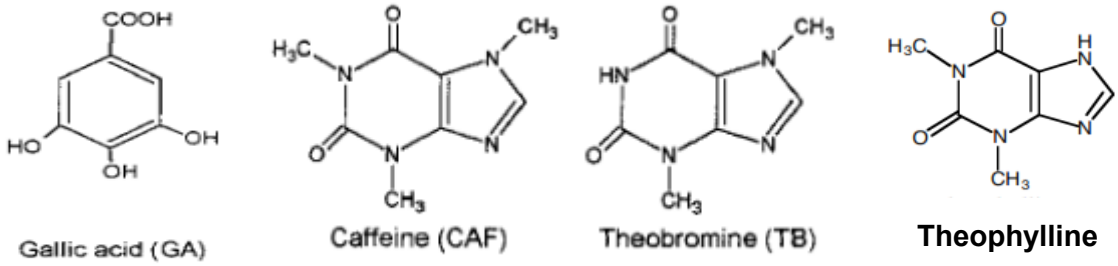


Figure 3.1 Structure of Gallic Acid and some Alkaloids (Caffeine, Theobromine and Theophylline) of tea.

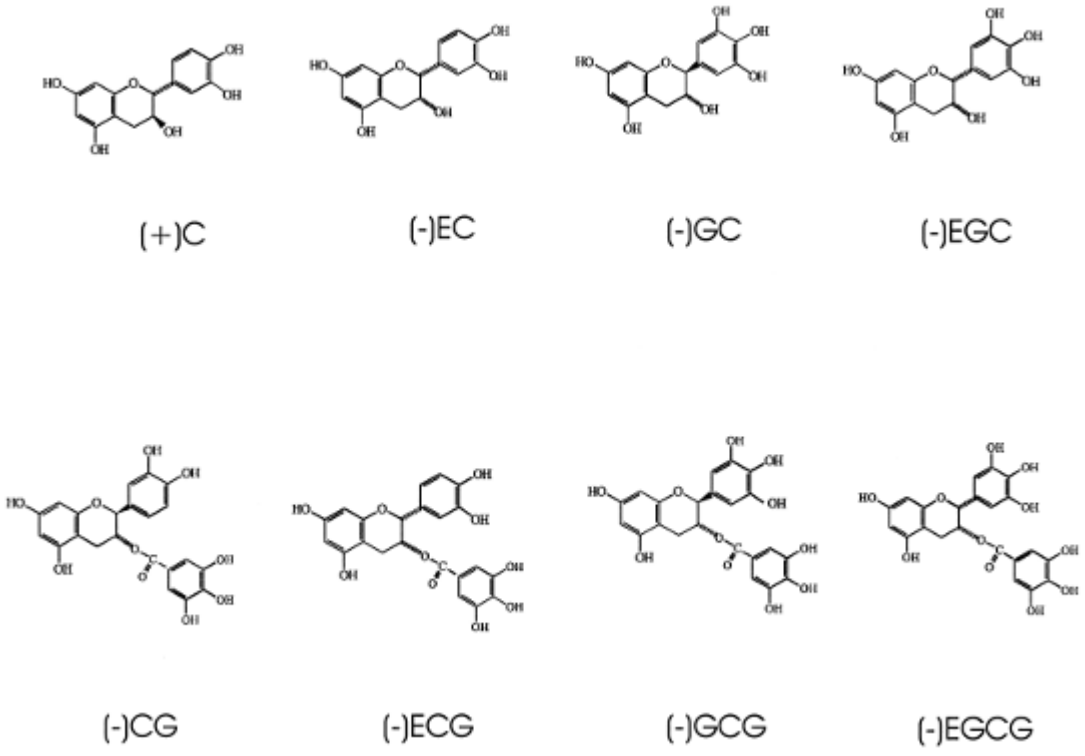


Figure 3.2 Structures of eight catechins from green tea. (+)-Catechin (C); (-)-epicatechin (EC); (-)-gallocatechin (GC); (-)-epigallocatechin (EGC); (-)-catechin gallate (CG); (-)-epicatechin gallate (ECG); (-)-gallocatechin gallate (GCG); (-)-epigallocatechin gallate (EGCG).

3.1.2. Tea Processing and its Relation to Biochemical Compounds

Depending on the manufacturing process, teas are classified into three major types: non-fermented green tea (produced by drying and steaming the fresh leaves and thus, no fermentation, i.e., oxidation, occurs); semi-fermented oolong tea (produced when the fresh leaves are subjected to a partial fermentation stage before drying); and fermented black and red (pu-erh) teas (which undergo a full fermentation stage before drying and steaming, although the fermentation of black tea is oxidation and that of pu-erh tea is attained using microorganisms) (Zuo *et al.* 2002). A flow chart of different tea processing is presented in **Figure 3.3**.

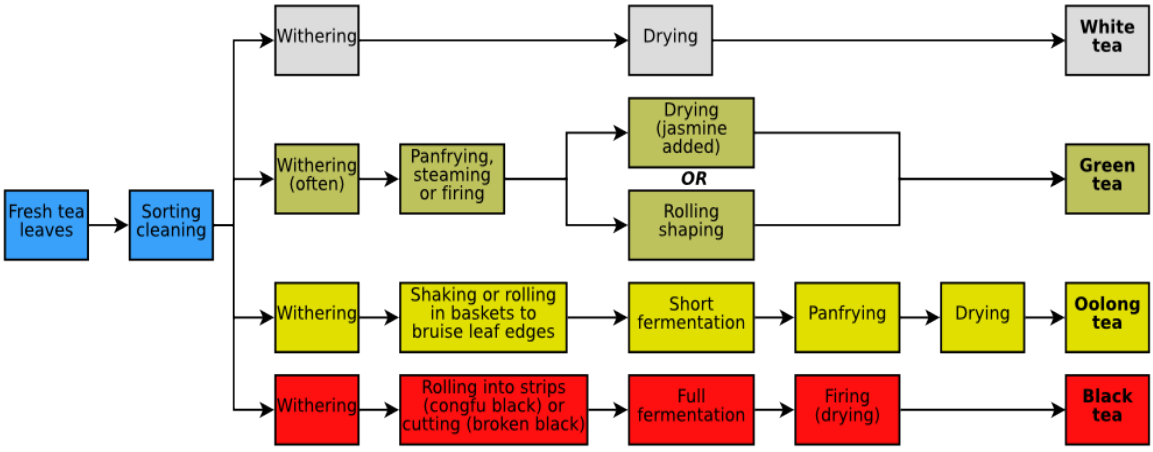


Figure 3.3 A schematic diagram of different tea processing (Adopted fromWikipedia)

3.1.3. Biochemical Changes during the Black Tea Processing

Biochemical constituents such as polyphenols, catechins and polyphenol oxidase (PPO) of tea are intrinsically related to black tea quality. In recent years, knowledge on biochemical components and the changes that take place during oxidation (fermentation) has increased noticeably. Biochemical analysis has been generally constrained to the flush since it is the young crop shoots that are plucked and processed to produce black tea with distinctive aroma (Millin and Rustridge, 1967).

Cut, tear and curl (CTC) type of black tea manufacture involves withering, cutting, oxidation and drying. During withering, a decline in the moisture content and a slight augment in the substrate as well as enzyme levels are noticed. The overall quality and flavour of the final black tea depends on moisture content and the changes in the

biochemical constituents that occurs during withering of the leaves. After withering, the leaves pass through ‘‘rotor vane’’, where the leaves are cut into pieces. Sliced leaves are then cut four times in ragged stainless steel rollers revolving oppositely. During oxidation major biochemical changes takes place, leading to the formation of quality constituents and the characteristic flavour of black tea. Thus a crucial stage is influenced by factors such as temperature, humidity, oxygen level, time and finally the biochemical constituents of macerated leaf (dhool). After optimal oxidation of leaves, it is subjected to firing at 120^0C for 10–20 min. During this stage, the enzyme activity is blocked and almost completes removal of moisture results.

During oxidation, PPO interacts with phenolic compounds mainly catechins and their fractions such as epicatechin (EC), epicatechin gallate (ECG), epigallo catechin gallate (EGCG) and epigallo catechin (EGC) in the presence of oxygen which results in the development of golden yellow theaflavins (theaflavin, theaflavin-3 gallate, theaflavin-3′ gallate and theaflavin-3-3′ gallate) a product of condensation reaction between two molecules of *o*-quinones (Owuor *et al.*, 1998; Madanhire *et al.*, 1996).To comprehend the formation of quality constituents from potential fresh tea leaves, it is necessary that the enzyme completely converts the available substrates into products. PPO is generally found in association with chloroplast (Kato *et al.*, 1976) and in the epidermal cells (Oparin *et al*., 1950) due to its role in the initial oxidation of catechins to *o*-quinones which later, condenses into quality constituents (Wickramasinge *et al.*, 1967). Polyphenols and catechins are mainly localized in the vacuoles and palisade cells of leaves (Mahanta, 1988). During withering, moisture content of the leaves is reduced to about 60%. During cutting and oxidation, the intact cells of tea leaves get ruptured and the substrates (polyphenols and catechins) come in contact with the vacuolar enzyme. This results in the formation of quality constituents of black tea (Selvendran *et al*., 1976). Since biochemical constituents influence the black tea quality, we have attempted to identify superior quality clones using the bio-constituents of tea leaves prior to black tea manufacture.

3.1.4. Rationale and Objectives

Self incompatibility, long term of allogamy, and artificial selection make tea plant of high heterogeneity and broad genetic variation (Chen *et al.*, 1998). Tea genetic resources are

presently one of the most valuable and fundamental materials for breeding, biotechnology, with valuable potential for the whole tea industry in the future. A great number of tea genetic resources have been collected and preserved in China (Chen *et al.*, 2002), Japan (Takeda, 2000), India, and Kenya, etc. Bangladesh Tea Research Institute (BTRI), has been focusing on tea genetic resources collection, conservation and evaluation using multidisciplinary approaches and utilization. About 516 accessions genetic resources from the tea growing area of Bangladesh and around the world have been collected and preserved in the germplasm of BTRI (Anonymous, 2011).

The polyphenols (catechins), caffeine, amino acids, and water extract (including inorganic elements, carbohydrates, lipids, etc) are the most important components for tea quality (Yamamoto *et al.*, 1998). Takeda (1994) reported the differences in caffeine and tannin contents between tea cultivars preserved in Japan national tea germplasm gardens. However, there is no systematic investigation of the main quality components of BTRI tea genetic resources, although there are some reports of improved clones.

In this chapter, the presence of tea components with antioxidant activity were being determined. The individual proportions of the catechins could be important in the determination of tea quality and genetic diversity (Owuor and McDowell, 1994). Therefore, there is need to study the relative expression of the individual tea catechins in different tea genotypes. The objectives of this part of the present study are to evaluate the cup quality and to analyze the different biochemical components related with organoleptic as well as nutritional quality of 12 tea genotypes of BTRI.

In this chapter, the experiment was carried out in depth biochemical characterization of 12 tea genotypes in Bangladesh. This investigation includes evaluation of the tea quality through organoleptic test, determination of total polyphenol, antioxident activity, determination and quantification of different biochemical compounds (gallic acid, theobromine, theophylline, epigallocatechin, caffeine, catechin, epicatechin, epigallocatechin gallet, gallocatechin gallet, epicatechin gallet and catechin gallet) using HPLC.

3.2. MATERIALS AND METHODS

3.2.1. Plant Materials

Tea shoots (two or three leaves and a bud) of 12 different cultivars coded as MZ/39, E/4, D/13, B2×T1, Br/2/97, SDL/1, Ph/9/4, Ph/9/25, Ph/9/40, BS-67, BT2 and BT5 were plucked from tea bushes at Institute's Tea Experimental Farm at BTRI, Srimangal, Bangladesh (altitude 21.95 m above the mean sea level).

3.2.2. Methods

3.2.2.1. Estimation of the cup quality of the tea by organoleptic test

Tea shoots consisting of two leaves and an active bud (500 g) were harvested from 20 bushes of each accession and black tea was produced in an environmental-controlled manufacturing system designed by Teacraft. Harvested shoots were withered in a withering cabinet. Withered leaves, after reaching the optimum withering stage (14 to 18 hours), were rolled for 15 minutes in a miniature roller and fed into a miniature spiral rotorvane to achieve a fine dhool. The macerated dhool was sieved through a 2.5 mm mesh and placed in a cabinet for a period of optimum fermentation time (2 ½ to 3 hours) at 20^0C. The inlet temperature of the miniature fluid bed dryer was set at 125^0C and fermented dhool was fed to it. The drying step was terminated when the outlet temperature reached 98^0C.

Made tea samples (20 gm from each accession) were sent for tasters' evaluation. Sensory evaluation made by two professional tea tasters with scores based on infused leaf colour, colour of liquor, briskness, strength and creaming down (thickening of the liquor) was used to determine the parameters related to quality of made tea. Each parameter was scored out of 10. Thus, the total tea tasting score was scored out of 50. The tea which was got total score 34 or above, 32 to below 34, 30 to below 32 and below 30, were remarked as excellent (E) tea, above average (AA) tea, average (A) tea and below average (BA) tea, respectively.

3.2.2.2. Biochemical analyses

Preparation of tea leaf extraction by sonication

Duration of the extraction: 2 days

Solvent used: 100% Methanol (Merck, Germany)

Volume of methanol used: 800ml

Apparatus:

- 12 conical flask for each tea genotype
- 12 glass beaker
- Ultrasound sonication Bath
- Rotary evaporator
- Filter paper (Whatmann 1)
- Aluminium foil

Procedure:

- A conical flask with aluminium foil cover was taken and washed thoroughly. The flask was rinsed with methanol and dried
- Then the fresh tea leaves (50 gm) was taken into the flask.
- After that 100% methanol (800 ml) was poured into the flask up to 1-inch height above the sample surface as it can sufficiently cover the sample surface.
- Then the flask with tea leaf sample was put onto the ultrasound sonication bath at 50^0C for 15 minutes.
- After sonication the soaked samples were rested for overnight.
- Then again sonication was performed as above and rested for overnight.
- After that the soaked sample was filtered and evaporated by rotary evaporator at 50^0C.
- The collected sample was taken to glass beaker.
- The tea leaf extract was concentrated by evaporating the solvent using a water bath at a temperature of 60^0C and kept under room temperature for further evaporation.

The above procedure was applied similarly for the above mentioned all fresh tea leaves samples of the tea cultivars.

3.2.2.3. Determination of total phenol (Velioglu et al., 1998)

Principle:

The content of total phenolic compounds in plant methanolic extracts was determined by Folin–Ciocalteu Reagent (FCR). The FCR actually measures a sample's reducing capacity. The exact chemical nature of the FC reagent is not known, but it is believed to contain heteropolyphosphotunstates - molybdates. Sequences of reversible one- or two-electron reduction reactions lead to blue species, possibly $(PMoW_{11}O_{40})^{4-}$. In essence, it is believed that the molybdenum is easier to be reduced in the complex and electron-transfer reaction occurs between reductants and Mo(VI):

$$\mathbf{Mo(VI) + e \rightarrow Mo}$$

Reagents:

- ✓ Folin – ciocalteu reagent (Sigma-Aldrich, India)
- ✓ Sodium carbonate (Na_2CO_3) (Sigma chemical company, USA)
- ✓ Methanol (Merck, Germany)
- ✓ Galic acid (Analytical or Reagent grade) (Wako pure chemicals LTD., Japan)

Experimental procedure:

1. 1.0 ml of plant extract or standard of different concentration was taken in a test tube.
2. 5 ml of Folin – ciocalteu (Diluted 10 fold) reagent solution was added into each test tube.
3. 4 ml of Sodium carbonate solution was added into the test tube.
4. For standard test tubes were incubated for 30 minutes at 20^0C to complete the reaction.
5. In case of test samples test tubes were incubated for 1 hour at 20^0C to complete the reaction.
6. Then the absorbance of the solution was measured at 765 nm using a spectrophotometer against blank.
7. A typical blank solution contained ethanol or methanol.

8. The Total content of phenolic compounds in plant methanol extracts in gallic acid equivalents (GAE) was calculated by the following formula equation C = (c x V)/m

   Where:

   C = total content of phenolic compounds, mg/g plant extract, in GAE;

   c = the concentration of gallic acid established from the calibration curve, mg/ml;

   V = the volume of extract, ml;

   m = the weight of pure plant methanolic extract, g.

3.2.2.4. Determination of flavonoid content (Wang et al., 2000)

Reagents:

- ✓ Aluminium Chloride ($AlCl_3$)
- ✓ Potassium Acetate
- ✓ Methanol (Merck, Germany)
- ✓ Quercetin (Analytical or Reagent grade)

Experimental procedure:

1. 1.0 ml of plant extract or standard of different concentration solution was taken in a test tube.
2. 3 ml of methanol was added into the test tube.
3. Then 200µl of 10% aluminium chloride solution was added into the test tube.
4. 200µl of 1M potassium acetate solution was supplemented into the test tube.
5. 5.6 ml of distilled water was added into the test tube.
6. Then the test tube was incubated for 30 minutes at room temperature to complete the reaction.
7. Then the absorbance of the solution was measured at 415 nm using a spectrophotometer against blank.
8. A typical blank solution contained methanol.
9. The Total content of flavonoid compounds in plant methanol extracts in quercetin equivalents was calculated by the following formula equation C = (c x V)/m

Where:

C = total content of flavonoid compounds, mg/g plant extract, in quercetin;

c = the concentration of quercetin established from the calibration curve, mg/ml;

V = the volume of extract, ml;

m = the weight of pure plant methanolic extract, g.

3.2.2.5. Determination of total antioxidant capacity

Principle:

The phosphomolybdenum method usually detects antioxidants such as ascorbic acid, some phenolics, α-tocopherol, and carotenoids. The phosphomolybdenum method (Prieto *et al.*, 1999) was based on the reduction of Mo(VI) to Mo(V) by the antioxidant compound and subsequent formation of a green phosphate/Mo(V) complex at acid pH.. In essence, it is believed that the molybdenum is easier to be reduced in the complex and electron-transfer reaction occurs between reductants and Mo(VI) and the formation of a green phosphate/Mo(V) complex with a maximal absorption at 695 nm.

$$\mathbf{Mo(VI) + e \rightarrow Mo}$$

Reagents:

- ✓ Concentrated H_2SO_4 (98%)
- ✓ Sodium Phosphate (Na_3PO_4)
- ✓ Ammonium Molybdate
- ✓ Ascorbic acid (Analytical or Reagent grade)

Experimental procedure:

1. 300µl of leaf extract or standard of different concentration solution was taken in a test tube.
2. 3 ml of reagent solution was added into the test tube.
3. the test tube was incubated at 95^0C for 90 minutes to complete the reaction.
4. Then the absorbance of the solution was measured at 695 nm using a spectrophotometer against blank after cooling to room temperature.

5. A typical blank solution contained 3 ml of reagent solution and the appropriate volume (300µl) of the same solvent used for the sample, and it was incubated under the same conditions as the rest of the samples solution.
6. The antioxidant activity is expressed as the number of equivalents of ascorbic acid .and was calculated by the following formula equation A = (c x V)/m

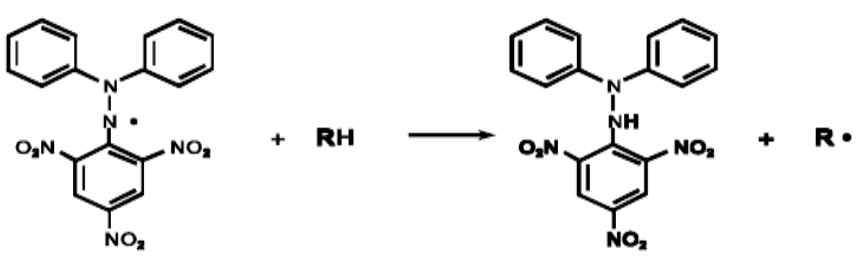
Where:

A = total content of Antioxidant compounds, mg/g plant extract, in Ascorbic acid;
c = the concentration of Ascorbic acid established from the calibration curve, mg/ml;
V = the volume of extract, ml;
m = the weight of pure plant methanolic extract, g.

3.2.2.6. DPPH (1,1-diphenyl-2-picrylhydrazyl) scavenging capacity assay (Brand-Williams et al., 1995)

Principle:

The 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) has been widely used to evaluate the free radical scavenging capacity of antioxidants. DPPH free radical is reduced to the corresponding hydrazine when it reacts with hydrogen donors. DPPH can make stable free radicals in aqueous or methanol solution. With this method it was possible to determine the antiradical power of an antioxidant activity by measurement of the decrease in the absorbance of DPPH at 519 nm. Resulting from a color change from purple to yellow the absorbance decreased when the DPPH was scavenged by an antioxidant, through donation of hydrogen to form a stable DPPH molecule. In the radical form this molecule had an absorbance at 519 nm which disappeared after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule.



DPPH
517 nm

Reagents:

- ✓ DPPH (1,1-diphenyl-2-picrylhydrazyl) (Sigma Aldrich, India)
- ✓ Methanol (Merck, Germany)
- ✓ Butylated hydroxy toluene (BHT) (Analytical or Reagent grade) (Merck, Germany)

Experimental procedure:

1. 200µl of plant extract or standard of different concentration solution was taken in a test tube.
2. 2 ml of reagent solution was supplemented into the test tube.
3. Then the test tube was incubated for 30 minutes to complete the reaction.
4. Then the absorbance of the solution was measured at 519 nm using a spectrophotometer against blank.
5. A typical blank solution contained ethanol or methanol.
6. The percentage (%) inhibition activity was calculated from the following equation

$$\%I = \{(A_o - A_1)/A_o\} \text{ X } 100$$

Where,

A_0 is the absorbance of the control, and

A_1 is the absorbance of the extract/standard.

7. Then % inhibitions were plotted against log concentration and from the graph IC_{50} was calculated.

3.2.2.7. Analysis of major tea alkaloids and catechin fraction using Reverse Phase High Pressure Liquid Chromatography (RP-HPLC)

Chromatography is an excellent technique for separating components form a mixture. Among different chromatographic techniques in our present investigation Reverse Phase High Pressure Liquid Chromatography (RP-HPLC) was used.

Gallic acid (GA) and major tea alkaloids viz., theobromine (TB), theophylline (TP), caffeine (CAF) as well as catechin fractions viz., (-)- epigallo catechin (EGC), (+)- catechin (C), (-)- epicatechin (EC), (-)- epigallo catechin gallet (EGCG), (-)-

gallocatechin gallet (GCG), (-)- epicatechin gallet (ECG) and (-)- catechin gallet (CG) were analyzed using RP-HPLC (Waters HPLC system, USA consisting of two 515 HPLC pump, pump control unit and 2489 UV/detector). The standards of gallic acid, theobromine, theophylline, caffeine, (-)- epigallo catechin, (+)- catechin, (-)- epicatechin, (-)- epigallo catechin gallet, (-)- gallocatechin gallet, (-)- epicatechin gallet and (-)- catechin gallet were obtain from Sigma-Aldrich, USA.

For reverse phase HPLC, Phenomenex C18 column (CA, USA) (Particle size: 5µm, Pore size: 300 Å, Dimension: 250 mm × 10 mm) was used as non-polar stationary phase. In mobile phase A contains 99.9% H_2O with 0.1% TFA and B contains 80% Acetonitrile with 0.1% TFA.

Preparation of buffer A and buffer B for reverse phase HPLC

One Liter of Buffer A was prepared using 999 ml of de-ionized water. 1 ml of TFA was added to it and mixed by swirling. It was then filtered through a 0.45µ PTFE membrane using a Sartorius vacuum buffer filtration system. Buffer was stored at room temperature until use. 80% acetonitrile was used as buffer B for the current experiment. To make one liter of buffer B 800 ml was acetonitrile was mixed with 199 ml of water and 1 ml of TFA was added to it. Buffer B was also filtered and stored in the same way as buffer A.

***Equilibration of the HPLC system and Column*:**

The whole system including the pump, tubing, and sample loop was washed with 100% buffer A. The C18 column was connected to the HPLC and first it was washed with 100% buffer B to remove any residual protein attached to it and until the base line monitored at 280nm was stable. Two Column volume (4ml × 2 = 8ml) of buffer B was needed to pass through the Column for complete washing. Then the column was equilibrated with three CV (4ml × 3 = 12 ml) of 100% buffer A. The flow rate was set as 1ml/min during equilibration. The equilibration was done until the base line became linear.

***Sample preparation and injection*:** 10 mg of each of the ethanol exteact of 12 cultivars were diluted in 10 ml of methanol and were filtered through 0.22 µm syringe

filter. 20µl of each of these extracts were injected into the HPLC system. Standards were also run in a volume of 20µl using the same HPLC protocol. 20µl sample loop was used in the experiments and the samples were subjected to C18 reverse phase HPLC column. Elution was monitored at UV absorption 280 nm.

***Gradient elution*:** For elution of proteins the concentration of buffer B was increased gradually. As the binding affinity of the proteins depends on the hydrophobicity, it is expected that the proteins which are loosely bound to the column (less hydrophobic and more polar) will elute in less amount of buffer B from the column whereas the more hydrophobic proteins will need more concentration of buffer B to release from the column, Initially 100% buffer is passed through the system and then gradually percentage of buffer A is decreased and buffer B is increased. Gradual increase of buffer B with the decrease of buffer A is maintained in a mixing chamber of HPLC system and pumped in the system through the column. For RP-HPLC purification of 12 tea cultivars we developed a protocol as follows:

100% A	: 1 CV
0 - 30% B	: 1 CV
30 - 80% B	: 5 CV
80 - 100%B	: 1 CV
100%B - 100%A	: 1CV
100% A	: 1CV
20% Ethanol	: 1 CV

3.3. RESULTS

In the present study 12 genotypes of tea [*Camellia sinensis* (L.) O. Kuntze] coded as MZ/39, E/4, D/13, B2×T1, Br/2/97, SDL/1, Ph/9/4, Ph/9/25, Ph/9/40, BS-67, BT2 and BT5 were screened for their cup quality by organoleptic evaluation, free radical scavenging and in vitro antioxidant properties. The antioxidant assay methods were total antioxidant capacity and DPPH (1, 1-diphenyl-2-picrylhydrazyl) scavenging capacity. In addition, the total phenolic and flavonoid contents of these genotypes were determined by comparing with gallic acid and quercetin respectively flowing Folin–Ciocalteu Reagent (FCR) and Flavonoid content determination method. Eleven phenolic and flavonoid standards were also compared with the chromatograms produced by the 12 tea genotypes extracts. The observed results are summarized in different tables and presented under different paragraph heads.

3.3.1. Cup Quality of the Selected 12 Tea Genotypes

The six qualititative characters viz., infusion, liquor colour, briskness, strength, creaming down and total tasting score were organoleptically tested on tea liquor prepared from black tea with boiling water. The results are shown in **Table 3.2**.

Infusion

It appears from the **Table 3.2** that there is no significant differences in infusion among the cultivars. The highest infusion value was observed in D/13 (7.58±0.16) and the lowest infusion value was noted in Ph/9/40 (7.10±0.13). The total coefficient of variability percentage was found to be very low.

Liquor colour

Table 3.2 reveals that there is no significant difference in liquor colour among the 12 cultivars. The brightest liquor colour was recorded in the genotype D/13 followed by Ph/9/4, BS-67, MZ/39, SDL/1 and E/4. On the other hand, the lowest value was shown by Ph/9/40 followed by Br2/97, BT5, B2×T1, BT2 and Ph/9/25. The total coefficient of variability percentage was recorded to be very low.

Briskness of the liquor

Table 3.2 shows that the highly significant difference in briskness of the liquor among the 12 tea genotypes. The highest brisk attribute was recorded for D/13 and

Ph/9/4 BT5, Ph/9/25, and BS-67. The lowest attribute of liquor briskness was recorded for Ph/9/40 followed by B2×T1, BT2, SDL/1, E/4, Br2/97 and MZ/39.
The total coefficient of variability percentage was very low.
The value of least significant differences at 5% and 1% level was 0.23 and 0.32, respectively.

Strength of the liquor

It appears from the **Table 3.2** that there was no significant difference in the strength of the liquor among the 12 tea clones. The highest mean of the strength attributes was found in BS-67 followed by D/13, Ph/9/4, BT5 and B2×T1. The lowest mean of the liquor strength was obtained by Ph/9/40 followed by Br2/97, Ph/9/25, BT2, SDL/1, E/4 and MZ/39.The total coefficient of variability percentage was found to be very low.

Creaming down of the liquor

Table 3.2 reveals the highly significant difference in creaming down of the liquor among the 12 cultivars of tea. The highest creaming down of the liquor was recorded for the genotype D/13 followed by the genotypes MZ/39, Ph/9/4, Br2/97 and SDL/1. The lowest attribute of creaming down was noted in the genotype Ph/9/25 followed by the genotypes Ph/9/40, BT2, B2×T1, E/4, BS-67 and BT5.

Total tasting score

The significant difference in total tasting score was found among the 12 genotypes of the tea (**Table 3.2**). Total tasting score was the highest in the genotype D/13 followed by Ph/9/4, MZ/39, BS-67, SDL/1, BT5 and E/4. The lowest of the total tasting score was recorded for Ph/9/40 followed by Ph/9/25, BT2, B2×T1 and Br2/97.
The total coefficient of variability percentage was 2.00.

The results in **Table 3.2** shows that teas made from the cultivar Ph/9/40 was average (A) quality whereas teas made from rest of the genotypes were all of above average (AA).

Table 3.2 Cup quality of the tea of 12 genotypes.

Genotypes	Infusion (10)	Liquor Colour (10)	Briskness (10)	Strength (10)	Creaming Down (10)	Total Tasting Score (50)	Remarks
MZ/39	7.38±0.29	7.58±0.15	7.48±0.23	7.38±0.25	3.12±0.06	32.95±0.89	AA
E/4	7.42±0.31	7.57±0.16	7.45±0.13	7.37±0.09	2.87±0.24	32.67±0.38	AA
D/13	7.58±0.16	7.75±0.08	7.60±0.15	7.55±0.10	3.25±0.11	33.73±0.42	AA
B2×T1	7.15±0.43	7.55±0.28	7.30±0.15	7.40±0.20	2.82±0.07	32.22±1.11	AA
Br2/97	7.20±0.10	7.47±0.10	7.47±0.14	7.23±0.06	3.05±0.13	32.42±0.21	AA
SDL/1	7.43±0.09	7.58±0.04	7.45±0.18	7.37±0.07	3.03±0.41	32.87±0.19	AA
BT2	7.23±0.10	7.55±0.14	7.40±0.10	7.32±0.07	2.52±0.29	32.02±0.10	AA
Ph/9/4	7.22±0.26	7.73±0.19	7.60±0.23	7.43±0.20	3.07±0.13	33.05±0.75	AA
Ph/9/25	7.17±0.31	7.55±0.20	7.53±0.22	7.27±0.11	2.45±0.16	31.97±0.68	AA
Ph/9/40	7.10±0.13	7.38±0.24	7.05±0.08	7.18±0.16	2.47±0.17	31.18±0.39	A
BS-67	7.27±0.16	7.67±0.16	7.52±0.04	7.58±0.10	2.88±0.21	32.92±0.25	AA
BT-5	7.35±0.21	7.50±0.33	7.55±0.09	7.43±0.70	2.93±0.09	32.77±0.52	AA
CV%	2.84	2.25	1.85	2.35	8.30	2.00	-
LSD P≤0.05	NS	NS	0.23	NS	0.40	1.10	-
LSD P≤0.01	NS	NS	0.32	NS	0.55	NS	-

AA = Above average, A = Average, NS = Non-significant

3.3.2. Analysis of Biochemical Properties of Selected Tea Genotypes

In the present study 12 tea genotypes (MZ/39, E/4, D/13, B2 × T1, Br/2/97, SDL/1, BT2, Ph/9/4, Ph/9/25, Ph/9/40, BS-67 and BT5) were screened for their free radical scavenging and in vitro antioxidant properties. The total phenolic and flavonoid contents of these plants were determined by comparing with gallic acid and quercetin respectively following Folin–Ciocalteu Reagent (FCR) and Flavonoid content determination method. The observed results are summarized in the subsequent paragraphs.

3.3.2.1. Total phenolic compound and flavonoid assay

The content of total phenolic and flavonoid in the methanolic plant extracts were determined using the Folin–Ciocalteu assay. The total amount of phenol and flavonoid were quantified from the regression equation of calibration curve ($y = 0.013x + 0.127$, $r^2 = 0.988$) and is expressed as gallic acid equivalents (GAE). The results presented in **Table 3.3** show that the amount of total phenolic compounds was significant different among the tea genotypes. Among the 12 tea genotypes, the highest amount of phenolics (GAE) was found in BT5 (3.88.76 mg/g GAE). Higher quantity of phenolics (more than 300 mg/g GAE) were also observed in the tea genotype SDL/1 and B2×T1. The phenolic content was the lowest in the genotype Br2/97.

Total flavonoid content were also significantly different in different tea genotypes. Among the 12 tea genotypes, the highest total flavonoid content was recorded in the genotype BS-67 (195.40 mg/g, in Quercetin). Total flavonoid content was also in the genotypes SDL/1, Br2/97, MZ/39 and BT5. The lowest amount of flavonoid was recorded in the genotype Ph/9/40.

Table 3.3 Total amount of plant phenolic compound and flavonoids of methanolic extract of the fresh leaves of 12 tea genotypes.

Clones	Total Phenol mg/g plant extract (in GAE)	Total Flavonoid mg/g plant extract (in Quercetin)
MZ/39	203.46 ± 0.22	167.33 ± 0.20
E/4	257.39 ± 0.37	145.72 ± 0.19
D/13	224.63 ± 0.42	91.83 ± 0.14
B2×T1	312.34 ± 0.26	92.30 ± 0.18
Br2/97	125.32 ± 0.39	175.51 ± 0.20
SDL/1	354.70 ± 0.43	180.25 ± 0.22
BT2	273.23 ± 0.29	88.36 ± 0.13
Ph/9/4	257.39 ± 0.36	50.51 ± 0.12
Ph/9/25	164.87 ± 0.18	37.75 ± 0.11
Ph/9/40	280.87 ± 0.36	110.36 ± 0.24
BS-67	178.33 ± 0.26	195.40 ± 0.19
BT-5	388.76 ± 0.47	140.81 ± 0.32
CV%	0.15	0.08
LSD P≤0.05	0.64	0.17
LSD P≤0.01	0.87	0.23

Each value is expressed as mean ± SD (n = 3).

3.3.2.2. Total antioxidant capacity

The total antioxidant capacity of the methanol extract of the fresh leaves of 12 tea genotypes were determined by using the method developed by Prieto *et al.* (1999).

The phosphomolybdenum method usually detects antioxidants such as ascorbic acid, some phenolics, a-tocopherol, and carotenoids. The phosphomolybdenum method was based on the reduction of Mo(VI) to Mo(V) by the antioxidant compound. The total antioxidant capacity determined as equivalent to ascorbic acid of the methanolic extract of fresh leaves of 12 tea genotypes were significantly different. Among the 12 genotypes the highest total antioxidant capacity was recorded in BT5 (910.39 mg/g) followed by Ph/9/25 (832.40 mg/g), Br2/97 (782.73 mg/g) and SDL/1 (704.51 mg/g). The lowest antioxidant capacity was recorded 312.30 mg/g in the genotype MZ/39.

The antioxidant capacity of the tea genotypes are in the order: BT5 > Ph/9/25 > Br2/97 > SDL/1 > Ph/9/4 > Ph/9/40 > BS-67 > E/4 > BT2 > D/13 > B2×T1 > MZ/39.

Table 3.4 Total antioxidant capacity of methanolic extract of the fresh leaves of 12 tea genotypes.

Clones	Used plant part	Total antioxidant capacity (Equivalent to ascorbic acid mg/g plant extract)
MZ/39	Two or three leaves and a bud	312.30 ± 0.26
E/4	-do-	455.40 ± 0.41
D/13	-do-	392.16 ± 0.25
B2×T1	-do-	346.12 ± 0.29
Br2/97	-do-	782.73 ± 0.59
SDL/1	-do-	704.51 ± 0.45
BT2	-do-	437.39 ± 0.26
Ph/9/4	-do-	661.38 ± 0.37
Ph/9/25	-do-	832.40 ± 0.41
Ph/9/40	-do-	581.39 ± 0.48
BS-67	-do-	553.67 ± 0.37
BT-5	-do-	910.39 ± 0.47
CV%	-	0.03
LSD P≤0.05	-	0.31
LSD P≤0.01	-	0.42

Each value is expressed as mean ± SD (n = 3).

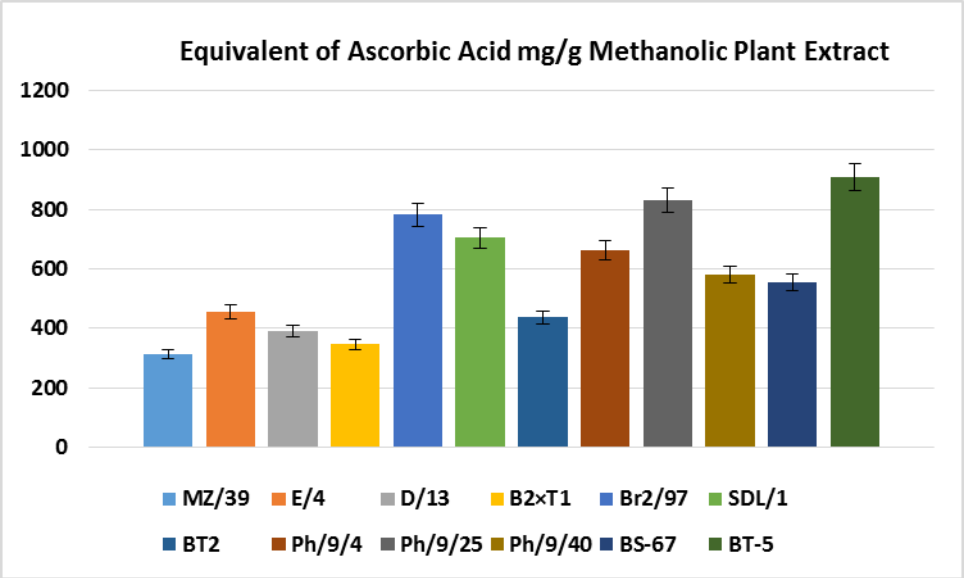


Figure 3.4 Graphical representation of total antioxideent capacity of methanolic extract of the fresh leaves of 12 tea genotypes.

3.3.2.3. DPPH radical scavenging activity

The model to scavenge the stable DPPH radical is a widely used method which is often used to determine the free radical scavenging ability of plant extract. The antioxidant activities of the leaf extract of 12 genotypes was determined by DPPH radical scavenging assay of butylated hydroxy toluene (BHT) standard. The results of DPPH radical scavenging assays of butylated hydroxy toluene (BHT) standard is given in **Table 3.5** and **Figure 3.5.**

This experiment reveals that the most potent activity was found in methanolic extract of genotypes BT5, Ph/9/25 and Br2/97 leaves with IC_{50} value of 39 µg/ml, 40.5 µg/ml and 42.5 µg/ml, respectively. And the methanolic extracts of SDL/1, Ph/9/4, BT2, D/13, MZ/39, Ph/9/40, BS-67 and E/4 also showed moderately free radical scavenging activity with IC_{50} value of 118.6 µg/ml, 126.5 µg/ml, 133.5 µg/ml, 151 µg/ml, 148.0 µg/ml, 149.0 µg/ml, 169.0 µg/ml and 187.5 µg/ml, respectively (**Figure 3.7)**. Only one sample named B2 × T1 of our investigation did not show IC_{50} within the concentration range of 25 – 200 µg/ml. This sample may require more than 200 µg/ml extract for showing IC_{50}. The result of DPPH radical scavenging assays of BHT standard and plant extracts are given in **Table 3.6** and **Figure 3.6**

The activity of the standard and different extractives decreases in the following order:

BHT > BT5 > Ph/9/25 > Br2/97 > SDL/1 > Ph/9/4 > BT2 > D/13 > MZ/39 > Ph/9/40 > BS-67 > E/4 > B2 × T1

Table 3.5 DPPH free radical scavenging activity of BHT standard (Synthetic antioxidant) at different concentrations.

Name of the Sample	Conc. (µg/ml)	% of Scavenging			% of Scavenging Mean ± STD	IC_{50} (µg/ml)
		A	B	c		
BHT	25	36.46	36.42	36.47	36.45 ± 0.026	35.6
	50	63.69	63.72	63.63	63.68 ± 0.046	
	100	88.51	88.47	88.53	88.50 ± 0.031	
	200	96.35	96.32	96.37	96.35 ± 0.026	

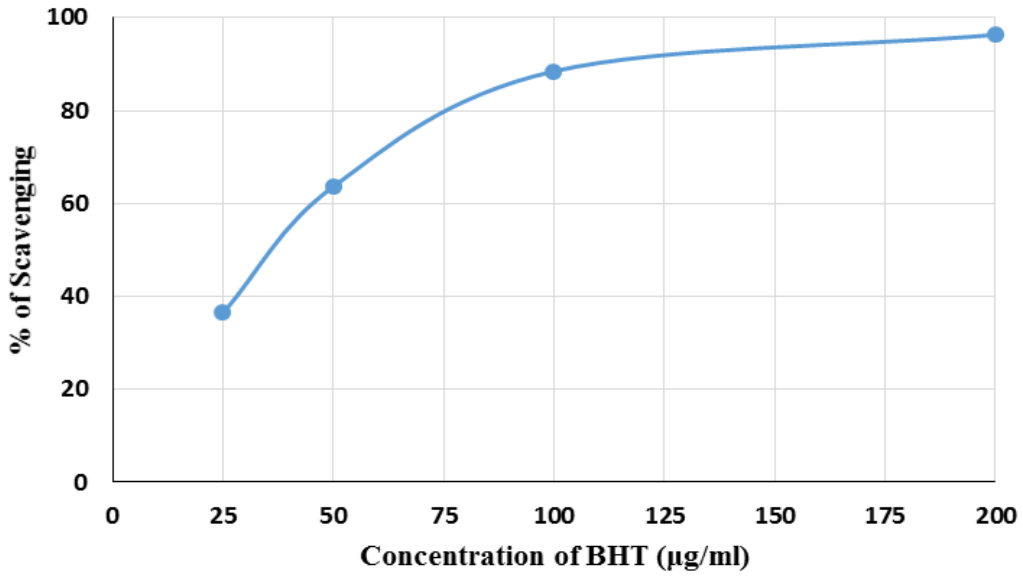


Figure 3.5 Determination of DPPH radical scavenging activity of BHT standard (synthetic antioxidant) at different concentrations.

Table 3.6 DPPH free radical scavenging activity of methanolic extracts of 12 tea genotypes in four different concentrations; Absorbance of each sample was taken in triplicated indicated as **a, b** and **c**.

Name of Samples	Conc. (µg/ml)	Absorbance			Absorbance Mean ± STD	% of Scavenging	IC_{50} (µg/ml)
		a	b	C			
MZ/39	25	1.423	1.457	1.489	1.456 ± 0.033	10.65	148.0
	50	1.409	1.461	1.357	1.409 ± 0.052	13.56	
	100	1.120	1.205	1.035	1.120 ± 0.085	31.29	
	200	0.497	0.534	0.460	0.497 ± 0.037	69.51	
E/4	25	1.428	1.495	1.482	1.468 ± 0.036	9.92	187.5
	50	1.449	1.408	1.467	1.441 ± 0.030	11.57	
	100	1.283	1.258	1.233	1.258 ± 0.025	22.82	
	200	0.928	0.753	0.578	0.753 ± 0.175	53.80	
D/13	25	1.529	1.345	1.437	1.437 ± 0.092	11.84	151.0
	50	1.433	1.143	1.288	1.288 ± 0.145	20.98	
	100	1.155	1.005	1.080	1.080 ± 0.075	33.74	
	200	0.803	0.297	0.550	0.550 ± 0.253	66.26	
B2 × T1	25	1.468	1.594	1.420	1.494 ± 0.090	8.34	-
	50	1.413	1.471	1.429	1.438 ± 0.030	11.80	
	100	1.263	1.309	1.355	1.309 ± 0.046	19.69	
	200	0.795	0.857	0.919	0.857 ± 0.062	47.42	
Br2/97	25	1.403	1.446	1.360	1.403 ± 0.043	13.93	42.5
	50	0.728	0.683	0.655	0.689 ± 0.037	57.75	
	100	0.513	0.495	0.462	0.490 ± 0.026	69.94	
	200	0.338	0.312	0.334	0.328 ± 0.014	79.88	
SDL/1	25	1.369	1.404	1.339	1.371 ± 0.033	15.91	118.6
	50	1.103	1.062	1.131	1.099 ± 0.035	32.60	
	100	1.050	0.920	0.753	0.908 ± 0.149	44.31	
	200	0.378	0.447	0.390	0.405 ± 0.037	75.15	

Name of Samples	Conc. (µg/ml)	Absorbance			Absorbance Mean ± STD	% of Scavenging	IC_{50} (µg/ml)
		a	b	C			
BT2	25	1.462	1.445	1.432	1.446 ± 0.015	11.27	133.5
	50	1.316	1.336	1.356	1.336 ± 0.020	18.04	
	100	1.144	1.162	0.680	0.995 ± 0.273	38.94	
	200	0.466	0.484	0.525	0.492 ± 0.030	69.84	
Ph/9/4	25	1.450	1.485	1.420	1.452 ± 0.033	10.94	126.5
	50	1.123	1.544	0.965	1.211 ± 0.299	25.73	
	100	1.021	0.891	0.956	0.956 ± 0.065	41.35	
	200	0.264	0.643	0.422	0.443 ± 0.190	72.82	
Ph/9/25	25	1.433	1.382	1.331	1.382 ± 0.051	15.21	40.5
	50	0.603	0.623	0.637	0.621 ± 0.017	61.90	
	100	0.438	0.458	0.415	0.437 ± 0.022	73.19	
	200	0.295	0.312	0.327	0.311 ± 0.016	80.90	
Ph/9/40	25	1.452	1.502	1.427	1.460 ± 0.038	10.41	149.0
	50	1.142	1.172	1.607	1.307 ± 0.260	19.82	
	100	0.918	1.103	1.288	1.103 ± 0.185	32.33	
	200	0.214	0.512	0.810	0.512 ± 0.298	68.59	
BS-67	25	1.415	1.501	1.492	1.469 ± 0.047	9.86	169.0
	50	1.176	1.499	1.422	1.366 ± 0.169	16.22	
	100	1.221	1.229	1.175	1.208 ± 0.029	25.87	
	200	0.509	0.535	0.872	0.639 ± 0.202	60.82	
BT5	25	1.186	1.425	1.398	1.336 ± 0.131	18.02	39.0
	50	0.568	0.581	0.598	0.582 ± 0.015	64.27	
	100	0.391	0.365	0.353	0.370 ± 0.019	77.32	
	200	0.254	0.267	0.275	0.265 ± 0.011	83.72	

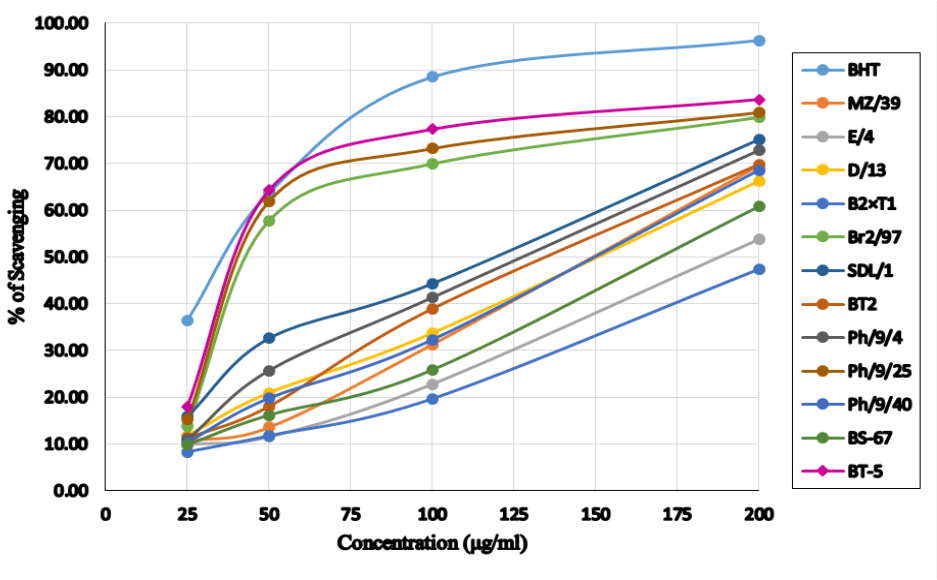


Figure 3.6 Determination of DPPH radical scavenging activity of methanolic extracts of 12 tea genotypes along with BHT as standard.

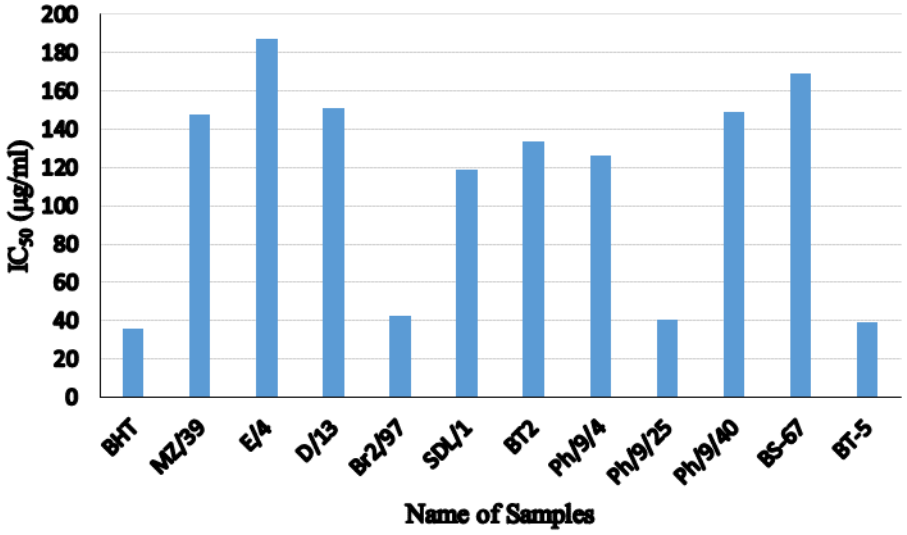


Figure 3.7 IC_{50} (µg/ml) values of methanolic extractives of 12 tea genotypes along with BHT as standard for DPPH radical scavenging activity.

3.3.2.4. Determination of gallic acid, four major alkaloids and catechin fraction through RP- HPLC

Eleven phenolic and flavonoid standards were compared with the chromatograms produced by the 12 tea genotypes leaf extracts. The chromatogram showed several numbers of peaks in different tea genotypes. Twelve peaks were identified in nine tea genotypes except D/13, B2×T1 and BS-67. In these three genotypes, 10 peaks were marked based on the standards which was run on the same LC system using the same chromatographic condition. Unwantedly, a peak supposed to be generated from methanol in the chromatogram during each run of HPLC with a retention time of 30.653 to 31.468 minutes. To confirm the peak was from methanol, 20 µl of methanol in the sample was injected and ran in HPLC. The same peak was observed in the chromatogram.

The results of the HPLC analysis of gallic acid (GA) and major tea alkaloids viz., theobromine (TB), theophylline (TP), caffeine (CAF) as well as catechin fractions viz., (-)- epigallo catechin (EGC), (+)- catechin (C), (-)- epicatechin (EC), (-)- epigallo catechin gallet (EGCG), (-)- gallocatechin gallet (GCG), (-)- epicatechin gallet (ECG) and (-)- catechin gallet (CG) for 12 tea genotypes are described separately below.

Tea genotype MZ/39: The chromatogram generated from the sample of the tea genotype MZ/39 shows 39 peaks (**Figure 3.8**). Among these, 12 peaks were marked based on the standards which were run on the same HPLC system using the same chromatographic condition. Rest of the peaks was unidentified. Among these 12 peaks gallic acid showed shortest retention time and came out of the column within 6.613 minutes, while methanol exhibited highest retain time and came out of the column within 31.468 minutes (**Table 3.7** and **Figure 3.8**).

Among the 11 flavonoid and alkaloid content the highest percent of peak area cover by caffeine (20.79%) followed by catechin (11.22%), gallocatechin gallet (9.49%), epicatechin (4.04%), gallic acid (3.78%) and epigallocatechin gallet (3.12%) (**Table 3.7**). The lowest percent of peak area cover by epigallocatechin (0.16%) followed by theophylline (0.81%), theobromine (1.09%), epicatechin gallet (1.19%) and catechin gallet (2.31%).

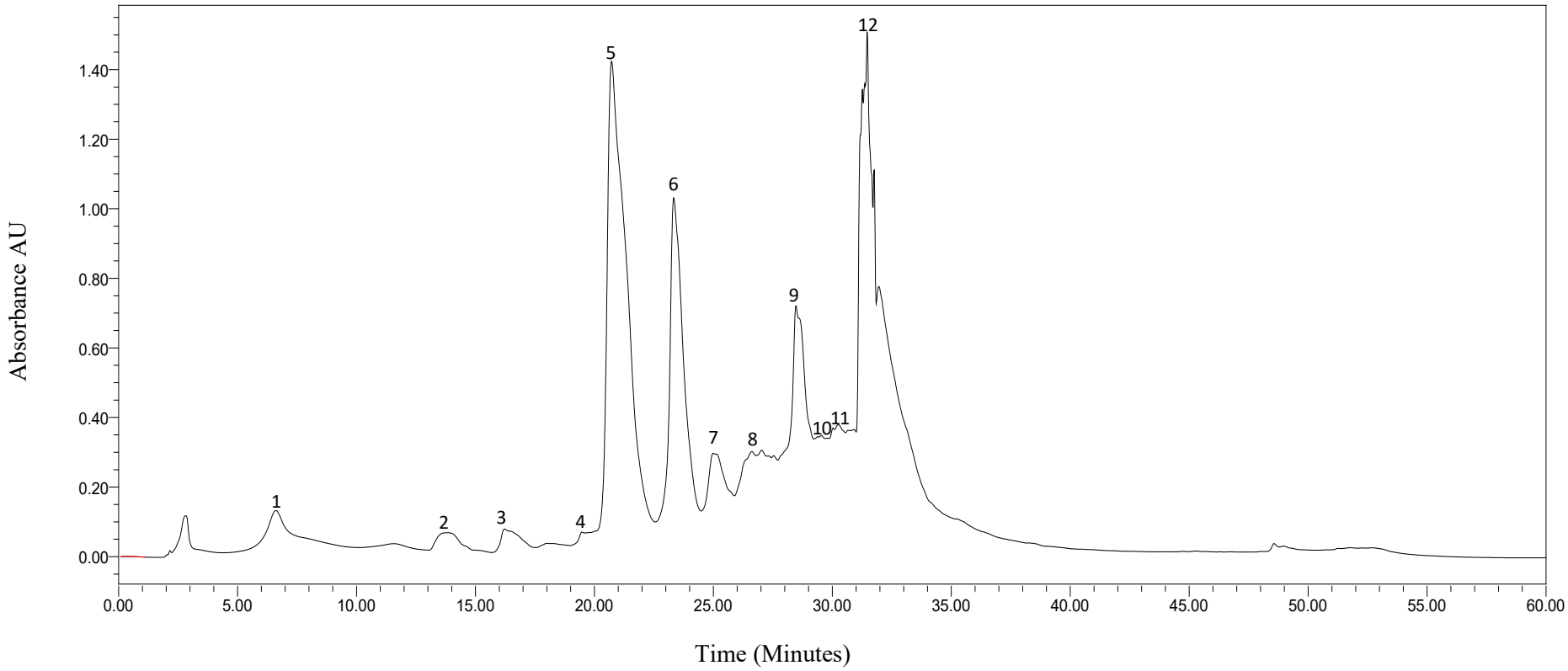


Figure 3.8 HPLC chromatogram of methanolic leaf extract of MZ/39 tea genotypes at 280 nm. X axis represents retention time and Y axis represents absorbance. Sample was run on Kromasil 100 C18 5µ 25 × 0.46 column using water as solvent A and 80% acetonitrile as solvent B with a flow rate of 1 ml/min. Major peaks were marked either by the standard ran using the same chromatographic condition or based on sequential peak profile of the major tea alkaloids reported earlier. Peaks (1) gallic acid; (2) theobromine; (3) theophylline; (4) epigallocatechin; (5) caffeine; (6) catechin; (7) epicatechin; (8) epigallocatechin gallet; (9) gallocatechin gallet; (10) epicatechin gallet; (11) catechin gallet and (12) methanol/unknown.

Table 3.7 Retention time and % of different peaks obtained in the HPLC of ethanolic leaf extract of genotype MZ/39. Sample was run on Kromasil 100 C18 5µ 25 × 0.46 column using water as solvent A and 80% acetonitrile as solvent B.

Peak Sl.	Marked Peak No.	Name	Retention Time	Area	Height	% Area
4	1	Gallic acid	6.613	15019258	129854	3.78
6	2	Theobromine	13.797	4305914	58899	1.09
7	3	Theophyline	16.223	3229187	63568	0.81
9	4	Epigallo catechin (EC)	19.477	642844	39037	0.16
10	5	Caffeine	20.725	82501070	1393604	20.79
11	6	Catechin	23.339	44522943	1000361	11.22
12	7	Epicatechin (EC)	25	16037679	265902	4.04
13	8	Epigallo catechin gallet (EGCG)	26.614	12385441	271045	3.12
17	9	Gallo catechin gallet (GCG)	28.476	37676887	691194	9.49
19	10	Epicatechin gallet (ECG)	29.542	4706445	317990	1.19
22	11	Catechin gallet (CG)	30.26	9149855	348498	2.31
26	12	Methanol	31.468	30960777	1476175	7.8

Tea genotype E/4: The chromatogram generated from the leaf extract of the tea genotype E/4 shows 40 peaks. Among these, 12 peaks were identified based on the standards that was run on the same LC system using the same chromatographic condition (**Figure 3.9**). Rest of the peaks were unidentified. Among these 12 peaks gallic acid showed shortest retain time and came out of the column within 9.011 minutes, while methanol exhibited highest retain time and came out of the column within 31.154 minutes (**Table 3.8**).

Among the 11 flavonoid and alkaloid content the highest percent of peak area cover by caffeine (18.97%) followed by gallocatechin gallet (9.97%), catechin (9.76%), epigallocatechin gallet (4.39%), gallic acid (3.95%) and epicatechin (3.24%). The lowest percent of peak area cover by theophylline (0.68%) followed by epigallocatechin (0.73%), epicatechin gallet (1.58%), theobromine (1.94%) and catechin gallet (2.97%) (**Table 3.8**).

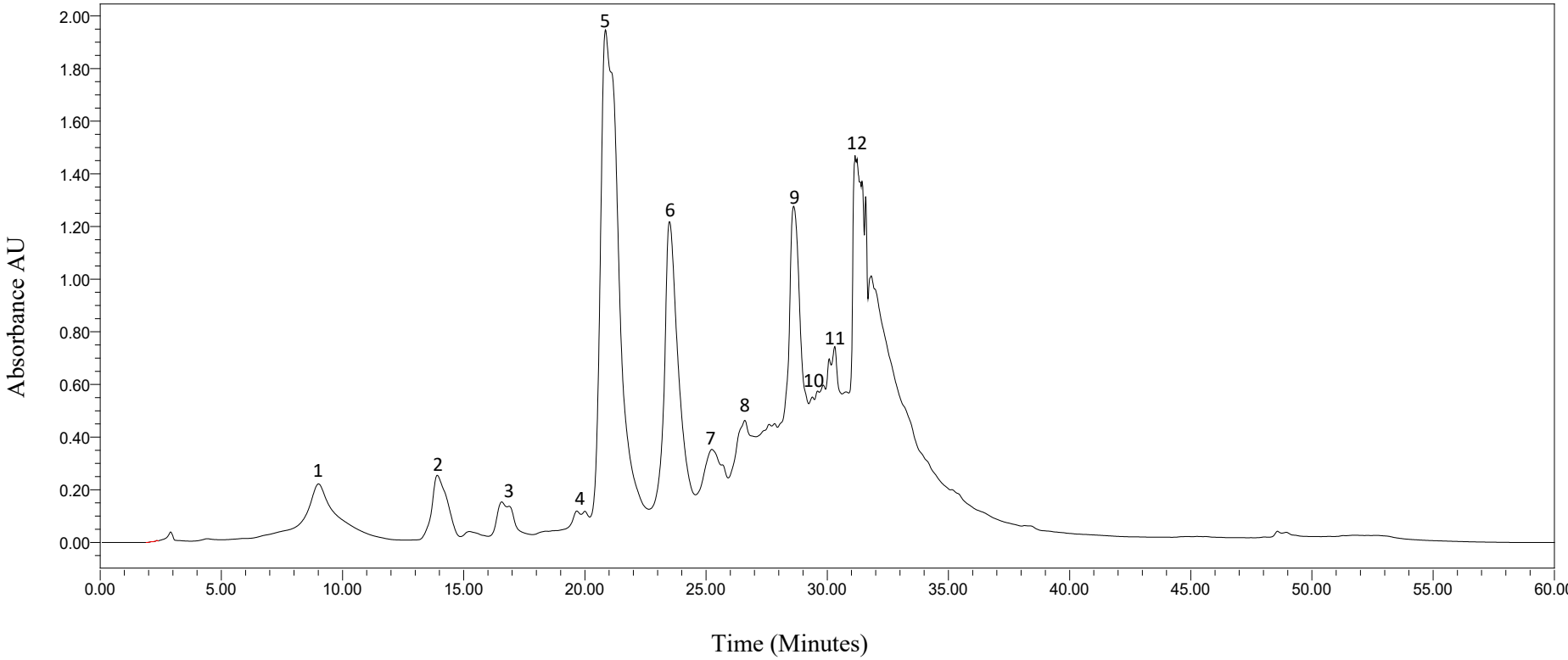


Figure 3.9 HPLC chromatogram of methanolic leaf extract of E/4 tea genotype at 280 nm. X axis represents retention time and Y axis represents absorbance. Sample was run on Kromasil 100 C18 5µ 25 × 0.46 column using water as solvent A and 80% acetonitrile as solvent B with a flow rate of 1 ml/min. Major peaks were marked either by the standard ran using the same chromatographic condition or based on sequential peak profile of the major tea alkaloids reported earlier. Peaks (1) gallic acid; (2) theobromine; (3) theophylline; (4) epigallocatechin; (5) caffeine; (6) catechin; (7) epicatechin; (8) epigallocatechin gallet; (9) gallocatechin gallet; (10) epicatechin gallet; (11) catechin gallet and (12) methanol/unknown.

Table 3.8 Retention time and % of different peaks obtained in the HPLC of methanolic leaf extract of E/4 tea genotype. Sample was run on Kromasil 100 C18 5µ 25 × 0.46 column using water as solvent A and 80% acetonitrile as solvent B.

Peak Sl.	Marked Peak No.	Name	Retention Time	Area	Height	% Area
5	1	Gallic acid	9.011	22465767	217117	3.95
6	2	Theobromine	13.911	11064772	245872	1.94
8	3	Theophyline	16.57	3857199	142772	0.68
11	4	Epigallocatechin (EGC)	19.667	4179120	105649	0.73
13	5	Caffeine	20.851	107937727	1932902	18.97
14	6	Catechin	23.492	55555245	1202515	9.76
15	7	Epicatechin (EC)	25.239	18440955	335442	3.24
17	8	Epigallocatechin gallet (EGCG)	26.599	24992208	444667	4.39
20	9	Gallocatechin gallet (GCG)	28.616	56751044	1256303	9.97
23	10	Epicatechin gallet (ECG)	29.839	9012473	575830	1.58
25	11	Catechin gallet (CG)	30.309	16908365	722612	2.97
27	12	Methanol	31.154	41734465	1439745	7.34

Tea genotype D/13: The HPLC chromatogram of D/13 shows 33 peaks (**Figure 3.10**). Among these 10 peaks were marked based on the standards which were run on the same LC system using the same chromatographic condition. Rest of the peaks were unidentified. Among these 10 peaks theobromine showed shortest retain time and came out of the column within 14.717 minutes, while methanol exhibited highest retain time and came out of the column within 31.209 minutes (**Table 3.9**).

Table 3.9 also reveals that among the 9 flavonoid and alkaloid content the highest percent of peak area cover by catechin (15.74%) followed by caffeine (7.95%), gallocatechin gallet (4.82%), epigallocatechin gallet (4.27%), and epicatechin (4.2%). The lowest percent of peak area cover by epigallocatechin (0.45%) followed by theobromine (0.85%), catechin gallet (1.62%) and epicatechin gallet (1.67%). Gallic acid and theophylline are absent in D/13.

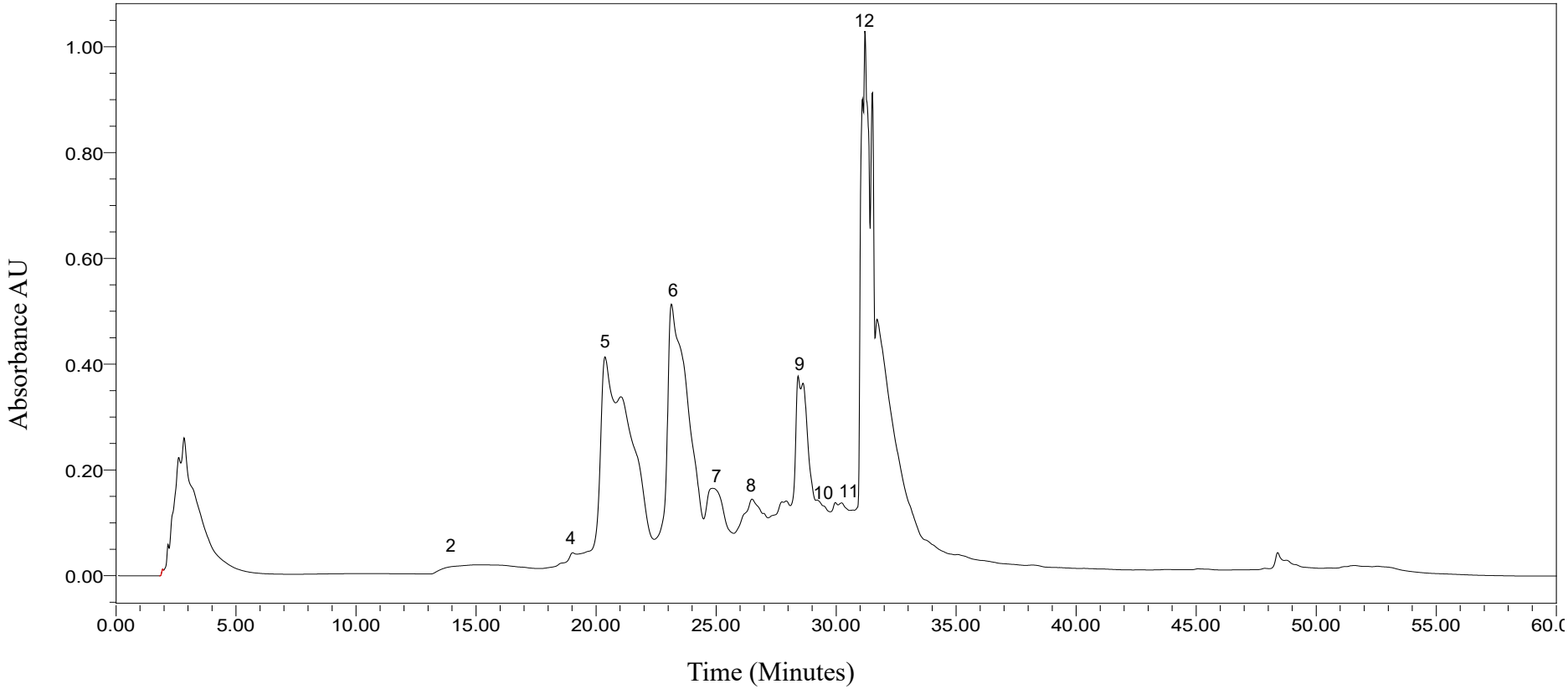


Figure 3.10 HPLC chromatogram of methanolic leaf extract of D/13 tea genotype at 280 nm. X axis represents retention time and Y axis represents absorbance. Sample was run on Kromasil 100 C18 5µ 25 × 0.46 column using water as solvent A and 80% acetonitrile as solvent B with a flow rate of 1 ml/min. Major peaks were marked either by the standard ran using the same chromatographic condition or based on sequential peak profile of the major tea alkaloids reported earlier. Peaks (2) theobromine; (3) theophylline; (4) epigallocatechin; (5) caffeine; (6) catechin; (7) epicatechin; (8) epigallocatechin gallet; (9) gallocatechin gallet; (10) epicatechin gallet; (11) catechin gallet and (12) methanol/unknown.

Table 3.9 Retention time and % of different peaks obtained in the HPLC of methanolic leaf extract of D/13 tea clone. Sample was run on Kromasil 100 C18 5µ 25 × 0.46 column using water as solvent A and 80% acetonitrile as solvent B.

Peak Sl.	Marked Peak No.	Name	Retention Time	Area	Height	% Area
5	2	Theobromine	14.717	1709452	11113	0.85
6	4	Epigallocatechin (EGC)	19.027	905814	29389	0.45
7	5	Caffeine	20.376	16044287	399453	7.95
9	6	Catechin	23.139	31751336	498169	15.74
10	7	Epicatechin (EC)	24.859	8477755	148703	4.2
11	8	Epigallocatechin gallet (EGCG)	26.496	8606254	127779	4.27
15	9	Gallocatechin gallet (GCG)	28.62	9716871	346147	4.82
16	10	Epicatechin gallet (ECG)	29.25	3372038	124192	1.67
18	11	Catechin gallet (CG)	30.222	3270176	119095	1.62
21	12	Methanol	31.209	13823318	1009271	6.85

Tea genotype B2×T1: The HPLC chromatogram of B2×T1 (**Figure 3.11**) shows 38 peaks, among these 10 peaks were identified based on the standards run on the same LC system using the same chromatographic condition. Rest of the peaks were unidentified. Among these 10 peaks theobromine showed shortest retention time and came out of the column within 14.617 minutes, while methanol exhibited highest retain time and came out of the column within 31.303 minutes (**Table 3.10**).

Among the 9 flavonoid and alkaloid contents the highest percent of peak area cover by caffeine (10.72%) followed by epigallocatechin (7.41%), catechin (5.90%) and epicatechin (4.23%). The lowest percent of peak area cover by theobromine (0.29%) followed by catechin gallet (1.81%), epicatechin gallet (2.53%), gallocatechin gallet (2.93%) and epicgallocatechin gallet (3.3%). Gallic acid and theophylline are also absent in B2×T1.

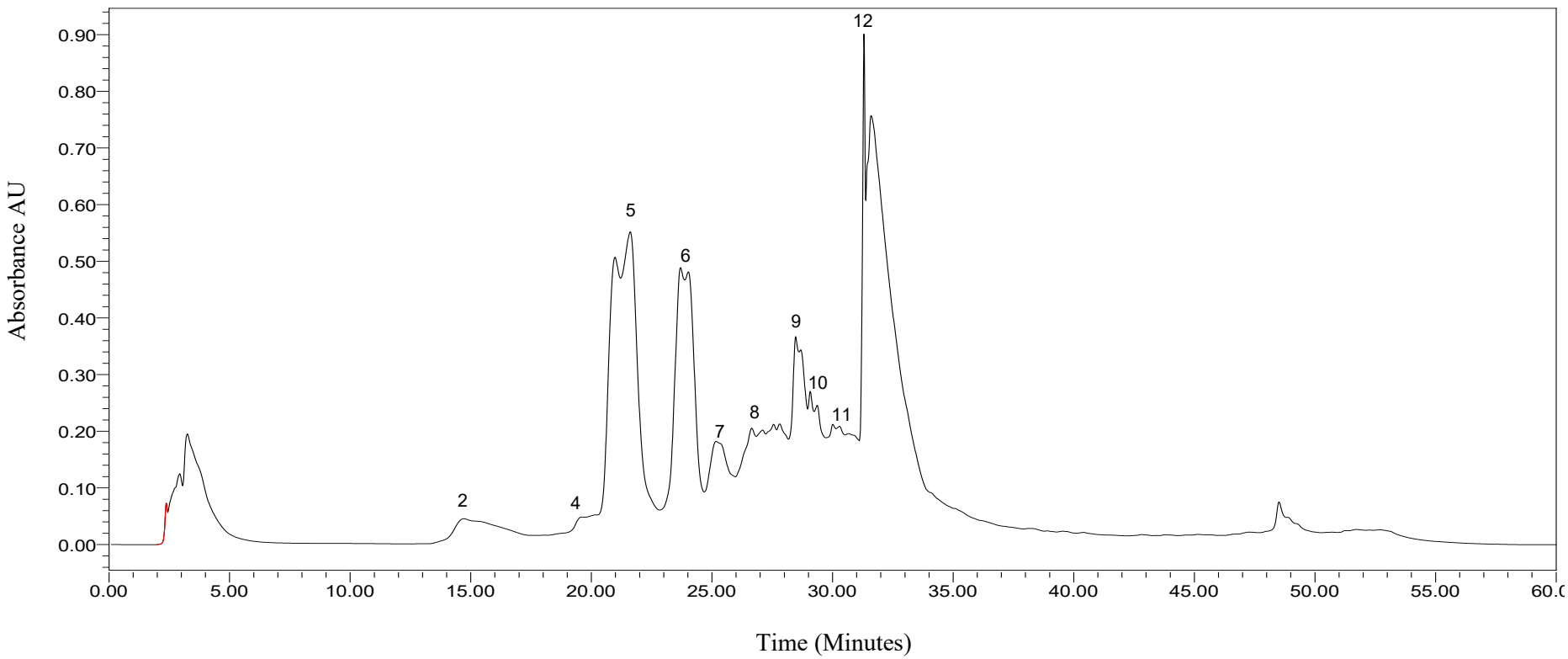


Figure 3.11 HPLC chromatogram of methanolic leaf extract of B2×T1 tea genotype at 280 nm. X axis represents retention time and Y axis represents absorbance. Sample was run on Kromasil 100 C18 5µ 25 × 0.46 column using water as solvent A and 80% acetonitrile as solvent B with a flow rate of 1 ml/min. Major peaks were marked either by the standard ran using the same chromatographic condition or based on sequential peak profile of the major tea alkaloids reported earlier. Peaks (2) theobromine; (4) epigallocatechin; (5) caffeine; (6) catechin; (7) epicatechin; (8) epigallocatechin gallet; (9) gallocatechin gallet; (10) epicatechin gallet; (11) catechin gallet and (12) methanol/unknown.

Table 3.10 Retention time and % of different peaks obtained in the HPLC of methanolic leaf extract of B2×T1 tea genotype. Sample was run on Kromasil 100 C18 5µ 25 × 0.46 column using water as solvent A and 80% acetonitrile as solvent B.

Peak Sl.	Marked Peak No.	Name	Retention Time	Area	Height	% Area
4	2	Theobromine	14.617	680461	18542	0.29
6	4	Epigallocatechin (EGC)	20.982	17487998	490655	7.41
7	5	Caffeine	21.608	25290508	535614	10.72
8	6	Catechin	23.7	13921553	472031	5.9
10	7	Epicatechin (EC)	25.172	9972868	165489	4.23
11	8	Epigallocatechin gallet (EGCG)	26.646	7790526	188750	3.3
15	9	Gallo catechin gallet (GCG)	28.47	6913475	349827	2.93
18	10	Epicatechin gallet (ECG)	29.359	5961790	229226	2.53
20	11	Catechin gallet (CG)	30.281	4264650	192015	1.81
22	12	Methanol	31.303	8406217	880432	3.56

Tea genotype Br2/97: The chromatogram of Br2/97 (**Figure 3.12**) shows a total of 40 peaks among which, 12 peaks were recognized based on the standards that was run on the same LC system using the same chromatographic condition. Rest of the peaks were unidentified. Among these 12 peaks gallic acid showed shortest retain time and came out of the column within 8.649 minutes, while methanol exhibited highest retain time and came out of the column within 31.406 minutes (**Table 3.11**).

Table 3.11 reveals that among the 11 flavonoid and alkaloid content the highest percent of peak area cover by caffeine (7.24%) followed by gallocatechin gallet (6.74%), catechin (4.4%), gallic acid (4.08%), and epicatechin (3.95%). The lowest percent of peak area cover by epigallocatechin (0.60%) followed by catechin gallet (1.11%), theophylline (1.19%) epicatechin gallet (1.38%), theobromine (2.98%) and epigallocatechin gallet (3.09%).

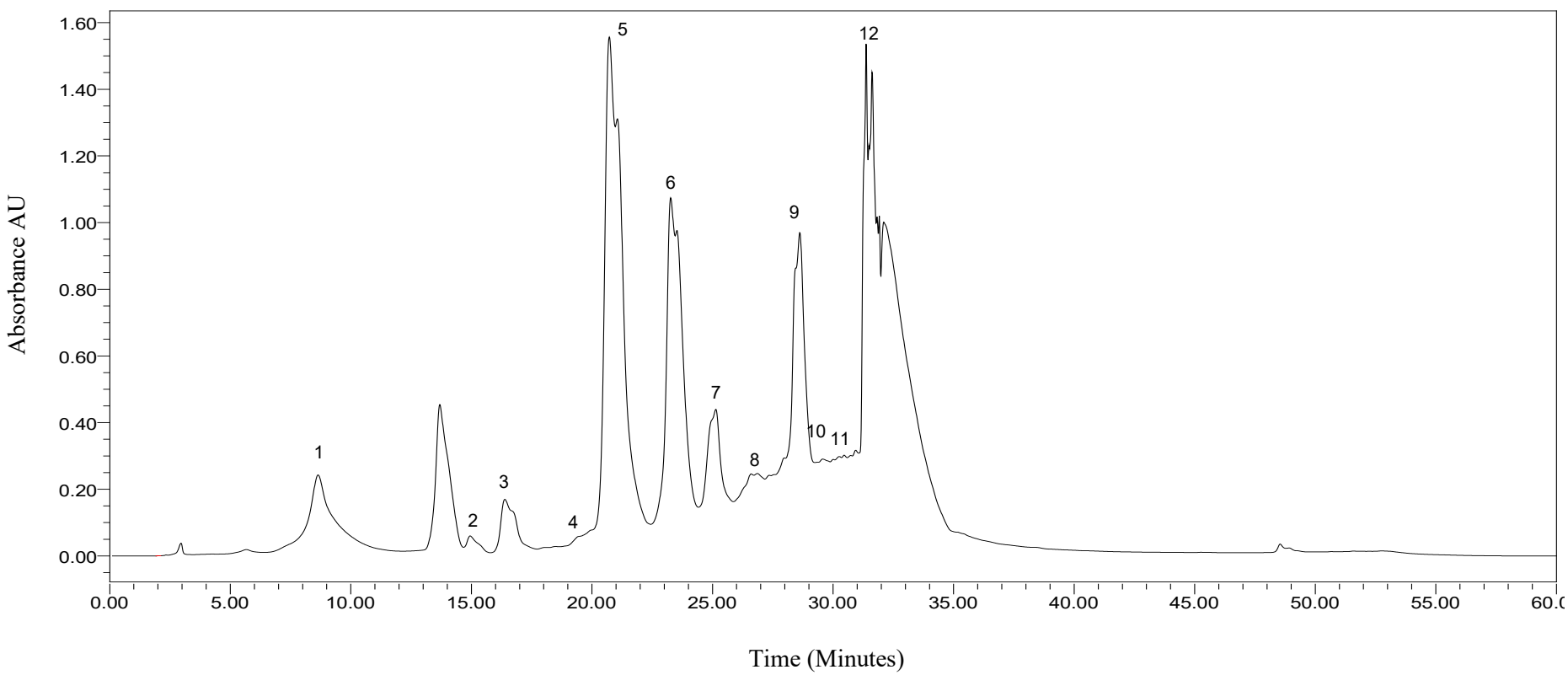


Figure 3.12 HPLC chromatogram of methanolic leaf extract of Br/2/97 tea genotype at 280 nm. X axis represents retention time and Y axis represents absorbance. Sample was run on Kromasil 100 C18 5µ 25 × 0.46 column using water as solvent A and 80% acetonitrile as solvent B with a flow rate of 1 ml/min. Major peaks were marked either by the standard ran using the same chromatographic condition or based on sequential peak profile of the major tea alkaloids reported earlier. Peaks (1) gallic acid; (2) theobromine; (3) theophylline; (4) epigallocatechin; (5) caffeine; (6) catechin; (7) epicatechin; (8) epigallocatechin gallet; (9) gallocatechin gallet; (10) epicatechin gallet; (11) catechin gallet and (12) methanol/unknown.

Table 3.11 Retention time and % of different peaks obtained in the HPLC of methanolic leaf extract of Br2/97 tea genotype. Sample was run on Kromasil 100 C18 5µ 25 × 0.46 column using water as solvent A and 80% acetonitrile as solvent B.

Peak Sl.	Marked Peak No.	Name	Retention Time	Area	Height	% Area
5	1	Gallic acid	8.649	12531904	160037	4.08
6	2	Theobromine	13.699	9142031	235140	2.98
8	3	Theophyline	16.399	3651760	85139	1.19
10	4	Epigallocatechin (EGC)	19.91	1829391	36216	0.6
11	5	Caffeine	20.73	22227920	819938	7.24
13	6	Catechin	23.278	13521012	527654	4.4
15	7	Epicatechin (EC)	25.145	12116931	221165	3.95
16	8	Epigallocatechin gallet (EGCG)	26.565	9493785	181050	3.09
21	9	Gallocatechin gallet (GCG)	28.626	20709400	473609	6.74
23	10	Epicatechin gallet (ECG)	29.546	4233905	186179	1.38
28	11	Catechin gallet (CG)	30.934	3419266	192729	1.11
30	12	Methanol	31.406	22815638	1458560	7.43

Tea genotype SDL/1: The HPLC chromatogram of SDL/1 (**Figure 3.13**) exhibits altogether 39 peaks, out of which 12 peaks were identified based on the standards that was run on the same LC system using the same chromatographic condition. Rest of the peaks were unidentified. Among the 12 peaks gallic acid showed shortest retain time and came out of the column within 6.922 minutes, while methanol exhibited highest retain time and came out of the column within 31.393 minutes (**Table 3.12**).

Out of the 11 flavonoid and alkaloid contents, the highest percent of peak area cover by caffeine (18.03%) followed by catechin gallet (10.18%), gallocatechin gallet (3.27) and epicatechin (3.01%). The lowest percent of peak area cover by epigallocatechin (0.04%) followed by theophylline (0.85%), theobromine (1.42%), epigallo catechin (2.0%), gallic acid (2.09%), catechin gallet (2.58%) and epigallocatechin gallet (2.63%).

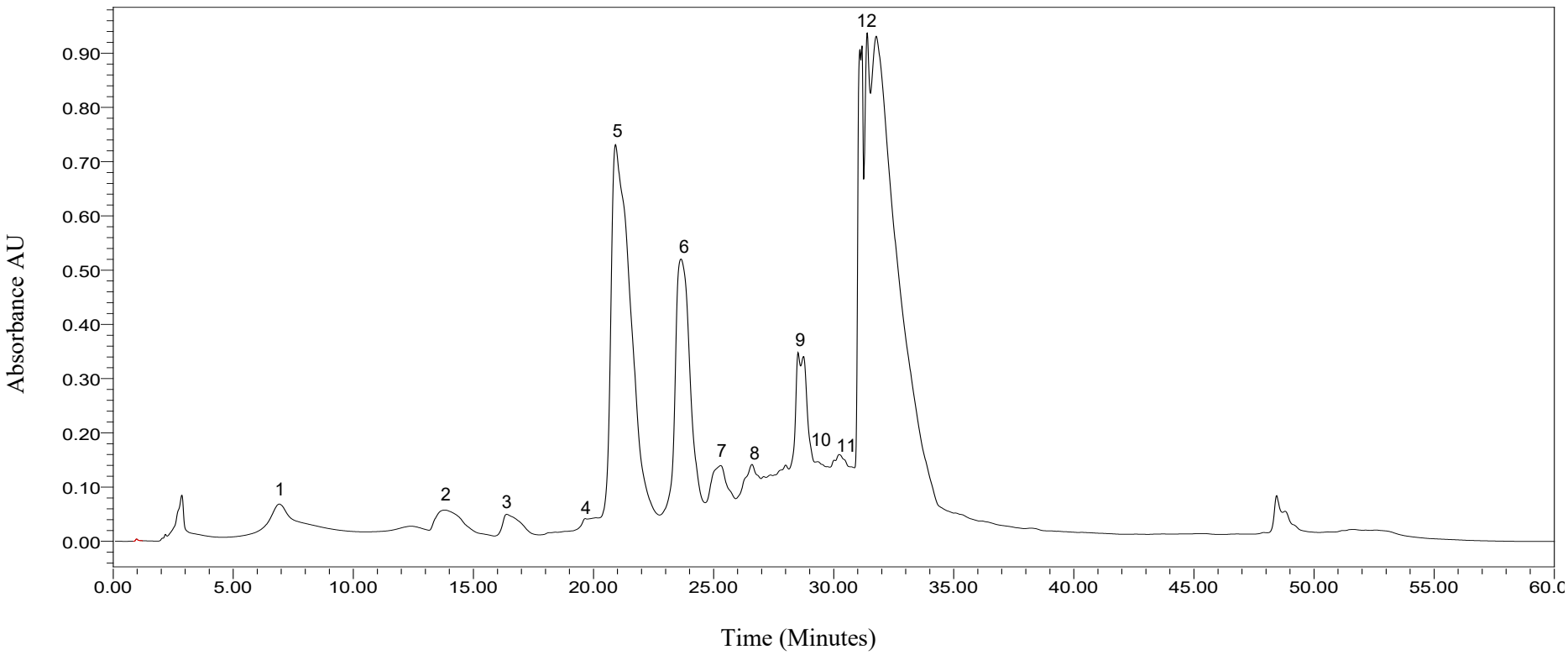


Figure 3.13 HPLC chromatogram of methanolic leaf extract of SDL/1 genotype at 280 nm. X axis represents retention time and Y axis represents absorbance. Sample was run on Kromasil 100 C18 5µ 25 × 0.46 column using water as solvent A and 80% acetonitrile as solvent B with a flow rate of 1 ml/min. Major peaks were marked either by the standard ran using the same chromatographic condition or based on sequential peak profile of the major tea alkaloids reported earlier. Peaks (1) gallic acid; (2) theobromine; (3) theophylline; (4) epigallocatechin; (5) caffeine; (6) catechin; (7) epicatechin; (8) epigallocatechin gallet; (9) gallocatechin gallet; (10) epicatechin gallet; (11) catechin gallet and (12) methanol/unknown.

Table 3.12 Retention time and % of different peaks obtained in the HPLC of methanolic leaf extract of SDL/1 genotypes. Sample was run on Kromasil 100 C18 5µ 25 × 0.46 column using water as solvent A and 80% acetonitrile as solvent B.

Peak Sl.	Marked Peak No.	Name	Retention Time	Area	Height	% Area
4	1	Gallic acid	6.922	5000207	57384	2.09
6	2	Theobromine	13.801	3408091	44135	1.42
7	3	Theophyline	16.386	2034746	39767	0.85
8	4	Epigallo catechin (EGC)	18.25	98004	5279	0.04
13	5	Caffeine	20.919	43205430	719553	18.03
14	6	Catechin	23.641	24389541	507623	10.18
15	7	Epicatechin (EC)	25.295	7204747	126092	3.01
16	8	Epigallo catechin gallet (EGCG)	26.598	6299084	127652	2.63
21	9	Gallocatechin gallet (GCG)	28.739	7845753	325708	3.27
22	10	Epigallocatechin (EGC)	29.335	4791199	131395	2
24	11	Ctechin gallet (CG)	30.233	6183712	144278	2.58
26	12	Methanol	31.393	14102218	922276	5.88

Tea genotype BT2: The chromatogram of BT2 (**Figure 3.14**) demonstrates total 41 peaks among which 12 peaks were marked based on the standards that was run on the same LC system using the same chromatographic condition. Rest of the peaks were unidentified. Among these 12 peaks gallic acid showed shortest retain time and came out of the column within 5.103 minutes, while methanol exhibited highest retain time and came out of the column within 31.006 minutes (**Table 3.13**).

Table 3.13 also presents that among the 11 flavonoid and alkaloid content the highest percent of peak area is covered by caffeine (17.48%) followed by catechin (9.44%), gallocatechin gallet (4.55%) and epicatechin (3.65%). The lowest percent of peak area cover by theophylline (0.01%) followed by epigallocatechin (0.53%), gallic acid (1.22%), epicatechin gallet (1.71%), theobromine (1.96%), catechin gallet (2.25%) and epigallocatechin gallet (2.28%).

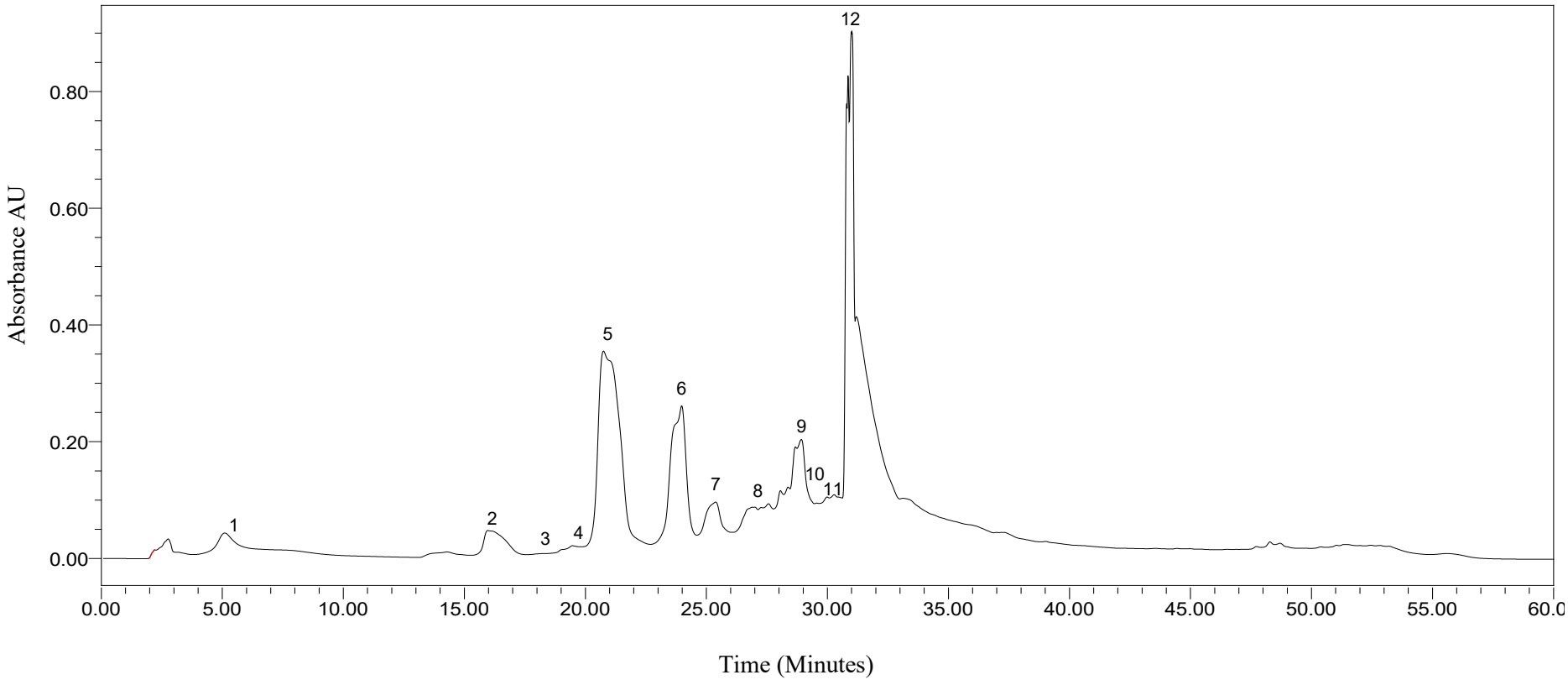


Figure 3.14 HPLC chromatogram of methanolic leaf extract of BT2 genotype at 280 nm. X axis represents retention time and Y axis represents absorbance. Sample was run on Kromasil 100 C18 5µ 25 × 0.46 column using water as solvent A and 80% acetonitrile as solvent B with a flow rate of 1 ml/min. Major peaks were marked either by the standard ran using the same chromatographic condition or based on sequential peak profile of the major tea alkaloids reported earlier. Peaks (1) gallic acid; (2) theobromine; (3) theophylline; (4) epigallocatechin; (5) caffeine; (6) catechin; (7) epicatechin; (8) epigallocatechin gallet; (9) gallocatechin gallet; (10) epicatechin gallet; (11) catechin gallet and (12) methanol/unknown.

Table 3.13 Retention time and % of different peaks obtained in the HPLC of methanolic leaf extract of BT2 genotype. Sample was run on Kromasil 100 C18 5µ 25 × 0.46 column using water as solvent A and 80% acetonitrile as solvent B.

Peak Sl.	Marked Peak No.	Name	Retention Time	Area	Height	% Area
3	1	Gallic acid	5.103	1570725	32316	1.22
6	2	Theobromine	15.974	2509084	42049	1.96
7	3	Theophyline	18.083	14342	1106	0.01
8	4	Epigallocatechin (EGC)	19.473	676057	13935	0.53
9	5	Caffeine	20.747	22421994	346550	17.48
10	6	Catechin	23.978	12112128	250587	9.44
11	7	Epicatehin (EC)	25.385	4687858	84557	3.65
12	8	Epigallocatechin gallet (EGCG)	26.892	2930429	74804	2.28
19	9	Gallocatechin gallet (GCG)	28.918	5837048	189395	4.55
21	10	Epicatechin gallet (ECG)	29.983	2192812	89975	1.71
22	11	Catechin gallet (CG)	30.283	2885734	93643	2.25
24	12	Methanol	31.004	11020497	887017	8.59

Tea genotype Ph/9/4: The HPLC chromatogram of Ph/9/4 shows total 37 peaks (**Figure 3.15**). Among these 12 peaks were recognized based on the standards that was run on the same LC system using the same chromatographic condition. Rest of the peaks were unidentified. Among these 12 peaks gallic acid showed shortest retain time and came out of the column within 8.787 minutes, while methanol exhibited highest retain time and came out of the column within 31.396 minutes (**Table 3.14**).

It is also revealed from the **Table 3.14** that among the 11 flavonoid and alkaloid content the highest percent of peak area cover by caffeine (17.92%) followed by catechin (11.19%), gallocatechin gallet (4.15%) and epicatechin (4.01%). The lowest percent of peak area cover by theophylline (0.62%) followed by epigallo catechin (0.86%), epicatechin gallet (1.45%), catechin gallet (1.85), theobromine (2.51%), gallic acid (2.94%) and epigallocatechin gallet (2.86%).

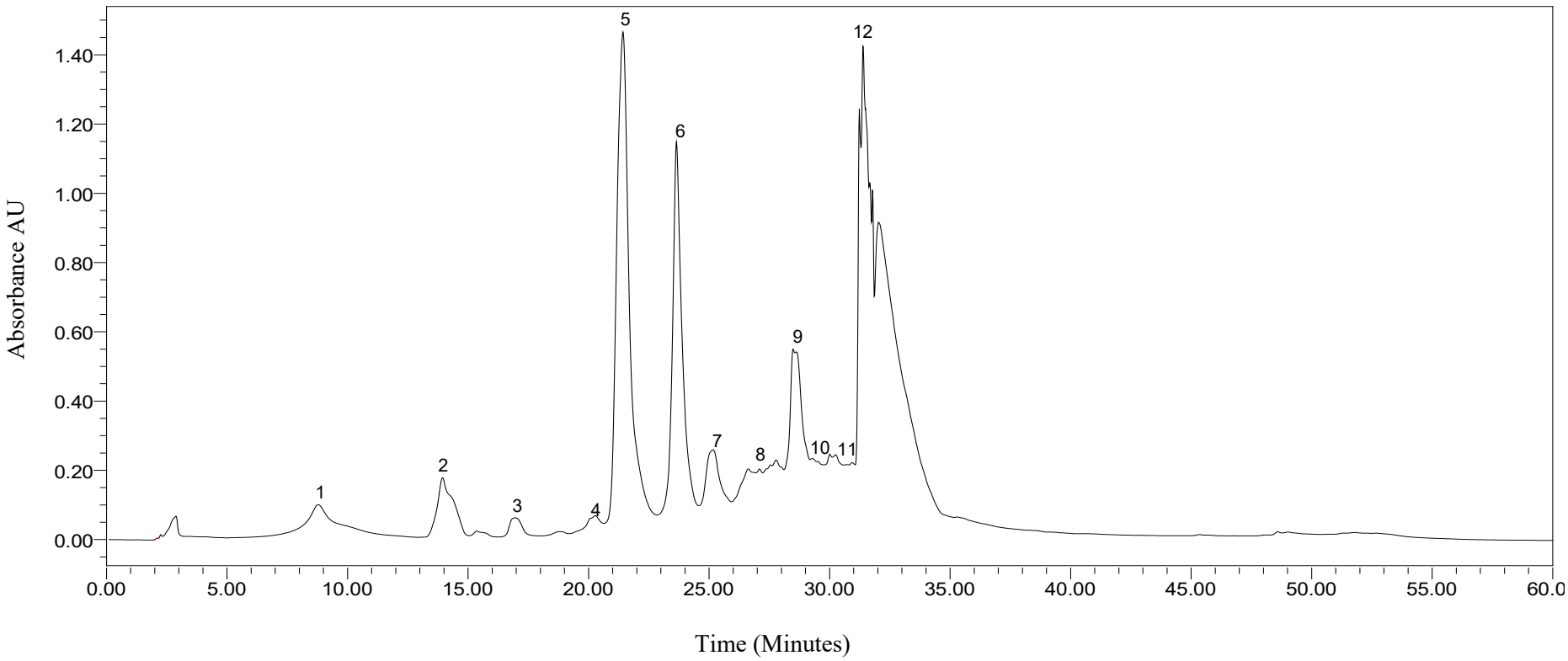


Figure 3.15 HPLC chromatogram of methanolic leaf extract of Ph/9/4 tea genotype at 280 nm. X axis represents retention time and Y axis represents absorbance. Sample was run on Kromasil 100 C18 5µ 25 × 0.46 column using water as solvent A and 80% acetonitrile as solvent B with a flow rate of 1 ml/min. Major peaks were marked either by the standard ran using the same chromatographic condition or based on sequential peak profile of the major tea alkaloids reported earlier. Peaks (1) gallic acid; (2) theobromine; (3) theophylline; (4) epigallocatechin; (5) caffeine; (6) catechin; (7) epicatechin; (8) epigallocatechin gallet; (9) gallocatechin gallet; (10) epicatechin gallet; (11) catechin gallet and (12) methanol/unknown.

Table 3.14 Retention time and % of different peaks obtained in the HPLC of methanolic leaf extract of Ph/9/4 tea genotype. Sample was run on Kromasil 100 C18 5µ 25 × 0.46 column using water as solvent A and 80% acetonitrile as solvent B.

Peak Sl.	Marked Peak No.	Name	Retention Time	Area	Height	% Area
4	1	Gallic acid	8.787	9353259	93792	2.94
5	2	Theobromine	13.941	7973604	171887	2.51
7	3	Theophyline	16.964	1977102	54934	0.62
9	4	Epigallo catechin (EGC)	20.28	2734828	57841	0.86
10	5	Caffeine	21.43	57033166	1453950	17.92
11	6	Catechin	23.648	35607553	1134794	11.19
12	7	Epicatechin (EC)	25.164	12758210	244052	4.01
13	8	Epigallocatechin gallet (EGCG)	26.625	9109310	187097	2.86
18	9	Gallocatechin gallet (GCG)	28.623	13209002	523831	4.15
19	10	Epicatechin gallet (ECG)	29.28	4629771	214825	1.45
22	11	Catechin gallet (CG)	30.249	5886280	225122	1.85
26	12	Methanol	31.396	29204603	1405846	9.18

Tea genotype Ph/9/25: The HPLC chromatogram of Ph/9/25 (**Figure 3.16**) shows total 44 peaks, out of which 12 peaks were identified based on the standards run on the same LC system using the same chromatographic condition. Rest of the peaks were unidentified. Among 12 peaks, gallic acid showed shortest retain time and came out of the column within 8.198 minutes, while methanol exhibited highest retain time and came out of the column within 31.316 minutes (**Table 3.15**).

It is shown from the **Table 3.15** that among the 11 flavonoid and alkaloid content the highest percent of peak area cover by caffeine (20.6%) followed by catechin (14.20%), gallocatechin gallet (4.31%), gallic acid (4.25%), theobromine (3.54%) and epicatechin (2.83%). The lowest percent of peak area cover by epigallo catechin (0.91%) followed by theophylline (1.23%), epicatechin gallet (1.37%), catechin gallet (1.64%) and epigallocatechin gallet (2.48%).

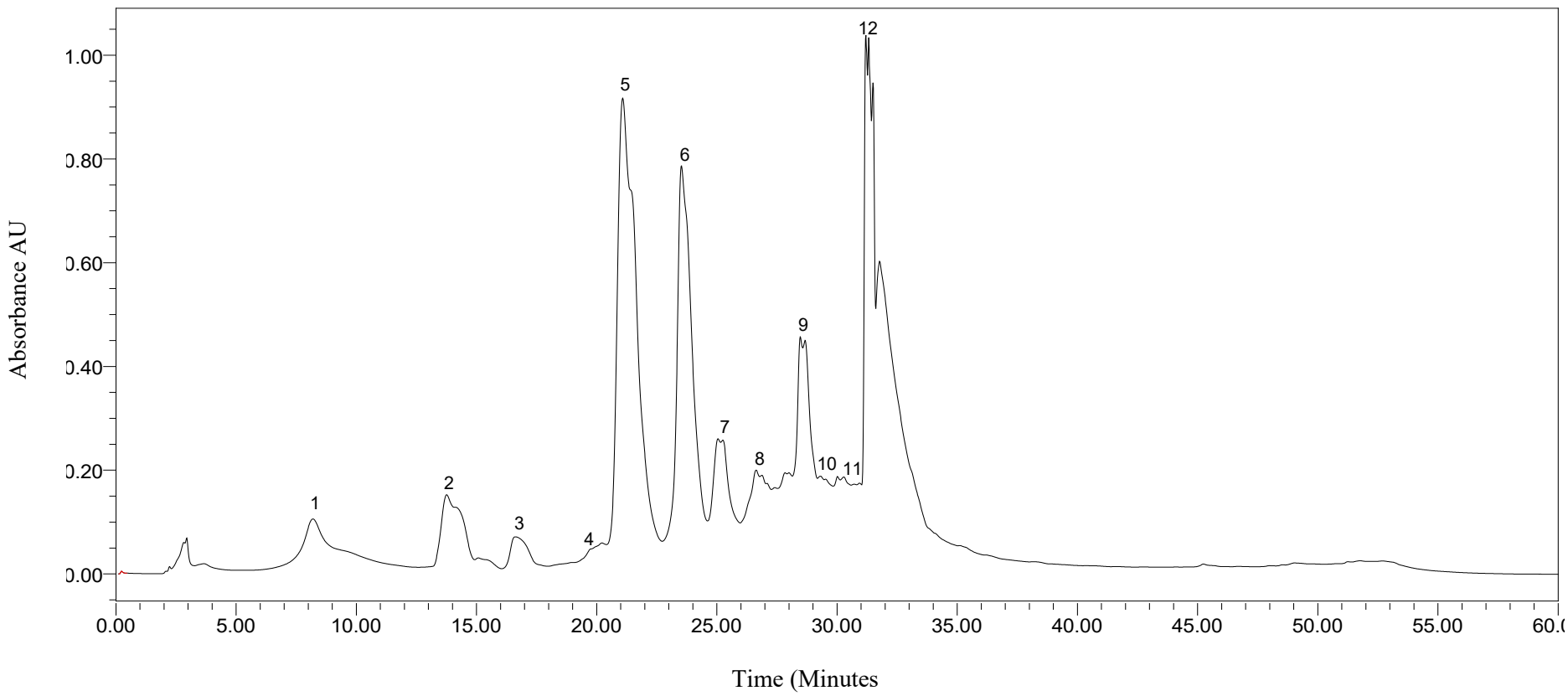


Figure 3.16 HPLC chromatogram of methanolic leaf extract of Ph/9/25 tea genotype at 280 nm. X axis represents retention time and Y axis represents absorbance. Sample was run on Kromasil 100 C18 5µ 25 × 0.46 column using water as solvent A and 80% acetonitrile as solvent B with a flow rate of 1 ml/min. Major peaks were marked either by the standard ran using the same chromatographic condition or based on sequential peak profile of the major tea alkaloids reported earlier. Peaks (1) gallic acid; (2) theobromine; (3) theophylline; (4) epigallocatechin; (5) caffeine; (6) catechin; (7) epicatechin; (8) epigallocatechin gallet; (9) gallocatechin gallet; (10) epicatechin gallet; (11) catechin gallet and (12) methanol/unknown.

Table 3.15 Retention time and % of different peaks obtained in the HPLC of methanolic leaf extract of genotype Ph/9/25. Sample was run on Kromasil 100 C18 5µ 25 × 0.46 column using water as solvent A and 80% acetonitrile as solvent B.

Peak Sl.	Marked Peak No.	Name	Retention Time	Area	Height	% Area
6	1	Gallic acid	8.198	10549416	97801	4.25
7	2	Theobromine	13.76	8801400	143059	3.54
9	3	Theophyline	16.625	3044912	60679	1.23
11	4	Epigallo catechin (EGC)	20.223	2260157	44245	0.91
12	5	Caffeine	21.083	51142327	900606	20.6
13	6	Catechin	23.53	35255944	766713	14.2
15	7	Epicatechin (EC)	25.245	7023432	235844	2.83
16	8	Epigallo catechin gallet(EGCG)	26.632	6147573	176230	2.48
23	9	Gallo catechin gallet (GCG)	28.672	10706996	423511	4.31
25	10	Epicatechin gallet	29.526	3400583	154791	1.37
27	11	Catechin gallet	30.28	4065406	158144	1.64
31	12	Methanol	31.316	9366027	998521	3.77

Tea genotype Ph/9/40: The chromatogram of Ph/9/40 (**Figure 3.17**) exhibits total 44 peaks. Among them 12 peaks were marked based on the standards that was run on the same LC system using the same chromatographic condition. Rest of the peaks were unidentified. Among these 12 peaks gallic acid showed shortest retain time and came out of the column within 4.666 minutes, while methanol exhibited highest retain time and came out of the column within 30.653 minutes (**Table 3.16**).

Among the 11 flavonoid and alkaloid content the highest percent of peak area cover by caffeine (21.22%) followed by catechin (13.31%), gallocatechin gallet (7.03%), epicatechin (2.86) and gallic acid (2.57%). The lowest percent of peak area cover by theophylline (0.1%) followed by theobromine (0.83%), epicatechin gallet (0.94%), epigallocatechin (1.1%), catechin gallet (1.54%) and epigallocatechin gallet (2.07%) (**Table 3.16**).

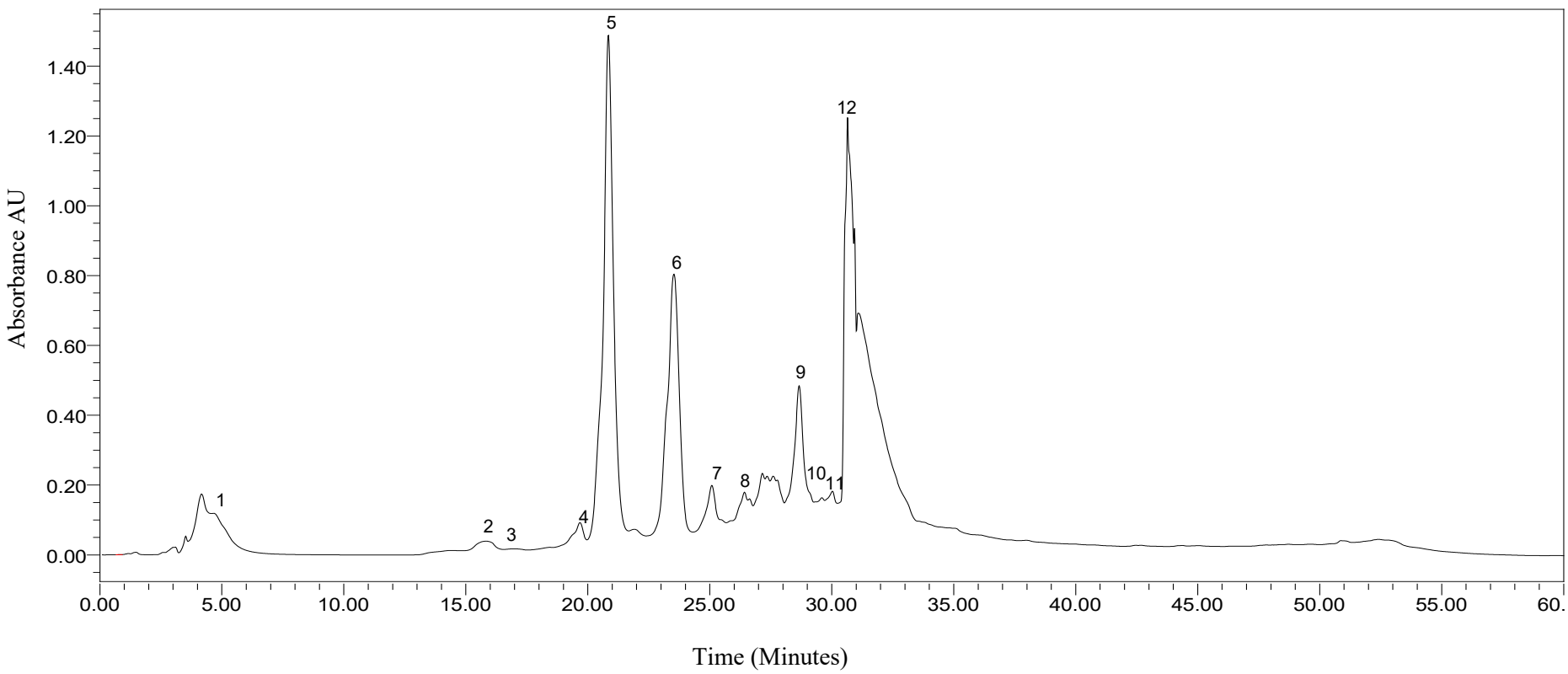


Figure 3.17 HPLC chromatogram of methanolic leaf extract of Ph/9/40 genotype at 280 nm. X axis represents retention time and Y axis represents absorbance. Sample was run on Kromasil 100 C18 5µ 25 × 0.46 column using water as solvent A and 80% acetonitrile as solvent B with a flow rate of 1 ml/min. Major peaks were marked either by the standard ran using the same chromatographic condition or based on sequential peak profile of the major tea alkaloids reported earlier. Peaks (1) gallic acid; (2) theobromine; (3) theophylline; (4) epigallocatechin; (5) caffeine; (6) catechin; (7) epicatechin; (8) epigallocatechin gallet; (9) gallocatechin gallet; (10) epicatechin gallet; (11) catechin gallet and (12) methanol/unknown.

Table 3.16 Retention time and % of different peaks obtained in the HPLC of methanolic leaf extract of the genotype Ph/9/40. Sample was run on Kromasil 100 C18 5µ 25 × 0.46 column using water as solvent A and 80% acetonitrile as solvent B.

Peak Sl.	Marked Peak No.	Name	Retention Time	Area	Height	% Area
9	1	Gallic acid	4.666	5426742	117788	2.57
11	2	Theobromine	15.847	1746810	30525	0.83
12	3	Theophyline	16.85	211825	5343	0.1
14	4	Epigallocatechin (EGC)	19.678	2318872	70635	1.1
15	5	Caffeine	20.843	44788453	1463425	21.22
17	6	Catechin	23.526	28080630	768346	13.31
18	7	Epicatechin (EC)	25.083	6034315	157317	2.86
19	8	Epigallocatechin gallet (EGCG)	26.42	4370583	132832	2.07
25	9	Gallocatechin gallet (GCG)	28.663	14841740	429728	7.03
27	10	Epicatechin gallet (ECG)	29.595	1992538	105403	0.94
28	11	Catechin gallet (CG)	30.026	3256221	122537	1.54
29	12	Methanol	30.653	30604392	1183110	14.5

Tea genotype BS-67: The chromatogram of BS-67 demonstrates (**Figure 3.18**) the lowest 27 peaks in BS-67. Among 27 peaks, 10 peaks were identified based on the standards run on the same LC system using the same chromatographic condition. Rest of the peaks were unknown. Among these 10 peaks theobromine showed shortest retain time and came out of the column within 14.25 minutes, while methanol exhibited highest retain time and came out of the column within 30.874 minutes (**Table 3.17**).

Among the 9 flavonoid and alkaloid content the highest percent of peak area cover by gallocatechin gallet (11.54%) followed by catechin (6.8%), caffeine (5.91) and epigallocatechin gallet (4.11). The lowest percent of peak area cover by theobromine (0.25%) followed by epigallocatechin (0.29%), epicatechin gallet (0.87%), catechin gallet (1.00%) and epicatechin (3.13%). Gallic acid and theophylline are absent in BS-67.

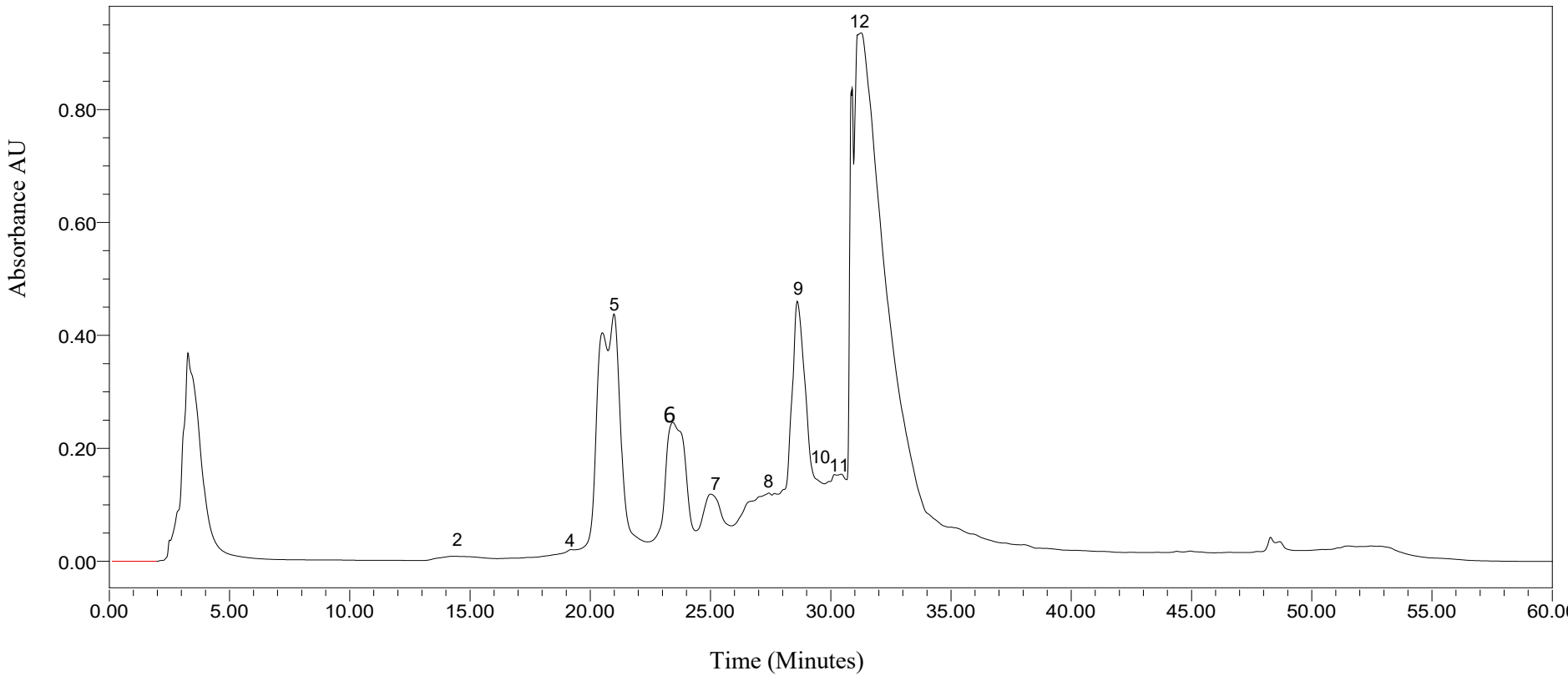


Figure 3.18 HPLC chromatogram of methanolic leaf extract of BS-67 tea genotype at 280 nm. X axis represents retention time and Y axis represents absorbance. Sample was run on Kromasil 100 C18 5µ 25 × 0.46 column using water as solvent A and 80% acetonitrile as solvent B with a flow rate of 1 ml/min. Major peaks were marked either by the standard ran using the same chromatographic condition or based on sequential peak profile of the major tea alkaloids reported earlier. Peaks (2) theobromine; (4) epigallocatechin; (5) caffeine; (6) catechin; (7) epicatechin; (8) epigallocatechin gallet; (9) gallocatechin gallet; (10) epicatechin gallet; (11) catechin gallet and (12) methanol/unknown.

Table 3.17 Retention time and % of different peaks obtained in the HPLC of methanolic leaf extract of BS-67 tea genotype. Sample was run on Kromasil 100 C18 5µ 25 × 0.46 column using water as solvent A and 80% acetonitrile as solvent B.

Peak Sl.	Marked Peak No.	Name	Retention Time	Area	Height	% Area
4	2	Theobromine	14.25	546938	5847	0.25
5	4	Epigallocatechin (EGC)	19.207	648018	14102	0.29
6	5	Caffeine	20.505	13071341	397796	5.91
8	6	Catechin	23.434	15047777	238157	6.8
9	7	Epicatechin (EC)	25.011	6938023	110245	3.13
10	8	Epigallocatechin gallet (EGCG)	27.43	9092898	111222	4.11
12	9	Gallocatechin gallet (GCG)	28.607	25541846	450805	11.54
13	10	Epicatechin gallet (ECG)	29.938	1936453	130920	0.87
14	11	Catechin gallet (CG)	30.154	2223444	143147	1
16	12	Methanol	30.874	9178801	828326	4.15

Tea genotype BT5: The HPLC chromatogram of BT5 (**Figure 3.19**) shows total 43 peaks, among which 12 peaks were identified based on the standards that was run on the same LC system using the same chromatographic condition. Rest of the peaks were unidentified. Among the 12 peaks, gallic acid showed shortest retain time and came out of the column within 7.943 minutes, while methanol exhibited highest retain time and came out of the column within 31.336 minutes (**Table 3.18**).

It is exposed from the **Table 3.18** that among the 11 flavonoid and alkaloid content the highest percent of peak area cover by caffeine (13.3%) followed by catechin (12.4%), epigallocatechin gallet (4.38%), epicatechin (4.05%), gallocatechin gallet (3.46%) and gallic acid (2.59%). The lowest percent of peak area cover by theobromine (0.65%) followed by theophylline (0.73%), epigallocatechin (0.79%), catechin gallet (1.9%) and epicatechin gallet (1.96%).

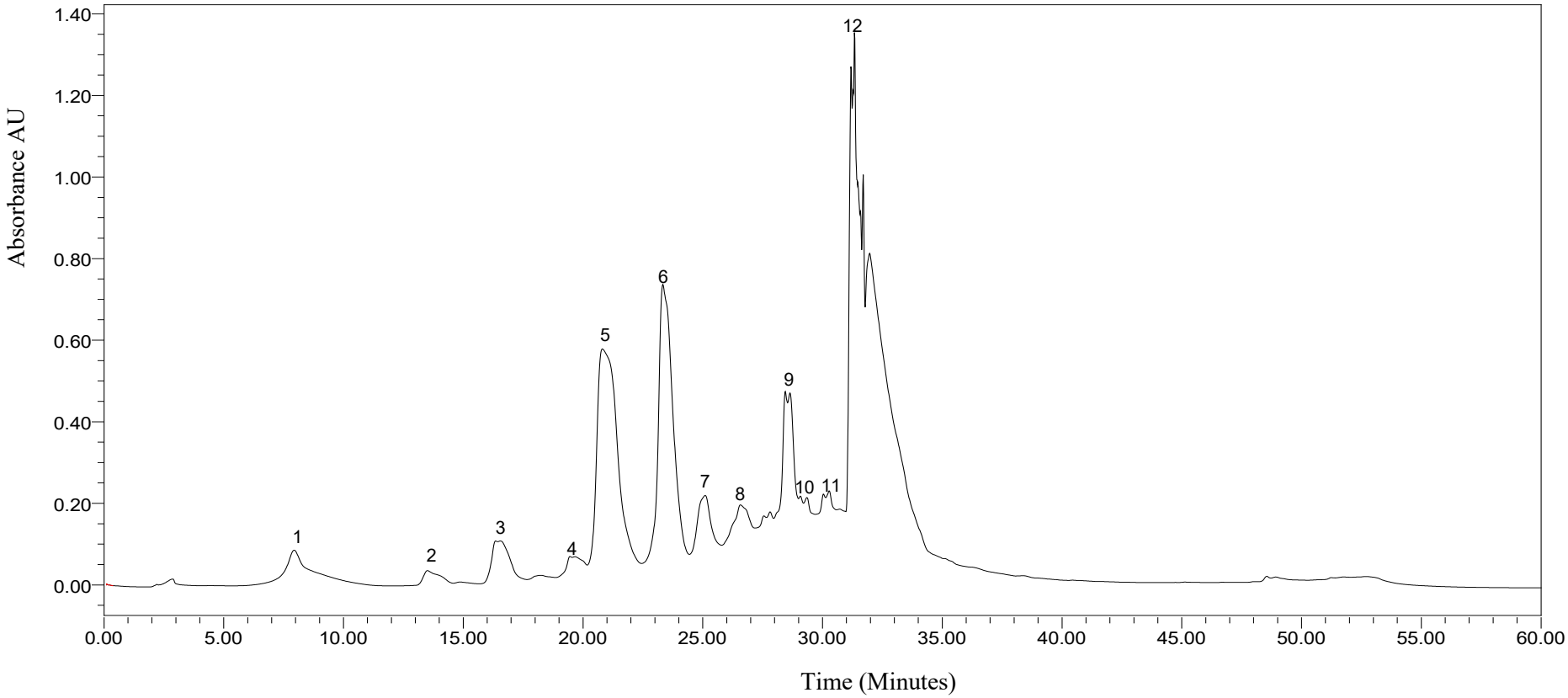


Figure 3.19 HPLC chromatogram of methanolic leaf extract of BT5 tea genotype at 280 nm. X axis represents retention time and Y axis represents absorbance. Sample was run on Kromasil 100 C18 5µ 25 × 0.46 column using water as solvent A and 80% acetonitrile as solvent B with a flow rate of 1 ml/min. Major peaks were marked either by the standard ran using the same chromatographic condition or based on sequential peak profile of the major tea alkaloids reported earlier. Peaks (1) gallic acid; (2) theobromine; (3) theophylline; (4) epigallocatechin; (5) caffeine; (6) catechin; (7) epicatechin; (8) epigallocatechin gallet; (9) gallocatechin gallet; (10) epicatechin gallet; (11) catechin gallet and (12) methanol/unknown.

Table 3.18 Retention time and % of different peaks obtained in the HPLC of methanolic leaf extract of BT5 tea genotype. Sample was run on Kromasil 100 C18 5µ 25 × 0.46 column using water as solvent A and 80% acetonitrile as solvent B.

Peak Sl.	Marked Peak No.	Name	Retention Time	Area	Height	% Area
4	1	Gallic acid	7.943	7180392	87044	2.59
5	2	Theobromine	13.511	1800403	36254	0.65
7	3	Theophylline	16.358	2025616	106869	0.73
11	4	Epigallocatechin (EGC)	19.664	2181404	66069	0.79
12	5	Caffaine	20.814	36928114	574395	13.3
13	6	Catechin	23.338	34426736	731009	12.4
14	7	Epicatechin (EC)	25.098	11246046	212386	4.05
15	8	Epigallocatechin gallet (EGCG)	26.577	12160240	188119	4.38
19	9	Gallocatechin gallet (GCG)	28.639	9601019	460936	3.46
21	10	Epicatechin gallet (ECG)	29.346	5429689	204231	1.96
23	11	Catechin gallet (CG)	30.275	5281396	219913	1.9
26	12	Methanol	31.336	25192503	1334475	9.08

Comparative study of HPLC analysis

In the present study 11 biochemical components viz., gallic acid (GA), theobromine (TB), theophylline (TP), caffeine (CAF), epigallocatechin (EGC), catechin (C), epicatechin (EC), epigallocatechin gallet (EGCG), gallocatechin gallet (GCG), epicatechin gallet (ECG) and catechin gallet (CG) were analyzed in 12 different tea genotypes through HPLC in order to find out the qualitative difference among those tea genotypes. The results which were obtained is summarized in **Table 3.19**.

Among the 12 genotypes, Ph/9/25 showed the highest percentage of gallic acid (**Table 3.19**) followed by Br2/97, E/4 and MZ/39. On the other hand, BT2 showed the lowest amount of gallic acid followed by SDL/1, Ph/9/40, BT5 and Ph/9/4. Gallic acid was totally absent in D/13, B2×T1 and BS-67.

Among the 12 tea genotypes, Ph/9/25 showed the highest performance with the value of 3.54% of theobromine followed by Br2/97 and Ph/9/4, whereas, genotype BS-67 showed the lowest amount of theobromine content followed by B2×T1, BT5, Ph/9/40, D/13, MZ/39, SDL/1, E/4 and BT2.

The tea genotype Ph/9/25 showed the highest amount of theophylline content followed by Br2/97, MZ/39 and BT5. The genotype BT2 showed the lowest theophylline content followed by Ph/9/40, Ph/9/4 and E/4. The genotype D/13, B2×T1 and BS-67 were free from theophylline content.

Out of 12 tea genotypes, Ph/9/40 showed the highest caffeine content followed by MZ/39, Ph/9/25, E/4, SDL/1 and Ph/9/4, whereas the genotype BS-67 showed the lowest amount of caffeine content followed by Br2/97, D/13, B2×T1, BT5 and BT2.

The tea genotype B2×T1 exhibited the highest amount of epigallo catechin followed by Ph/9/40, Ph/9/25, Ph/9/4, BT5 and E/4 whereas, SDL/1 showed the lowest content of epigallo catechin.

The tea genotype D/13 showed the highest content of catechin followed by Ph/9/25, Ph/9/40, BT5, MZ/39 and Ph/9/4.The genotype Br2/97 showed the lowest content of catechin followed by B2×T1, BS-67, BT2, E/4 and SDL/1.

Table 3.19 shows the tea genotype B2×T1 showed the highest content of epicatechin (4.23%) followed by D/13, BT5, MZ/39, Ph/9/4 and Br2/97, while the lowest epicatechin was found in the Ph/9/25.

The genotype E/4 exhibited the highest content of epigallo catechin gallet followed by BT5, D/13 and BS-67 whereas, the genotype Ph/9/40 showed the lowest content of epigallo catechin gallet followed by BT2, Ph/9/25, SDL/1, Ph/9/4, Br2/97, MZ/39 and B2×T1 (**Table 3.19**)..

Table 3.19 shows the tea genotype BS-67 exhibited the highest content of gallocatechin gallet followed by E/4, MZ/39, Ph/9/40 and Br2/97 whereas, the genotype B2×T1 showed the lowest content of gallocatechin gallet followed by SDL/1, BT5, Ph/9/4, Ph/9/25, BT2 and D/13.

The tea genotype B2×T1 showed the highest content of epicatechin gallet followed by SDL/1, BT5, BT2, D/13 and E/4, while the genotype BS-67 showed the lowest content of epicatechin gallet followed by Ph/9/40, MZ/39, Ph/9/25, Br2/97 and Ph/9/4.**Table 3.19** shows genotypethe highest content of catechin gallet was found in the genotype E/4 followed by SDL/1, MZ/39 and BT2. The genotype BS-67 exhibited the lowest content of catechin gallet followed by Br2/97, Ph/9/40, D/13, and Ph/9/25, B2 × T1, Ph/9/4 and BT5.

Table 3.19 A comparative study of gallic acid (GC), theobromine (TB), theophylline (TP), caffeine (CAF), (-)- epigallo catechin (EGC), (+)-catechin (C), (-)- epicatechin (EC), (-)- epigallo catechin gallet (EGCG), (-)- gallocatechin gallet (GCG), (-)- epicatechin gallet (ECG) and (-)- catechin gallet (CG) of 12 tea geotypes.

Genotypes	GA (%)	Distribution of alkaloids (%)			Total alkaloids (%)	Distribution of catechin fractions (%)							Total catechins (%)
		TB	TP	CAF		EGC	C	EC	EGCG	GCG	ECG	CG	
MZ/39	3.78	1.09	0.81	20.79	22.69	0.16	11.22	4.04	3.12	9.49	1.19	2.31	31.53
E/4	3.95	1.94	0.68	18.97	21.59	0.73	9.76	3.24	4.39	9.97	1.58	2.97	32.64
D/13	-	0.85	-	7.95	8.80	0.45	15.74	4.20	4.27	4.82	1.67	1.62	32.77
B2×T1	-	0.29	-	10.72	11.01	7.41	5.90	4.23	3.30	2.93	2.53	1.81	28.11
Br2/97	4.08	2.98	1.19	7.24	11.41	0.60	4.40	3.95	3.09	6.74	1.38	1.11	21.27
SDL/1	2.09	1.42	0.85	18.03	20.30	0.04	10.18	3.01	2.63	3.27	2.00	2.58	23.71
BT2	1.22	1.96	0.01	17.48	19.45	0.53	9.44	3.65	2.28	4.55	1.71	2.25	24.41
Ph/9/4	2.94	2.51	0.62	17.92	21.05	0.86	11.19	4.01	2.86	4.15	1.45	1.85	26.37
Ph/9/25	4.25	3.54	1.23	20.60	25.37	0.91	14.20	2.83	2.48	4.31	1.37	1.64	27.74
Ph/9/40	2.57	0.83	0.10	21.22	22.15	1.10	13.31	2.86	2.07	7.03	0.94	1.54	28.85
BS-67	-	0.25	-	5.91	6.16	0.29	6.80	3.13	4.11	11.54	0.87	1.00	27.74
BT-5	2.59	0.65	0.73	13.30	14.68	0.79	12.40	4.05	4.38	3.46	1.96	1.90	28.94

3.4. DISCUSSIONS

Morphological traits have been given importance for characterizing the germplasm. Recently, the biochemical content offered a new arena in plant improvement programme where trait specific characters played an important role in marker assisted selection. So far, baseline data on biochemical contents for the tea germplasms in BTRI are not available. However, considering the prevailing situation a holistic approach is required to identify the promising selection in perennial crop like tea.

Cup quality of the 12 tea genotypes

Overall quality of black tea should be evaluated by professional tea tasters and the prices will depend on the tasters' score. High market value is guaranteed with the opinion of tea tasters, whereas low tasters' scores indicate the poor quality of made tea. According to the tasters' quality score, MZ/39, E/4, D/13, B2 × T1, Br/2/97, SDL/1, BT2, Ph/9/4, Ph/9/25, BS-67 and BT5 can be categorized as good (above average) quality accessions whereas Ph/9/40 as average quality accessions. Those categorizations are in agreement with the results of previous studies to a greater extent (Kirthisinghe *et al.,* 1968; Alam and Haque, 2001; Anonymous, 2002), although certain accessions were not qualified to be in the same quality category.

Total phenolic compound and flavonoid assay

The systematic literature collection, pertaining to this investigation indicates that the plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators. Therefore, it is worthwhile to determine their total amount in the plants chosen for the study. Flavonoids as one of the most diverse and widespread group of natural compounds, are likely to be the most important natural phenolics (Kumaran *et al.,* 2007). These compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties. Therefore, the content of both groups of phenolics is also determined in the extracts (**Table 3.3**).

Phenolic compounds are commonly found in both edible and inedible plants, and they have been reported to have multiple biological effects, including antioxidant activity. The antioxidant activity of phenolic compounds is mainly due to their redox

properties, which can play an important role in adsorbing and neutralising free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Osawa, 1994). Crude extracts of fruits, herbs, vegetables, cereals, nuts, and other plant materials rich in phenolics are increasingly of interest in the food industry. The importance of the antioxidant constituents of plant materials in the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists, food manufacturers, and consumers (Loliger 1991). It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when up to 1.0 g was daily ingested from a diet rich in fruits and vegetables (Tanaka *et al.,* 1988).

Phenolic compounds form one of the main classes of secondary metabolites. They display a large range of structures and are responsible for the major organoleptic characteristics of plant-derived foods and beverages, particularly color and taste properties. They also contribute to the nutritional qualities of fruits and vegetables. Among these compounds, flavonoids constitute one of the most ubiquitous groups of plant phenolics. Owing to their importance in food organoleptic properties and human health, a better understanding of their structures and biological activities indicates their potentials as therapeutic agents and also for predicting and controlling food quality. Due to the variety of pharmacological activities in the mammalian body, flavonoids are more correctly referred as “nutraceuticals” (Tapas *et al.,* 2008).

In the present study the content of total phenolics in the methanolic leaf (two leaves and one bud) extracts of 12 tea genotypes were determined by Folin–Ciocalteu assay. The result of the present study reveals that the presence of the highest amount of polyphenols in BT5 (388.76 mg/g GAE) and the lowest in Br2/97 (125.32 mg/g GAE). The content of flavonoids (mg/g), in quercetin equivalents were varied from 37.5 to 195.40. The highest amounts of flavonoids were found in extracts of BS-67 (195.40) followed by SDL/1, Br2/97, MZ/39, E/4, and BT5, whereas the leaf extracts of the rest of the genotypes were possessed remarkably lower amounts of these compounds. Similar results were obtained by Karori *et al.* (2007) in tea, Sultana (2013) in *Moringa oleifera* and *Ficus recemosa* and Alam (2012) in *Withania somnifera*.

Total antioxidant capacity

The phosphomolybdenum method usually detects antioxidants such as ascorbic acid, some phenolics, a-tocopherol, and carotenoids. The phosphomolybdenum method was based on the reduction of Mo(VI) to Mo(V) by the antioxidant compound. In essence, it is believed that the molybdenum is easier to be reduced in the complex and electron-transfer reaction occurs between reductants and Mo(VI) and the formation of a green phosphate/Mo(V) complex with a maximal absorption at 695 nm. The assay is successfully used to quantify vitamin E in seeds and, being simple and independent of other antioxidant measurements commonly employed, it was decided to extend its application to plant extracts. Moreover, it is a quantitative one, since the antioxidant activity is expressed as the number of equivalents of ascorbic acid. The present study reveals that the antioxidant activity of the extract is in the increasing trend with the increasing concentration of the plant extract. The antioxidant activity of the tea genotypes are in the order: BT5 > Ph/9/25 > Br2/97 > SDL/1 > Ph/9/4 > Ph/9/40 > BS-67 > E/4 > BT2 > D/13 > B2×T1 > MZ/39. Similar result were obtain by Karori *et al.* (2007) in different types of tea product, Alam (2012) in *Withania somnifera* and Sultana (2013) in *Moringa oleifera* and *Ficus recemosa*.

DPPH radical scavenging activity

DPPH free radical is reduced to the corresponding hydrazine when it reacts with hydrogen donors. Free radicals are known to be a major factor in biological damages, and DPPH has been used to evaluate the free radical scavenging activity of natural antioxidants. The 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) has been widely used to evaluate the free radical scavenging capacity of antioxidants (Brand-Williams *et al.,* 1995; Espin *et al.,* 2000; Yu, 2001). It is well known that free radicals are able to induce lipid per oxidation. DPPH can make stable free radicals in aqueous or methanol solution. The determination of scavenging stable DPPH was a very fast method to evaluate the antioxidant activity of the extracts. With this method it was possible to determine the antiradical power of an antioxidant activity by measurement of the decrease in the absorbance of DPPH at 517 nm. Resulting from a colour change from purple to yellow the absorbance decreased when the DPPH was scavenged by an antioxidant, through donation of hydrogen to form a stable DPPH molecule. In the

radical form this molecule had an absorbance at 517 nm which disappeared after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule (Matthaus, 2002). The scavenging effect of methanol extracts and standards with the DPPH radical is in the following order (according to IC_{50} value):

BHT > BT5 > Ph/9/25 > Br2/97 > SDL/1 > Ph/9/4 > BT2 > D/13 > MZ/39 > Ph/9/40 > BS-67 > E/4 > B2 × T1

The reduction capability of the DPPH radical is determined by the decrease in its absorbance at 517 nm, induced by antioxidants. In order to evaluate the antioxidant potency through free radical scavenging by the test samples, the change of optical density of DPPH radicals was monitored. **Figure 3.6** showed the decrease in absorbance of DPPH radical due to the scavenging ability of soluble solids in different concentrations of plant extract and standard BHT.

The DPPH radical scavenging activities of the total extracts of the tea genotypes and of BHT are shown in **Table 3.6** and **Figure 3.6** and **3.7**. The most potent activity was found in methanolic extract of genotypes BT5 leaves with IC_{50} value of 39 µg/ml followed by leaf extract of Ph/9/25 and Br2/97. And the methanolic extracts of SDL/1, Ph/9/4, BT2, D/13, MZ/39, Ph/9/40, BS-67 and E/4 also showed moderately free radical scavenging activity. Only one sample named B2 × T1 of the present investigation did not show IC_{50} within the concentration range of 25 – 200 µg/ml. This sample may require more than 200 µg/ml extract for showing IC_{50}. The experimental data of these tea genotypes reveal that all these extracts are likely to have the effect of scavenging free radical. Similar result were obtain by Karori *et al.* (2007) in different types of tea product, Alam (2012) in *Withania somnifera* and Sultana (2013) in *Moringa oleifera* and *Ficus recemosa*.

From **Figure 3.6** we also observe that a dose–response relationship is found in the DPPH radical scavenging activity; the activity increased as the concentration increased for each individual tea genotypes. The involvement of free radicals, especially their increased production, appears to be a feature of most, if not all human diseases, including cardiovascular disease and cancer. It has been found that cysteine,

glutathione, ascorbic acid, tocopherol, flavonoids, tannins, and aromatic amines (p-phenylene diamine, p-aminophenol, etc.), reduce and decolourise DPPH by their hydrogen donating ability. Phenolic compounds of the plant extracts are probably involved in their antiradical activity.

Determination of gallic acid, four major alkaloids and catechin fraction through RP- HPLC

Eleven phenolic and flavonoid standards were compared with the chromatograms produced by the unknown 12 tea genotypes extracts. The chromatogram showed several numbers of peaks in different tea genotypes. There were 12 peaks were identified in all tea genotypes except D/13, B2×T1 and BS-67. In these three genotypes, 10 peaks were marked based on the standards that was run on the same LC system using the same chromatographic condition.

In the present study 11 biochemical characters such as gallic acid (GA), theobromine (TB), theophylline (TP), caffeine (CAF), (-)-epigallocatechin (EGC), (+)-catechin (C), (-)-epicatechin (EC), (-)-epigallocatechin gallet (EGCG), (-)-gallocatechin gallet (GCG), (-)-epicatechin gallet (ECG) and (-)-catechin gallet (CG) were analyzed in 12 different tea genotypes through HPLC in order to find out the qualitative difference among those tea genotypes.

Though, BTRI clones released for cultivation or commercial exploitation they are belonging to one geographical origin within North-East part (e.g. Sylhet) of Bangladesh. Most of them were identified from different tea estate in the country or selected from hybrid progenies. These clones or genotypes contained variable quantum of gallic acid, total alkaloids and total catechins (**Table 3.19**). Variations are not only observed in their phenological but also in genetic characteristics (Aziz *et al.*, 2011 and Sarwar *et al.*, 2002). Tea genotypes of the present investigation exhibited wide variation in their phenotypic characteristics.

Among the 12 genotypes, Ph/9/25 showed the highest percentage of gallic acid followed by Br2/97, E/4 and MZ/39. BT2 performed lowest percent of gallic acid followed by SDL/1, Ph/9/40, BT5 and Ph/9/4. Gallic acid is totally absent in D/13, B2×T1 and BS-67.

In respect of total alkaloid content (theophylline, theobromine and caffeine), Ph/9/25 exhibited the highest percent distribution followed by MZ/39, Ph/9/40, E/4, Ph/9/4 and SDL/1. Genotype BS-67 showed the lowest distribution of total alkaloids followed by D/13, B2×T1, Br2/97, BT5 and BT2. The highest amount caffeine content was recorded in Ph/9/40 followed by MZ/39 and Ph/9/25. The caffeine content was the lowest in BS-67.

The quantity of the catechins content was also varied with different tea genotypes. Although there was a marginal variation in amount of catechin fractions, total catechin contents seems to be very high in D/13 followed by E/4 and MZ/39. While Br2/97 registered lower values of total catechins and their fractions. Results obtained in this study confirmed the earlier studies on biochemical and metabolic functions of tea clones (Ranganath and Marimuthu, 1992; Rajkumar *et al.*, 1993 and Saravanan *et al.*, 2005).

Results presented here using catechins content of 12 genotypes can form a basis for further exploitation of identification of potential or premium quality mother bushes from the existing tea germplasm. In this study, high values of catechin ratio used along with other characteristics like caffeine content as a marker to identify the superior quality clone.

3.5. SUMMARY

Present study was carried out for the analyses of the major biochemical and nutritional components of 12 selected tea genotypes of BTRI. This investigation includes evaluation of the tea quality through organoleptic test, determination of total polyphenol, antioxident activity, determination and quantification of different biochemical compounds (gallic acid, theobromine, theophylline, epigallocatechin, caffeine, catechin, epicatechin, epigallocatechin gallet, gallocatechin gallet, epicatechin gallet and catechin gallet) using HPLC.

Present study reveals that the significant difference in total tea quality tasting score among the 12 genotypes of tea. According to the tasters' quality score, MZ/39, E/4, D/13, B2 × T1, Br/2/97, SDL/1, BT2, Ph/9/4, Ph/9/25, BS-67 and BT5 could be categorized as good (above average) quality accessions whereas Ph/9/40 as average.

The results on biochemical analyses showed that the amount of total phenolic compounds was significantly different among the tea genotypes. Among the 12 genotypes, the highest amount of phenolics was found in BT5 and the lowest in genotype Br2/97. Total flavonoid contents were also significantly different among the tea genotypes. Among the 12 tea genotypes, the highest total flavonoid content was recorded in the genotype BS-67.

The content of phenolics in the leaf extracts correlates with the antioxidant activity. The total antioxidant capacity determined as equivalent to ascorbic acid of the methanolic extract of fresh leaves of 12 tea genotypes were significantly different. Among the 12 genotypes the highest total antioxidant capacity was recorded in BT5. The antioxidant capacity of the tea genotypes are in the order: BT5 > Ph/9/25 > Br2/97 > SDL/1 > Ph/9/4 > Ph/9/40 > BS-67 > E/4 > BT2 > D/13 > B2×T1 > MZ/39.

In DPPH assay free radical scavenging activity of the extracts were evaluated comparing with butylated hydroxytoluene (BHT) at 519 nm. Among all the selected genotypes BT5 showed the highest antioxidant activity followed by the genotypes Ph/9/25 and Br2/97 leaves with IC_{50} value of 39 µg/ml, 40.5 µg/ml and 42.5 µg/ml, respectively.

Present study shows that these biochemical components viz., gallic acid, theobromine, theophylline, caffeine, epigallocatechin, catechin, epicatechin, epigallocatechin gallet, gallocatechin gallet, epicatechin galle tand catechin gallet analyzed in 12 different tea genotypes through HPLC were quantitatively different those tea genotypes. Results reveal that among the 12 genotypes, Ph/9/25 showed the highest percentage of gallic acid whereas, BT2 performed lowest percent of gallic acid. On the other hand, Gallic acid is totally absent in D/13, B2×T1 and BS-67.

Similarly, in respect of total alkaloid content, Ph/9/25 exhibited the highest percent distribution and the genotype BS-67 showed the lowest distribution of total alkaloids. It was also revealed that the quantity of the catechins content was also varied with different tea genotypes. Although there was a marginal variation in amount of catechin fractions, total catechin contents seems to be very high in D/13 among the 12 tea genotype, while Br2/97 registered lower values of total catechins and their fractions.

Results presented here using catechins content of 12 genotypes can form a basis for further exploitation of identification of potential or premium quality mother bushes from the existing tea germplasm. In this study, high values of catechin ratio used along with other characteristics like caffeine content as a marker to identify the superior quality clone. In this respect MZ/39 and E/4 was found to be the most suitable tea genotype that deserve commercial exploitation.

Chapter 4

ANALYSIS OF GENETIC DIVERSITY IN TWELVE TEA GENOTYPES WITH RAPD MARKER

4.1. INTRODUCTION

The tea plant [Camellia sinensis (L) O. Kuntze], which belongs to section *Thea*, genus *Camellia*, family Theaceace, originated in southwestern China, Yunnan province (Hashimoto and Takasi 1978; Yu 1986). It is the most commercially and ecologically important species in the genus. It is believed that all of the tea plants around the world were directly or indirectly introduced from China. Tea contributes greatly to wealth and job opportunities in several Asian, African and South American countries, including Bangladesh, China, India, Sri Lanka, Kenya, etc. Self-incompatibility and long-term of allogamy make the tea plant to be highly heterogeneous and consequently broad genetic variation. Tea genetic resource is presently one of the most valuable fundamental materials for tea breeding with valuable potential for the whole tea industry in the future. There are a numbers of genetic resources, including the tea plant and its allied species and varieties in genus *Camellia* have been collected and preserved in Bangladesh (Rashid, 1983; Alam *et al.* 1997), China (Chen and Yamaguchi 2002), Japan (Takeda 2000), India, Kenya, etc. The success of tea genetic resource collection, preservation, exploitation, utilization, present and long-term breeding programs depend largely on the knowledge and understanding of the genetic background, diversity, relationship and identification.

Randomly amplified polymorphic DNA (RAPD) (Williams *et al.* 1990), which combined the advantages of low technical input with almost unlimited marker numbers, has proven quite useful in woody plant DNA diversity, genetic relationships and identification studies, such as in apple (*Malus*) (Landry *et al.* 1994), coffee (*Cofea*), (Orozec-Castillo *et al.* 1996) 1996), spruce (*Picea*) (Khasa and Dancik 1996), date palm (*Phoenix dactylifera*) (Sedra *et al.* 1998), hazelnut (*Corylus avellana*) (Galderisi *et al.* 1999), olive (*Olea europaea*) (Belaj *et al.* 2001), pecan

(*Carya illinoensis*) (Conner and Wood 2001), and in Indian cashew (*Anacarditum occidentale*) (Dhanaraj *et al.* 2002). More recently, it has also been used in tea for the investigation of genetic relationships (Wachira *et al.* 1995; Lee *et al.* 1995), identification of parentage (Tanaka and Yamaguchi 1996; Tanaka *et al.* 2001), evaluation of DNA polymorphism (Kaundun *et al.* 2000; Park et al. 2002), detection of genetic stability (Chen *et al.* 1999) and integrity (Mondal and Chand 2002). RAPD marker has also been used in genetic linkage mapping (Hackett *et al.* 2000), reconstructing and assessing the phylogeny of tea plant and its allied species in section *Thea* (Chen and Yamaguchi 2002) and genus *Camellia* (Wachira *et al.* 1997). However, the genetic diversity, relationship and molecular identification of Bangladeshi tea genetic resources using RAPD markers has not been reported yet. These are of critical importance for various tea genetic resource and breeding programs, both for Bangladesh and other tea growing countries.

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.

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).

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 counterpart, 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 into 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.

4.1.1. Objectives

The objectives of this study were to determine the genetic diversity and to evaluate the genetic relationship of the typical Bangladeshi elite tea genetic resources preserved in Bangladesh Tea Research Institute (BTRI) with RAPD markers.

4.2. MATERIALS AND METHODS

4.2.1. Plant Material

Twelve tea genotypes were used as planting material for the present study. These genotypes were collected from the different tea estate of Bangladesh and preserved as *in situ* at BTRI's germplasm. Among the genotypes BT2, BT5 and B2×T1 (recently released as BT17) are using as commercial tea cultivars in the country's tea estates. Detail of the 12 tea genotypes are shown in **Table 2.1** (**Chapter 2**).

4.2.2. Genomic DNA Isolation

Tender unfold leaf samples from 12 tea genotypes were collected from clonally propagated plants and stored at -80^{0}C for DNA extraction. The total genomic DNA was extracted from the stored leaf samples using the modified CTAB method (Murray and Thompson, 1980) and purified according to standard method (Sambrook and Russell, 2001). Concentration of the purified genomic DNA in each case was adjusted to 10 ng/µL in different aliquots and stored at -20^{0}C for use in PCR amplification.

4.2.3. PCR Amplification for RAPD

Initially 100 random decamer primers of kits A, B, C, D (Operon Technologies, Inc., USA) were used for PCR amplification in order to select appropriate primers that generate recognizable polymorphic bands. On the basis of PCR performance 10 primers out of 100 were selected and used for RAPD generation (**Table 4.1**). PCR reactions were performed in a 25 µL reaction mixture containing 10 µL template DNA (30 ng), 2.5 µL 10× PCR buffer [500 m*M* KCl, 100 m*M* Tris-HCl (pH 8.0), 15 m*M* $MgCl_2$, 0.1% Gelatin], 2 µL dNTPs mixture (100 n*M*), 5 µL of single 10-mer random primer (50 n*M*), 1 µL of 1 Unit Taq DNA polymerase and final volume was adjusted by adding sterile distilled water. A negative control with no DNA was included in each PCR run. The PCR amplification reactions were performed in a Gene Amp PCR System 2400 (Perkin-Elmer) programmed for an initial denaturation step of 94^{0}C for 4 minutes, followed by 35 cycles of 1 minute at 94^{0}C, 1 minute at 36^{0}C and 2 minutes at 72^{0}C. After the last cycle, a final step of 7 minutes at 72^{0}C was added to allow complete extension of all amplified fragments and reaction was ended at 4^{0}C forever. The PCR amplified products (RAPD) was size fractionated in 1%

agarose gel in 1× TAE buffer and visualized the DNA bands on UV-transilluminator after staining with ethidium bromide and photographed.

Table 4.1 Random primer used in the present study for screening

Primer Code	Sequence (5′ - 3′)	TM or annealing temperature (^{0}C)
OPA 7	GAA ACG GGT G	32
OPA 9	GGG TAA CGC C	34
OPA 10	GTG ATC GCC G	34
OPB 10	CTG CTG GGA C	34
OPB 13	TTC CCC CGC T	34
OPC 9	CTC ACC GTC C	34
OPC 17	TTC CCC CCA G	34
OPD 3	GTC GCC GTG A	34
OPD 8	GTG TGC CCC A	34
OPD 15	CAT CCG TGC T	32

4.2.4. Statistical Analysis

The genetic diversity of tea populations was analysed by DNA fingerprints. The DNA bands at the same molecular weight are known as common bands, which means that they came from the same DNA loci. The same DNA loci means that the band in DNA fingerprints was at one site without polymorphism. Bands at different molecular weights mean that they were special DNA bands with polymorphism (Wang *et al.*, 1996). The typical displayed band was marked as ‘1’, and the untypical and absent bands were marked as ‘0’. The DNA bands were all characterized according to their molecular weights. Data were computerized in 1/0 matrix form, and analysed by the software PAST (PAleontological STatistics). The size of the fragments (molecular weight in base pairs) were estimated by using 100 bp ladder markers, which was run along with the amplified products. A genetic dissimilarity matrix was calculated according to Squared Euclidean Distance which estimated all pair-wise differences in the amplification was done by Ward’s method using variance algorithm (Ward, 1963). A phylogenetic dendogram was constructed using PAST software.

4.3. RESULTS

Ten primers used viz., OPA-7, OPA-9, OPA-10, OPB-10, OPB-13, OPC-9, OPC-17, OPD-3, OPD8 and OPD-15 for the amplification of genomic DNA of 12 different tea genotype. The scored on total no. of bands, no. of monomorphic bands, no. of polymorphic bands, % of polymorphism and the range of band size. The results on these parameters are shown in **Table 4.2.**

It reveals from **Table 4.2** that primer OPA7 gave 7 different bands. Among these bands, 6 were polymorphic and 1 was monomorphic bands. Primer OPA-9 gave 5 different bands. Among these bands, 3 were polymorphic and 2 were monomorphic bands. Primer OPA10 generated 12 different bands. Out of these, 8 bands were polymorphic and 4 were monomorphic bands. Primer OPB10 gave 14 different bands and in them 12 were polymorphic and 2 were monomorphic bands. Primer OPB13 generated 13 different bands and among these 9 bands were polymorphic and 4 were monomorphic band. Primer OPC9 gave 6 different bands and among them, all were polymorphic. Primer OPC17 contributed 4 different bands and among them 1 was monomorphic and 3 were polymorphic. Among 9 different bands, 7 were polymorphic and 2 were monomorphic bands, which were contributed by OPD3 primers. Primer OPD8 gave 6 different bands and among these 4 were polymorphic and 2 were monomorphic bands. Primer OPD15 contributed 7 different RAPD bands and among those 6 were polymorphic and 1 was monomorphic bands. The 10 primers generated different bands with size ranging from 125-9000 bp. Out of the 83 bands, 64 scorable bands (77.11%) were found to be polymorphic and 19 bands (22.89%) were found to be monomorphic. These results gave an average of polymorphic bands per primer was shown in **Table 4.2**.

Table 4.2 Analysis of polymorphisms obtained with RAPD primers in clonal tea cultivars.

Primers	Total no. of RAPD bands (a)	No. of monomorphic bands	No. of polymorphic bands (b)	Polymorphism b/a × 100 (%)	Range of fragment size (bp)	
					Min.	Max.
OPA 7	7	1	6	85	125	5000
OPA 9	5	2	3	60	1000	5000
OPA 10	12	4	8	66.67	500	2300
OPB 10	14	2	12	85.71	500	2300
OPB 13	13	4	9	69.23	125	9000
OPC 9	6	0	6	100	1500	9000
OPC 17	4	1	3	75	1500	9000
OPD 3	9	2	7	77.78	1000	5000
OPD 8	6	2	4	66.67	500	5000
OPD 15	7	1	6	85.71	300	1600
Total	**83**	**19**	**64**	**771.77**	**-**	**-**
Average	**8.3**	**1.9**	**6.4**	**77.18**		

4.3.1. Genetic Diversity Analysis in 12 Tea Genotypes with RAPD Markers

4.3.1.1. Genetic diversity analysis with primer OPA7

The segregated RAPD bands amplified with primer OPA7 with 12 tea genotypes are shown in **Figure 4.1**.

Phylogenetic dendogram generated with the primer OPA7 shows that the 12 tea genotypes were categorized into three main groups when divided by the genetic distance of 1.8, the average genetic distance of all the 12 tea genotypes (**Figure 4.2**). Group A contained three genotypes, i.e., E/4, Ph/9/25 and BT5. Group B included six tea genotypes, i.e., MZ/39, D/13, Ph/9/40, B2×T1, SDL/1 and BS-67. These six genotypes are further divided into two sub-groups, i.e., S_1 and S_2. Sub-group S_1 was comprised MZ/39, D/13 and Ph/9/40. On the other hand, S_2 sub-group was covered B2×T1, SDL/1 and BS-67. Group C included three genotypes, i.e., Br2/97, BT2 and Ph/9/4. Among these three genotypes, Br2/97 and BT2 were covered under S_3 subgroup.

Table 4.3 shows the Jaccard's similarity coefficient matrix. This table reveals a wide range (0.25 to 1.00) of similarity among the tea genotypes. The similarity coefficient among the three genotypes (MZ/39, D/13 and Ph/9/40) of sub-group S_1 and the three genotypes (B2×T1, SDL/1 and BS-67) of sub-group S_2 are the highest, at 1.00 with 1.2 unit distance. The similarity coefficient between two genotypes (Br2/97 and BT2) of sub-group S_3 is also the highest, at 1.00 with 1.1 unit distance.

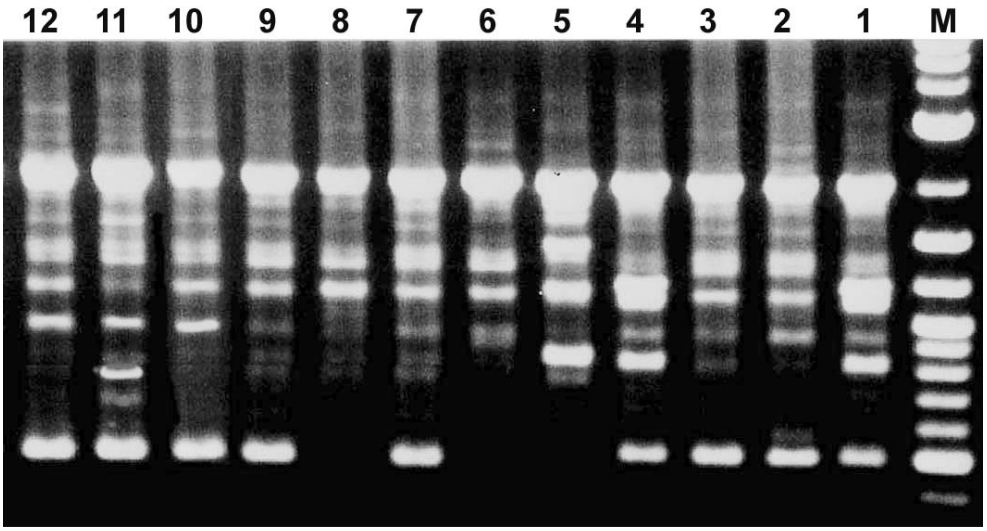


Figure 4.1 RAPD pattern amplified with primer OPA7 from genomic DNA of 12 tea genotypes. Lanes M= 1 kb size marker, 1= MZ/39, 2= E/4, 3= D/13, 4= B2×T1, 5= Br2/97, 6= SDL/1, 7= BT2, 8= Ph/9/4, 9= Ph/9/25, 10= Ph/9/40, 11= BS-67 and 12= BT5.

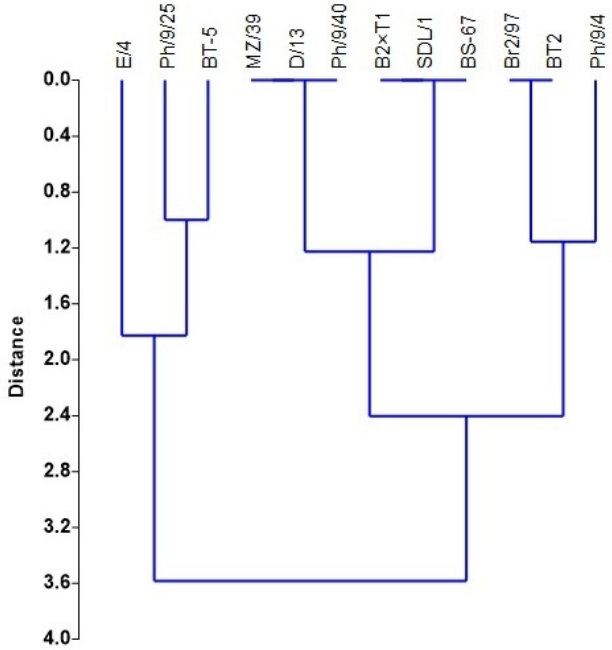


Figure 4.2 Phylogenetic dendogram generated from the banding pattern developed with primer OPA7 from the genomic DNA of 12 tea genotypes.

Table 4.3 Jaccard's similarity matrix of gel banding pattern generated by the primer OPA7 from the genomic DNA of 12 tea genotypes.

Genotypes	MZ/39	E/4	D/13	B2×T1	Br2/97	SDL/1	BT2	Ph/9/4	Ph/9/25	Ph/9/40	BS-67	BT-5
MZ/39	**1.00**											
E/4	0.57	**1.00**										
D/13	1.00	0.57	**1.00**									
B2×T1	0.80	0.43	0.80	**1.00**								
Br2/97	0.60	0.29	0.60	0.75	**1.00**							
SDL/1	0.80	0.43	0.80	1.00	0.75	**1.00**						
BT2	0.60	0.29	0.60	0.75	1.00	0.75	**1.00**					
Ph/9/4	0.43	0.38	0.43	0.50	0.60	0.50	0.60	**1.00**				
Ph/9/25	0.67	0.38	0.67	0.50	0.33	0.50	0.33	0.43	**1.00**			
Ph/9/40	1.00	0.57	1.00	0.80	0.60	0.80	0.60	0.43	0.67	**1.00**		
BS-67	0.80	0.43	0.80	1.00	0.75	1.00	0.75	0.50	0.50	0.80	**1.00**	
BT-5	0.67	0.57	0.67	0.50	0.33	0.50	0.33	0.25	0.67	0.67	0.50	**1.00**

4.3.1.2. Genetic diversity analysis by primer OPA9

The segregated RAPD bands amplified with primer OPA9 with 12 tea genotypes are shown in **Figure 4.3**.

Phylogenetic dendogram (**Figure 4.4**) shows that the 12 tea genotypes are categorized into three main groups when divided by the average genetic distance 1.4 of all the 12 tea genotypes. Group A contained two genotypes, i.e., MZ/39 and D/13. Group B included five tea genotypes, i.e., Ph/9/4, E/4, Ph/9/40, BS-67 and BT5. Among the five genotypes, four genotypes, i.e., E/4, Ph/9/40, BS-67 and BT5 were included under sub-group S_1. Group C comprised five genotypes, i.e., B2×T1, Br2/97, SDL/1, BT2 and Ph/9/25. Among these five genotypes, B2×T1, Br2/97, SDL/1 and BT2 were covered under S_2 sub-group.

Jaccard's similarity matrix calculated from the banding pattern generated by the primer OPA9 is shown in **Table 4.4**. The **Table 4.4** shows that a wide range (0.40 to 1.00) of similarity was noticed among the tea genotypes. The similarity coefficient among the four genotypes (E/4, Ph/9/40, BS-67 and BT5) of sub-group S_1 and the four genotypes (B2×T1, Br2/97, SDL/1 and BT2) of sub-group S_2 are the highest, at 1.00 with 0.9 unit distance.

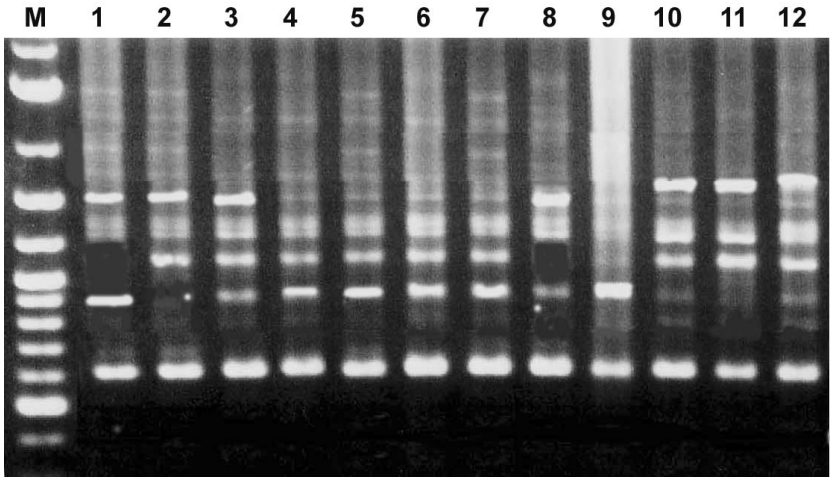


Figure 4.3 RAPD pattern amplified with primer OPA9 from the genomic DNA of tea genotypes. Lanes M= 1 kb size marker, 1= MZ/39, 2= E/4, 3= D/13, 4= B2×T1, 5= Br2/97, 6= SDL/1, 7= BT2, 8= Ph/9/4, 9= Ph/9/25, 10= Ph/9/40, 11= BS-67 and 12= BT5.

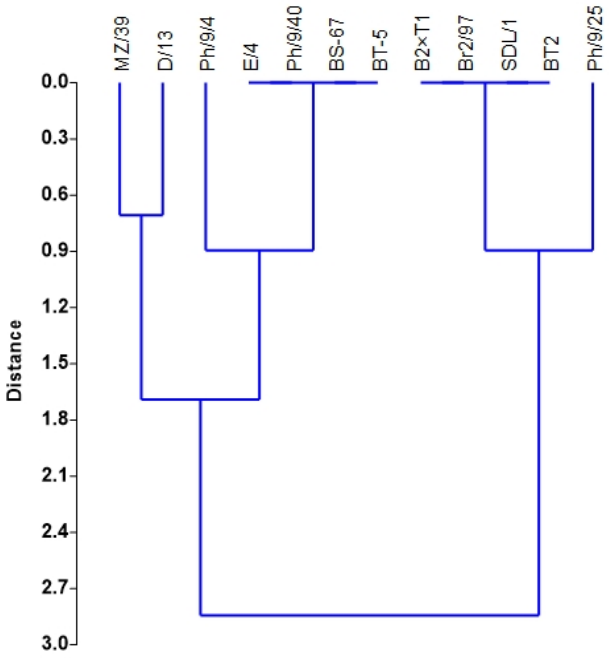


Figure 4.4 Phylogenetic dendogram generated from the banding pattern developed with the primer OPA9 from the genomic DNA of 12 tea genotypes.

Table 4.4 Jaccard's similarity matrix of gel banding patterns generated with the primer OPA9 from the genomic DNA of 12 tea genotypes.

Genotypes	MZ/39	E/4	D/13	B2×T1	Br2/97	SDL/1	BT2	Ph/9/4	Ph/9/25	Ph/9/40	BS-67	BT-5
MZ/39	**1.00**											
E/4	0.60	**1.00**										
D/13	0.80	0.80	**1.00**									
B2×T1	0.60	0.60	0.80	**1.00**								
Br2/97	0.60	0.60	0.80	1.00	**1.00**							
SDL/1	0.60	0.60	0.80	1.00	1.00	**1.00**						
BT2	0.60	0.60	0.80	1.00	1.00	1.00	**1.00**					
Ph/9/4	0.75	0.75	0.60	0.40	0.40	0.40	0.40	**1.00**				
Ph/9/25	0.75	0.40	0.60	0.75	0.75	0.75	0.75	0.50	**1.00**			
Ph/9/40	0.60	1.00	0.80	0.60	0.60	0.60	0.60	0.75	0.40	**1.00**		
BS-67	0.60	1.00	0.80	0.60	0.60	0.60	0.60	0.75	0.40	1.00	**1.00**	
BT-5	0.60	1.00	0.80	0.60	0.60	0.60	0.60	0.75	0.40	1.00	1.00	**1.00**

4.3.1.3. Genetic diversity analysis by primer OPA10

The segregated RAPD bands amplified with primer OPA10 with 12 tea genotype are shown in **Figure 4.5**.

Phylogenetic dendogram generated with the primer OPA10 shows that the 12 tea genotypes were categorized into two main groups when divided by the genetic distance of 2.7, the average genetic distance of all the 12 tea genotypes (**Figure 4.6**). Group A contained seven genotypes, i.e., B2×T1, SDL/1, E/4, Ph/9/25, D/13, Br2/97, and Ph/9/4. These seven genotypes were further divided into three sub-groups, i.e., S_1, S_2 and S_3. Sub-group S_1 comprised B2×T1 and SDL/1. On the other hand, S_2 sub-group was covered E/4 and Ph/9/25. And S_3 sub-group of group A was roofed by three genotypes such as D/13, Br2/97, and Ph/9/4. Group B included three genotypes, i.e., MZ/39, BT5, BT2, Ph/9/40 and BS-67. Among these five genotypes, MZ/39 and BT5 were covered under S_4 subgroup and BT2, Ph/9/40 and BS-67 were roofed under the sub-group S_5 of group B.

Table 4.5 shows the Jaccard's similarity coefficient matrix. This table reveals a wide range (0.42 to 1.00) of similarity among the tea genotypes. The similarity coefficient between the two genotypes (E/4 and Ph/9/25) of sub-group S_2 and among the three genotypes (D/13, Br2/97 and Ph/9/4) of sub-group S_3 are the highest, at 1.00 with 1.3 unit and 2.0 unit distance, respectively.

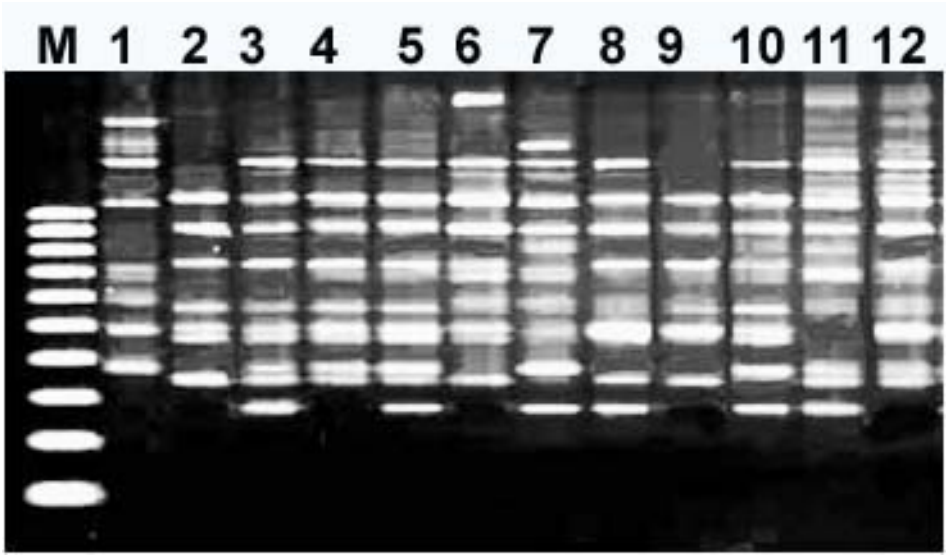


Figure 4.5 RAPD pattern amplified with primer OPA10 from genomic DNA of 12 tea genotypes. Lanes M= 100 bp size marker, 1= MZ/39, 2= E/4, 3= D/13, 4= B2×T1, 5= Br2/97, 6= SDL/1, 7= BT2, 8= Ph/9/4, 9= Ph/9/25, 10= Ph/9/40, 11= BS-67 and 12= BT5.

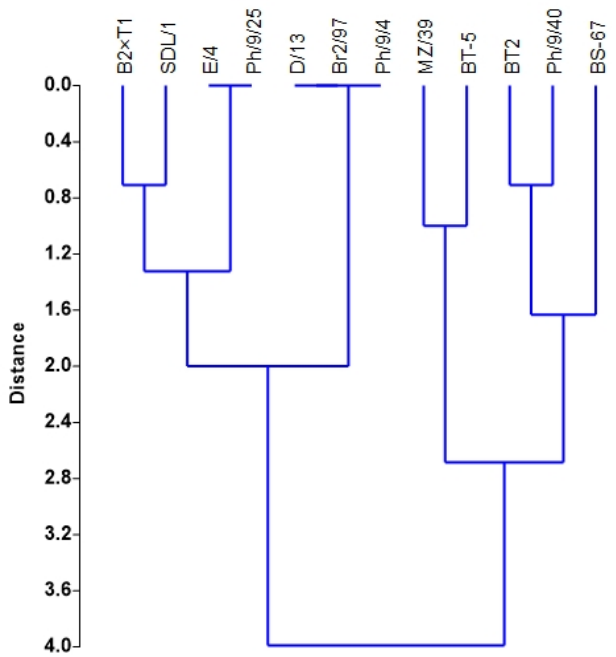


Figure 4.6 Phylogenetic dendogram generated from the banding pattern developed with primer OPA10 from the genomic DNA of 12 tea genotypes.

Table 4.5 Jaccard's similarity matrix of gel banding pattern generated by the primer OPA10 from the genomic DNA of 12 tea genotypes.

Genotypes	MZ/39	E/4	D/13	B2×T1	Br2/97	SDL/1	BT2	Ph/9/4	Ph/9/25	Ph/9/40	BS-67	BT-5
MZ/39	**1.00**											
E/4	0.56	**1.00**										
D/13	0.60	0.75	**1.00**									
B2×T1	0.67	0.86	0.88	**1.00**								
Br2/97	0.60	0.75	1.00	0.88	**1.00**							
SDL/1	0.60	0.75	0.78	0.88	0.78	**1.00**						
BT2	0.64	0.60	0.80	0.70	0.80	0.64	**1.00**					
Ph/9/4	0.60	0.75	1.00	0.88	1.00	0.78	0.80	**1.00**				
Ph/9/25	0.56	1.00	0.75	0.86	0.75	0.75	0.60	0.75	**1.00**			
Ph/9/40	0.55	0.67	0.89	0.78	0.89	0.70	0.90	0.89	0.67	**1.00**		
BS-67	0.58	0.42	0.58	0.50	0.58	0.58	0.75	0.58	0.42	0.67	**1.00**	
BT-5	0.80	0.60	0.64	0.70	0.64	0.80	0.67	0.64	0.60	0.58	0.75	**1.00**

4.3.1.4. Genetic diversity analysis by primer OPB10

The segregated of RAPD bands amplified with primer OPB10 with 12 tea genotypes are shown in **Figure 4.7**.

Phylogenetic dendogram (**Figure 4.8**) shows that the 12 tea genotypes were categorized into three main groups when divided by the average genetic distance 2.3 of all the 12 tea genotypes. Group A contained three genotypes, i.e., Br2/97, Ph/9/4 and Ph/9/25. Among these three, Ph/9/4 and Ph/9/25 were covered under the sub-group S_1. Group B comprised five genotypes such as E/4, D/13, B2×T1, SDL/1 and BS-67. These five genotypes were further divided into two sub-groups, i.e., S_2 and S_3. Sub-group S_2 included D/13 and B2×T1. On the other hand, S_3 sub-group was covered SDL/1 and BS-67. Group C included three genotypes, i.e., BT2, BT5 and Ph/9/40. Among these three genotypes, BT2 and BT5 were covered under S_4 subgroup. However, MZ/39 failed to be included in either A, B or C group.

Jaccard's similarity matrix calculated from the banding pattern generated by the primer OPB10 is shown in (**Table 4.6**). The **Table 4.6** shows that a wide range (0.21 to 1.00) of similarity was noticed among the tea genotypes. The similarity coefficient between the two genotypes (Ph/9/4 and Ph/9/25) of sub-group S_1 and the two genotypes (SDL/1 and BS-67) of sub-group S_3 are the highest, at 1.00 with 0.8 unit and 1.3 unit distance, respectively.

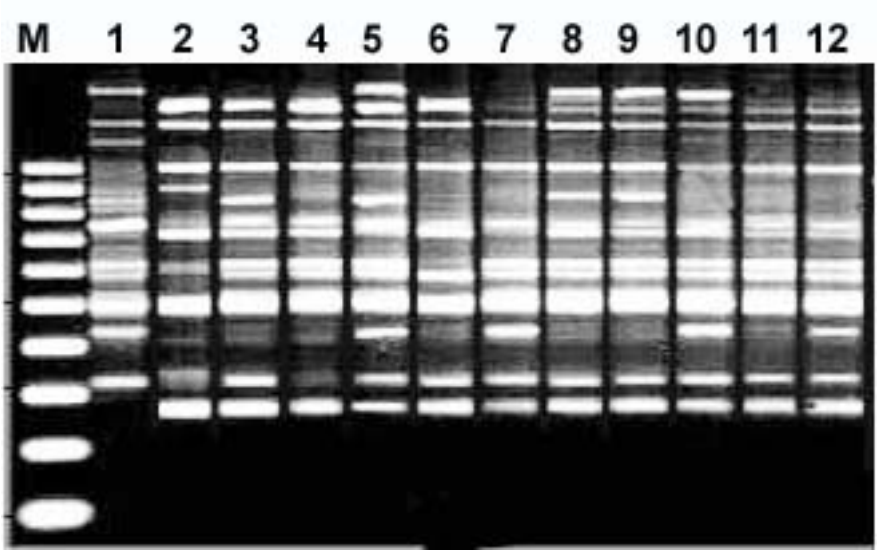


Figure 4.7 RAPD pattern amplified with primer OPB10 from the genomic DNA of tea genotypes. Lanes M= 100 bp size marker, 1= MZ/39, 2= E/4, 3= D/13, 4= B2×T1, 5= Br2/97, 6= SDL/1, 7= BT2, 8= Ph/9/4, 9= Ph/9/25, 10= Ph/9/40, 11= BS-67 and 12= BT5.

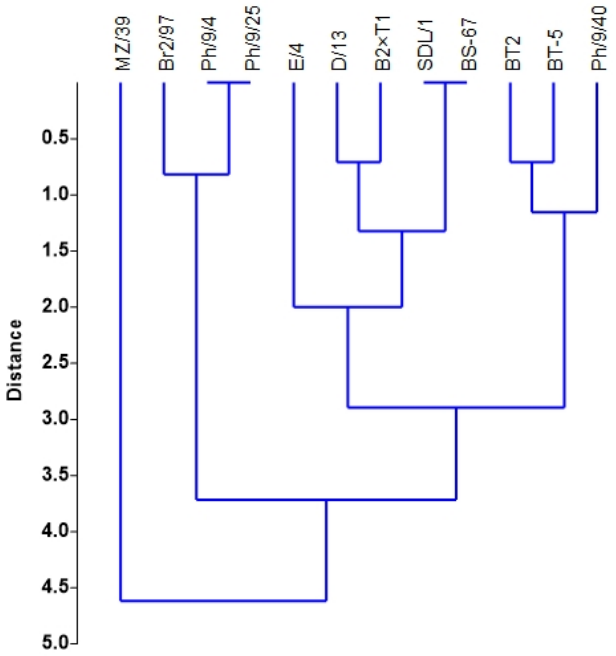


Figure 4.8 Phylogenetic dendogram generated from the banding pattern development with the primer OPB10 from the genomic DNA of 12 tea genotypes.

Table 4. 6 Jaccard's similarity matrix of gel banding patterns generated with primer OPB10 from the genomic DNA of 12 tea genotypes.

Genotypes	MZ/39	E/4	D/13	B2×T1	Br2/97	SDL/1	BT2	Ph/9/4	Ph/9/25	Ph/9/40	BS-67	BT-5
MZ/39	**1.00**											
E/4	0.23	**1.00**										
D/13	0.29	0.60	**1.00**									
B2×T1	0.21	0.67	0.89	**1.00**								
Br2/97	0.50	0.58	0.75	0.67	**1.00**							
SDL/1	0.21	0.67	0.89	0.78	0.67	**1.00**						
BT2	0.31	0.50	0.70	0.60	0.67	0.78	**1.00**					
Ph/9/4	0.43	0.64	0.82	0.73	0.92	0.73	0.58	**1.00**				
Ph/9/25	0.43	0.64	0.82	0.73	0.92	0.73	0.58	1.00	**1.00**			
Ph/9/40	0.36	0.55	0.73	0.64	0.83	0.80	0.80	0.75	0.75	**1.00**		
BS-67	0.21	0.67	0.89	0.78	0.67	1.00	0.78	0.73	0.73	0.80	**1.00**	
BT-5	0.29	0.60	0.80	0.70	0.75	0.89	0.89	0.67	0.67	0.90	0.89	**1.00**

4.3.1.5. Diversity analysis by primer OPB13

The segregated of RAPD bands amplified with primer OPB13 with 12 tea genotypes are shown in **Figure 4.9**.

Phylogenetic dendogram generated with the primer OPB13 shows that the 12 tea genotypes were categorized into three main groups when divided by the genetic distance of 2.8, the average genetic distance of all the 12 tea genotypes (**Figure 4.10**). Group A contained four genotypes, i.e., B2×T1, Br2/97, SDL/1 and Ph/9/4. These four genotypes were further divided into two sub groups, i.e., S_1 and S_2. Sub-group S_1 was included B2×T1 and Br2/97 genotypes, while S_2 was comprised SDL/1 and Ph/9/4. Group B contained six genotypes such as MZ/39, BS-67, D/13, Ph/9/40, E/4 and BT5. These six genotypes were further separated into three sub-groups, i.e., S_3, S_4 and S_5. Sub-group S_3 was included MZ/39 and BS-67, while sub-group S_4 was comprised D/13 and Ph/9/40. On the other hand, E/4 and BT5 were covered under S_5 sub-group of group B. Group C included two genotypes, i.e., BT2 and Ph/9/25.

Table 4.7 shows the Jaccard's similarity coefficient matrix. This table reveals a wide range (0.38 to 1.00) of similarity among the tea genotypes. The similarity coefficient between the two genotypes (B2×T1 and Br2/97) of sub-group S_1 and the two genotypes (SDL/1 and Ph/9/4) of sub-group S_2 are the highest, at 1.00 with 1.0 unit distance.

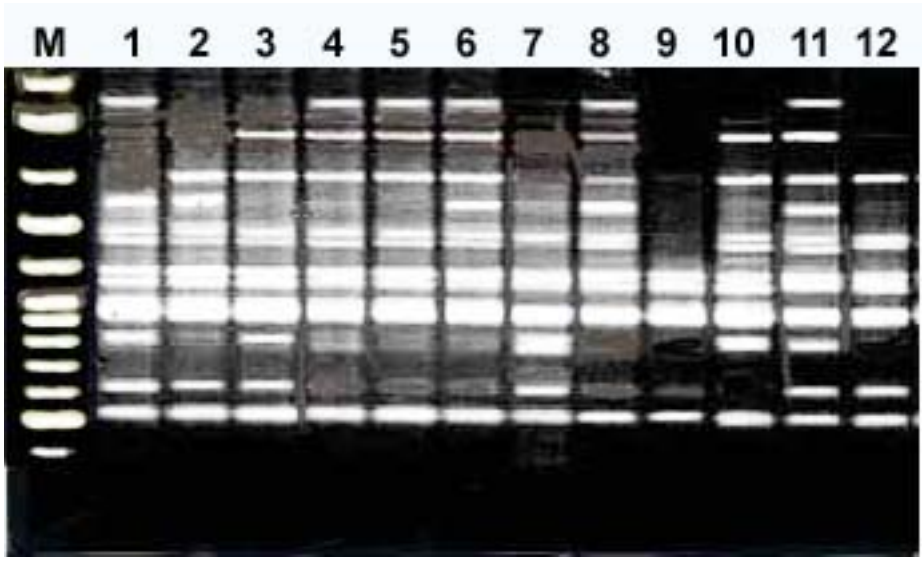


Figure 4.9 RAPD pattern amplified with primer OPB13 from genomic DNA of 12 tea genotypes. Lanes M= 1 kb size marker, 1= MZ/39, 2= E/4, 3= D/13, 4= B2×T1, 5= Br2/97, 6= SDL/1, 7= BT2, 8= Ph/9/4, 9= Ph/9/25, 10= Ph/9/40, 11= BS-67 and 12= BT5.

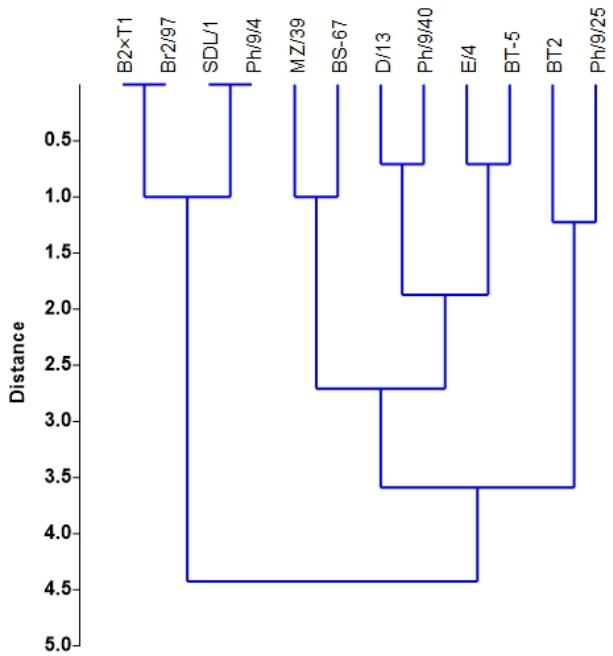


Figure 4.10 Phylogenetic dendogram generated from the banding pattern developed with primer OPB13 from the genomic DNA of 12 tea genotypes.

Table 4.7 Jaccard's similarity matrix of gel banding pattern generated by the primer OPB13 from the genomic DNA of 12 tea genotypes.

Genotypes	MZ/39	E/4	D/13	B2×T1	Br2/97	SDL/1	BT2	Ph/9/4	Ph/9/25		BS-67	BT-5
MZ/39	**1.00**											
E/4	0.75	**1.00**										
D/13	0.69	0.75	**1.00**									
B2×T1	0.62	0.67	0.75	**1.00**								
Br2/97	0.62	0.67	0.75	1.00	**1.00**							
SDL/1	0.69	0.75	0.69	0.91	0.91	**1.00**						
BT2	0.73	0.64	0.73	0.50	0.50	0.46	**1.00**	0.46				
Ph/9/4	0.69	0.75	0.69	0.91	0.91	1.00	0.46	**1.00**				
Ph/9/25	0.45	0.50	0.45	0.50	0.50	0.45	0.63	0.45	**1.00**			
Ph/9/40	0.62	0.67	0.91	0.82	0.82	0.75	0.64	0.75	0.50	**1.00**		
BS-67	0.85	0.77	0.85	0.77	0.77	0.85	0.62	0.85	0.38	0.77	**1.00**	
BT-5	0.67	0.90	0.82	0.73	0.73	0.67	0.70	0.67	0.56	0.73	0.69	**1.00**

4.3.1.6. Genetic diversity analysis by primer OPC9

The segregated of RAPD bands amplified with primer OPC9 with 12 tea genotypes are shown in **Figure 4.11**.

Phylogenetic dendogram (**Figure 4.12**) shows that the 12 tea genotypes were categorized into three main groups when divided by the average genetic distance 1.7 of all the 12 tea genotypes (**Figure 4.12**). Group A contained five genotypes, i.e., MZ/39, E/4, D/13, B2×T1 and Br2/97. These five genotypes were further divided into two sub groups, i.e., S_1 and S_2. Sub-group S_1 was included MZ/39 and E/4 genotypes, while S_2 was comprised D/13 and B2×T1. However, Br2/97 was failed to be included in either S_1 or S_2 sub-group. Group B contained four genotypes such as SDL/1, Ph/9/25, BT2 and Ph/9/4. These four genotypes were further separated into two sub-groups, i.e., S_3 and S_4. Sub-group S_3 was included SDL/1 and Ph/9/25, while sub-group S_4 was comprised BT2 and Ph/9/4. Group C included three genotypes, i.e., Ph/9/40, BS-67 and BT5. Among these three genotypes, Ph/9/40 and BS-67 were covered under S_5 sub-group of group C. BT5 was excluded of S_5 sub-group.

Table 4.8 shows the Jaccard's similarity coefficient matrix. This table reveals a wide range (0.0 to 1.00) of similarity among the tea genotypes. The similarity coefficient between the two genotypes (MZ/39 and E/4) of sub-group S_1 is the highest, at 1.00 with 0.9 unit distance. On the other hand, the similarity coefficient between the two genotypes (SDL/1 and Ph/9/4) of sub-group S_3 and between the two genotypes (Ph/9/40 and BS-67) are also the highest, at 1.00 with 1.0 unit distance.

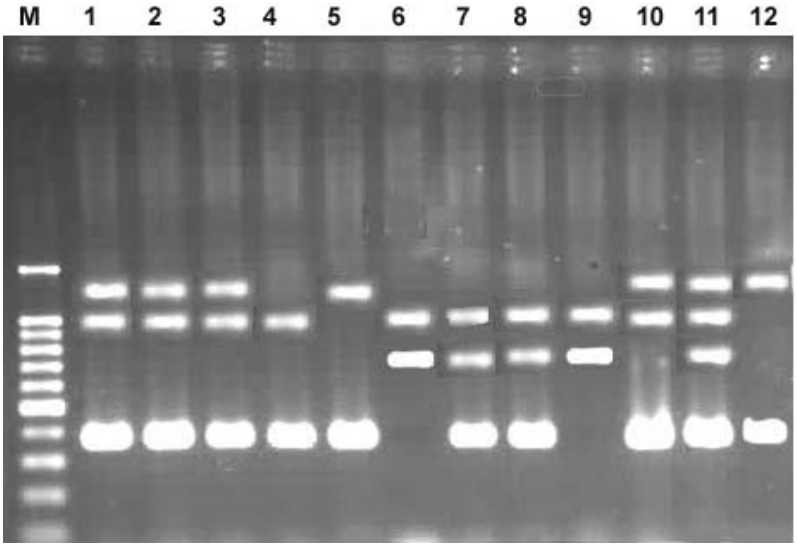


Figure 4.11 RAPD pattern amplified with primer OPC9 from the genomic DNA of 12 tea genotypes. Lanes M= 1 kb size marker, 1= MZ/39, 2= E/4, 3= D/13, 4= B2×T1, 5= Br2/97, 6= SDL/1, 7= BT2, 8= Ph/9/4, 9= Ph/9/25, 10= Ph/9/40, 11= BS-67 and 12= BT5

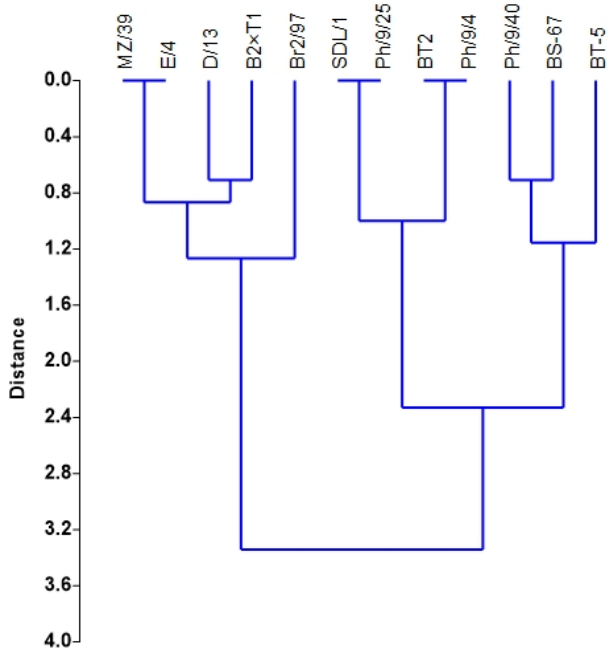


Figure 4.12 Phylogenetic dendogram generated from the banding pattern developed with the primer OPC9 from the genomic DNA of 12 tea genotypes.

Table 4.8 Jaccard's similarity matrix of gel banding patterns generated with primer OPC9 from the genomic DNA of 12 tea genotypes.

Genotypes	MZ/39	E/4	D/13	B2×T1	Br2/97	SDL/1	BT2	Ph/9/4	Ph/9/25	Ph/9/40	BS-67	BT-5
MZ/39	**1.00**											
E/4	1.00	**1.00**										
D/13	1.00	1.00	**1.00**									
B2×T1	0.67	0.67	0.67	**1.00**								
Br2/97	0.67	0.67	0.67	0.33	**1.00**							
SDL/1	0.25	0.25	0.25	0.33	0.00	**1.00**						
BT2	0.50	0.50	0.50	0.67	0.25	0.67	**1.00**					
Ph/9/4	0.50	0.50	0.50	0.67	0.25	0.67	1.00	**1.00**				
Ph/9/25	0.25	0.25	0.25	0.33	0.00	1.00	0.67	0.67	**1.00**			
Ph/9/40	0.50	0.50	0.50	0.67	0.25	0.25	0.50	0.50	0.25	**1.00**		
BS-67	0.40	0.40	0.40	0.50	0.20	0.50	0.75	0.75	0.50	0.75	**1.00**	
BT-5	0.25	0.25	0.25	0.33	0.33	0.00	0.25	0.25	0.00	0.67	0.50	**1.00**

4.3.1.7. Genetic diversity analysis by primer OPC17

The segregated RAPD bands amplified with primer OPC17 with 12 tea genotypes are shown in **Figure 4.13**.

Phylogenetic generated with the primer OPC17 shows that the 12 tea genotypes were categorized into two main groups when divided by the genetic distance of 1.6, the average genetic distance of all the 12 tea genotypes (**Figure 4.14**). Group A contained eight genotypes, i.e., D/13, B2×T1, MZ/39, Br2/97, SDL/1 and BT2. These eight genotypes were further divided into three sub groups, i.e., S_1, S_2 and S_3. Sub-group S_1 included D/13 and B2×T1 genotypes, while S_2 comprised MZ/39, Br2/97, SDL/1 and BT2. On the other hand, Ph/9/40 and BS-67 were covered under sub-group S_3 of of group A. Group B contained four genotypes such as E/4, Ph/9/4, Ph/9/25 and BT5. These four genotypes were further separated into two sub-groups, i.e., S_4 and S_5. Sub-group S_4 was comprised E/4 and Ph/9/4, while sub-group S_5 was comprised Ph/9/25 and BT5.

Table 4.9 shows the Jaccard's similarity coefficient matrix. This table reveals a wide range (0.25 to 1.00) of similarity among the tea genotypes. The similarity coefficient between the two genotypes (D/13 and B2×T1) of sub-group S_1 was the highest, at 1.00 with 1.6 unit distance. On the other hand, the similarity coefficient among the four genotypes (MZ/39, Br2/97, SDL/1 and BT2) of sub-group S_2 was the highest, at 1.00 with 0.9 unit distance. The similarity coefficient between the two genotypes (E/4 and Ph/9/4) of sub-group S_4 and between the two genotypes (Ph/9/25 and BT5) of sub-group S_5 were also the highest, at 1.00 with 1.0 unit distance.

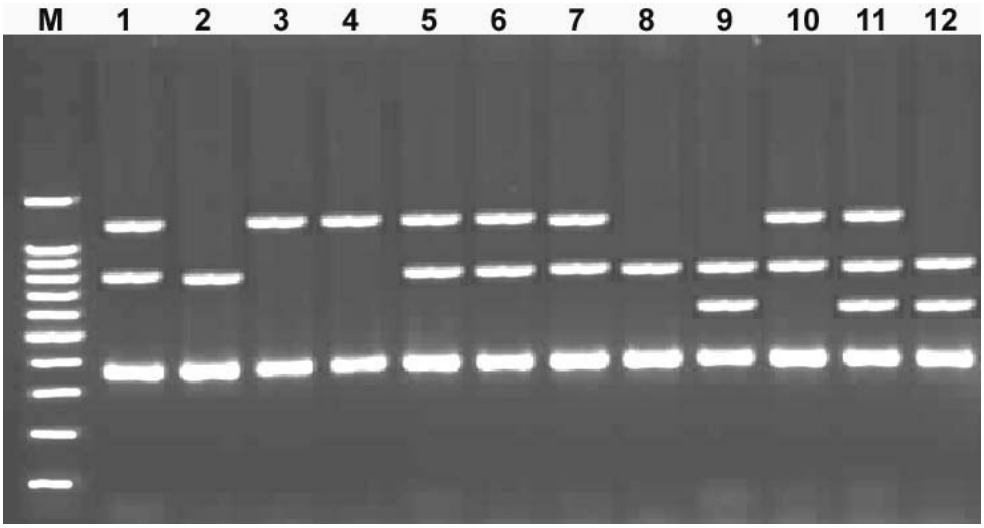


Figure 4.13 RAPD pattern amplified with primer OPC17 from genomic DNA of 12 tea genotypes. Lanes M= 1 kb size marker, 1= MZ/39, 2= E/4, 3= D/13, 4= B2×T1, 5= Br2/97, 6= SDL/1, 7= BT2, 8= Ph/9/4, 9= Ph/9/25, 10= Ph/9/40, 11= BS-67 and 12= BT5

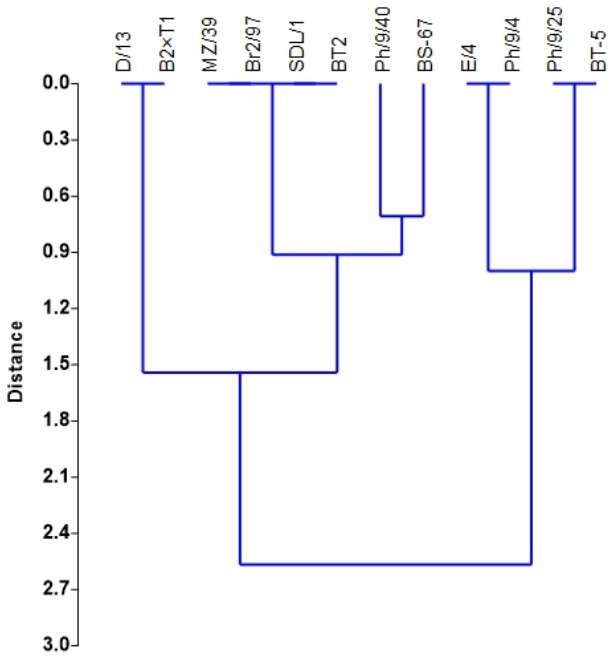


Figure 4.14 Phylogenetic dendogram generated from the banding pattern developed with primer OPC17 from the genomic DNA of 12 tea genotypes.

Table 4.9 Jaccard's similarity matrix of gel banding pattern generated by the primer OPC17 from the genomic DNA of 12 tea genotypes.

Genotypes	MZ/39	E/4	D/13	B2×T1	Br2/97	SDL/1	BT2	Ph/9/4	Ph/9/25	Ph/9/40	BS-67	BT-5
MZ/39	**1.00**											
E/4	0.67	**1.00**										
D/13	0.67	0.33	**1.00**									
B2×T1	0.67	0.33	1.00	**1.00**								
Br2/97	1.00	0.67	0.67	0.67	**1.00**							
SDL/1	1.00	0.67	0.67	0.67	1.00	**1.00**						
BT2	1.00	0.67	0.67	0.67	1.00	1.00	**1.00**					
Ph/9/4	0.67	1.00	0.33	0.33	0.67	0.67	0.67	**1.00**				
Ph/9/25	0.50	0.67	0.25	0.25	0.50	0.50	0.50	0.67	**1.00**			
Ph/9/40	1.00	0.67	0.67	0.67	1.00	1.00	1.00	0.67	0.50	**1.00**		
BS-67	0.75	0.50	0.50	0.50	0.75	0.75	0.75	0.50	0.75	0.75	**1.00**	
BT-5	0.50	0.67	0.25	0.25	0.50	0.50	0.50	0.67	1.00	0.50	0.75	**1.00**

4.3.1.8. Genetic diversity analysis by primer OPD3

The segregated RAPD bands amplified with primer OPD3 with 12 tea genotypes are shown in **Figure 4.15**.

Phylogenetic dendogram (**Figure 4.16**) shows that the 12 tea genotypes were categorized into two main groups when divided by the average genetic distance 1.9 of all the 12 tea genotypes. Group A contained five genotypes, i.e., MZ/39, E/4, BT2, Ph/9/4 and Ph/9/25. These five genotypes were further divided into two sub groups, i.e., S_1 and S_2. Sub-group S_1 was included MZ/39 and E/4 genotypes, while S_2 was comprised BT2, Ph/9/4 and Ph/9/25. Group B also contained five genotypes such as B2×T1, Br2/97, BS-67, Ph/9/40 and BT5. These five genotypes were further separated into two sub-groups, i.e., S_3 and S_5. Sub-group S_3 was included B2×T1, Br2/97 and BS-67, while sub-group S_4 was comprised Ph/9/40 and BT5. However, D/13 and SDL/1 failed to be included in either A or B group.

Jaccard's similarity matrix calculated from the banding pattern generated by the primer OPD3 is shown in **Table 4.10**. This table shows that a wide range (0.29 to 1.00) of similarity was noticed among the tea genotypes. The similarity coefficient between the two genotypes (MZ/39 and E/4) of sub-group S_1 is the highest, at 1.00 with 1.7 unit distance.

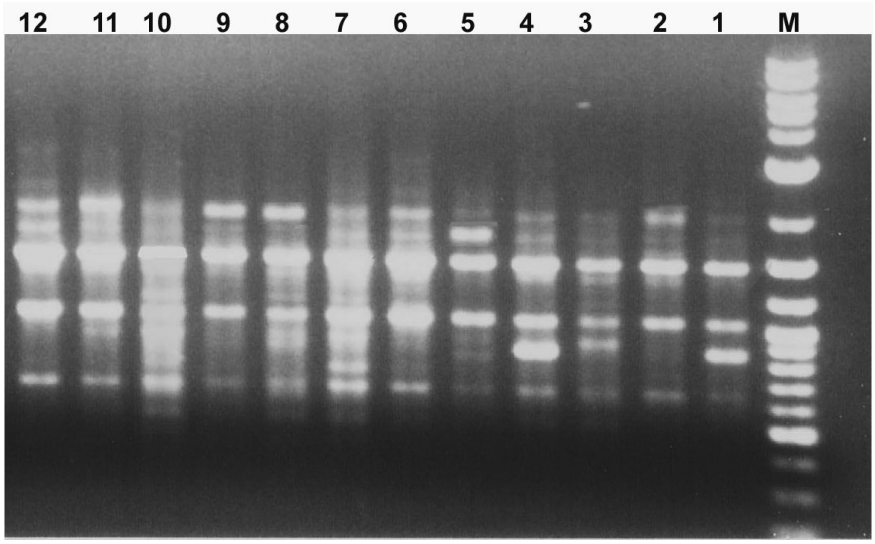


Figure 4.15 RAPD pattern amplified with primer OPD3 from the genomic DNA of 12 tea genotypes. Lanes M= 1 kb size marker, 1= MZ/39, 2= E/4, 3= D/13, 4= B2×T1, 5= Br2/97, 6= SDL/1, 7= BT2, 8= Ph/9/4, 9= Ph/9/25, 10= Ph/9/40, 11= BS-67 and 12= BT5

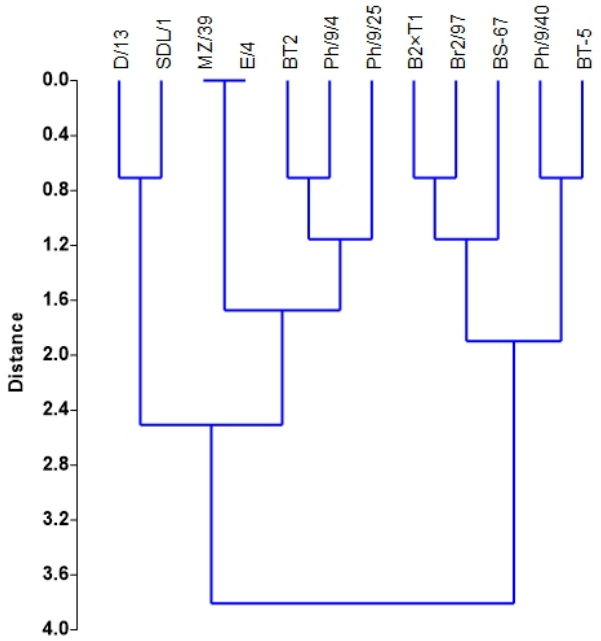


Figure 4.16 Phylogenetic dendogram generated from the banding pattern developed with the primer OPD3 from the genomic DNA of 12 tea genotypes.

Table 4.10 Jaccard's similarity matrix of gel banding patterns generated with primer OPD3 from the genomic DNA of 12 tea genotypes.

Genotypes	MZ/39	E/4	D/13	B2×T1	Br2/97	SDL/1	BT2	Ph/9/4	Ph/9/25	Ph/9/40	BS-67	BT-5
MZ/39	**1.00**											
E/4	1.00	**1.00**										
D/13	0.56	0.56	**1.00**									
B2×T1	0.50	0.50	0.38	**1.00**								
Br2/97	0.43	0.43	0.50	0.75	**1.00**							
SDL/1	0.63	0.63	0.88	0.43	0.57	**1.00**						
BT2	0.83	0.83	0.63	0.60	0.50	0.71	**1.00**					
Ph/9/4	0.67	0.67	0.50	0.75	0.60	0.57	0.80	**1.00**				
Ph/9/25	0.71	0.71	0.75	0.50	0.67	0.86	0.83	0.67	**1.00**			
Ph/9/40	0.43	0.43	0.50	0.40	0.60	0.57	0.50	0.33	0.67	**1.00**		
BS-67	0.67	0.67	0.50	0.75	0.60	0.57	0.80	0.60	0.67	0.60	**1.00**	
BT-5	0.29	0.29	0.38	0.50	0.75	0.43	0.33	0.40	0.50	0.75	0.40	**1.00**

4.3.1.9. Genetic diversity analysis by primer OPD8

The segregated RAPD bands amplified with primer OPD8 with 12 tea genotypes are shown in **Figure 4.17**.

Phylogenetic dendogram generated with the primer OPD8 shows that the 12 tea genotypes were categorized into three main groups when divided by the genetic distance of 1.7, the average genetic distance of all the 12 tea genotypes (**Figure 4.18**). Group A contained four genotypes, i.e., MZ/39, SDL/1, E/4 and Ph/9/4. These four genotypes were further divided into two sub groups, i.e., S_1 and S_2. Sub-group S_1 included MZ/39 and SDL/1genotypes, while S_2 comprised E/4 and Ph/9/4. Group B contained five genotypes such as D/13, B2×T1, Br2/97, BT2 and BT5. Among the five genotypes, D/13, B2×T1, Br2/97 and BT2 were covered under the sub-group S_3, while BT5 failed to be included in sub-group S_3. Group C included three genotypes, i.e., Ph/9/25, Ph/9/40 and BS-67. Among these three genotypes, Ph/9/40 and BS-67 were covered under S_4 sub-group, while Ph/9/25 was excluded of S_5 sub-group.

Table 4.11 shows the Jaccard's similarity coefficient matrix. This table reveals a wide range (0.33 to 1.00) of similarity among the tea genotypes. The similarity coefficient between the two genotypes (MZ/39 and SDL/1) of sub-group S_1 and the similarity coefficient among the four genotypes (D/13, B2×T1, Br2/97 and BT2) of sub-group S_3 were the highest, at 1.00 with 0.9 unit distance.

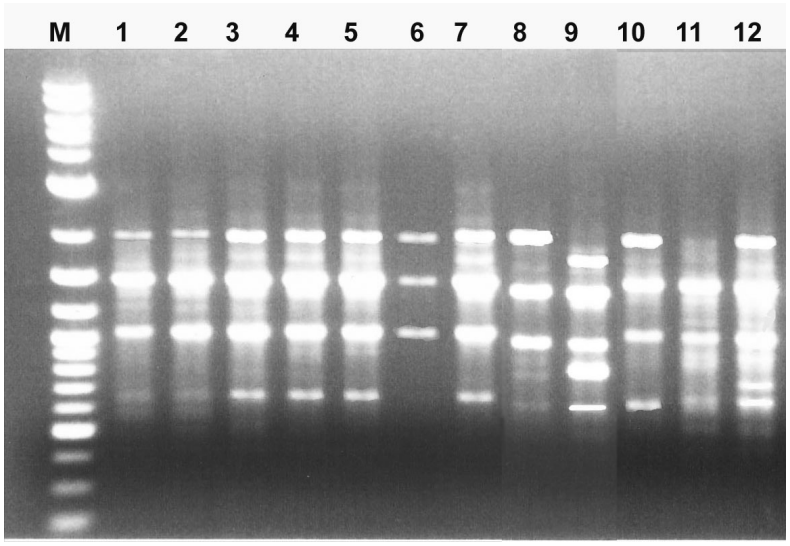


Figure 4.17 RAPD pattern amplified with primer OPD8 from genomic DNA of 12 tea genotypes. Lanes M= 1 kb size marker, 1= MZ/39, 2= E/4, 3= D/13, 4= B2×T1, 5= Br2/97, 6= SDL/1, 7= BT2, 8= Ph/9/4, 9= Ph/9/25, 10= Ph/9/40, 11= BS-67 and 12= BT5

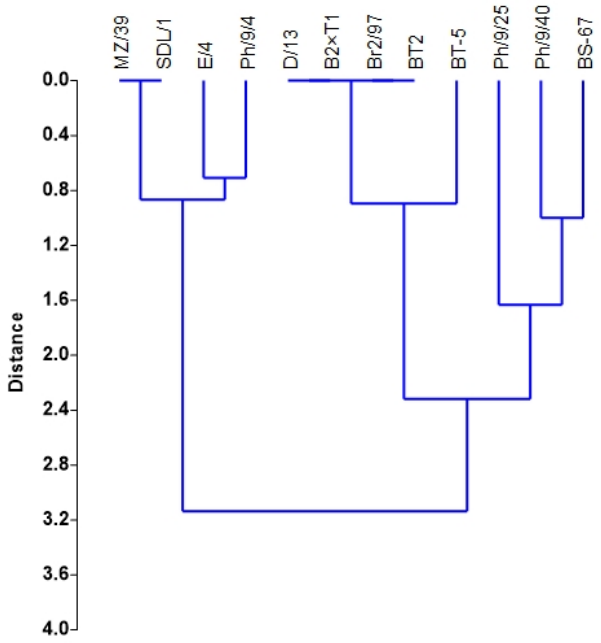


Figure 4.18 Phylogenetic dendogram generated from the banding patterns developed with primer OPD8 from the genomic DNA of 12 tea genotypes.

Table 4.11 Jaccard's similarity matrix of gel banding pattern generated by the primer OPD8 from the genomic DNA of 12 tea genotypes.

Genotypes	MZ/39	E/4	D/13	B2×T1	Br2/97	SDL/1	BT2	Ph/9/4	Ph/9/25	Ph/9/40	BS-67	BT-5
MZ/39	**1.00**											
E/4	0.75	**1.00**										
D/13	0.60	0.80	**1.00**									
B2×T1	0.60	0.80	1.00	**1.00**								
Br2/97	0.60	0.80	1.00	1.00	**1.00**							
SDL/1	1.00	0.75	0.60	0.60	0.60	**1.00**						
BT2	0.60	0.80	1.00	1.00	1.00	0.60	**1.00**					
Ph/9/4	1.00	0.75	0.60	0.60	0.60	1.00	0.60	**1.00**				
Ph/9/25	0.33	0.50	0.67	0.67	0.67	0.33	0.67	0.33	**1.00**			
Ph/9/40	0.75	0.60	0.80	0.80	0.80	0.75	0.80	0.75	0.50	**1.00**		
BS-67	0.40	0.33	0.50	0.50	0.50	0.40	0.50	0.40	0.50	0.60	**1.00**	
BT-5	0.50	0.67	0.83	0.83	0.83	0.50	0.83	0.50	0.57	0.67	0.67	**1.00**

4.3.1.10. Genotype diversity analysis by primer OPD15

The segregated RAPD bands amplified with primer OPD15 with 12 tea genotypes are shown in **Figure 4.19**.

Phylogenetic dendogram (**Figure 4.20**) shows that the 12 tea genotypes are categorized into two main groups when divided by the average genetic distance 2.4 of all the 12 tea genotypes. Group A contained five genotypes, i.e., MZ/39, BT2, E/4, Ph/9/4 and BT5. These five genotypes were further divided into two sub groups, i.e., S_1 and S_2. Sub-group S_1 was included MZ/39 and BT2 genotypes, while S_2 was comprised E/4, Ph/9/4 and BT5. Group B contained seven genotypes such as D/13, B2×T1, Br2/97, SDL/1, Ph/9/25, Ph/9/40 and BS-67. Among the seven genotypes, D/13, B2×T1, Br2/97 and SDL/1 were covered under the sub-group S_3, while Ph/9/25, Ph/9/40 and BS-67 were covered under the sub-group S_4.

Jaccard's similarity matrix calculated from the banding pattern generated by the primer OPD15 is shown in **Table 4.12**. This table shows that a wide range (0.17 to 1.00) of similarity was noticed among the tea genotypes. The similarity coefficient between the two genotypes (Ph/9/4 and BT5) of sub-group S_2 and the similarity coefficient among the two genotypes (D/13, B2×T1, Br2/97 and SDL/1) of sub-group S_3 were the highest, at 1.00 with 0.8 and 2.3 unit distance, respectively.

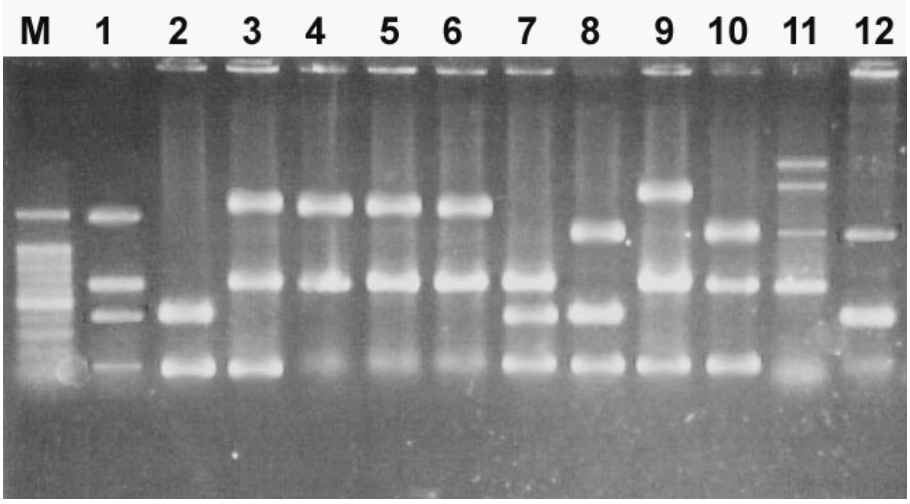


Figure 4.19 RAPD pattern amplified with primer OPD15 from genomic DNA of 12 tea genotypes. Lanes M= 1 kb size marker, 1= MZ/39, 2= E/4, 3= D/13, 4= B2×T1, 5= Br2/97, 6= SDL/1, 7= BT2, 8= Ph/9/4, 9= Ph/9/25, 10= Ph/9/40, 11= BS-67 and 12= BT5

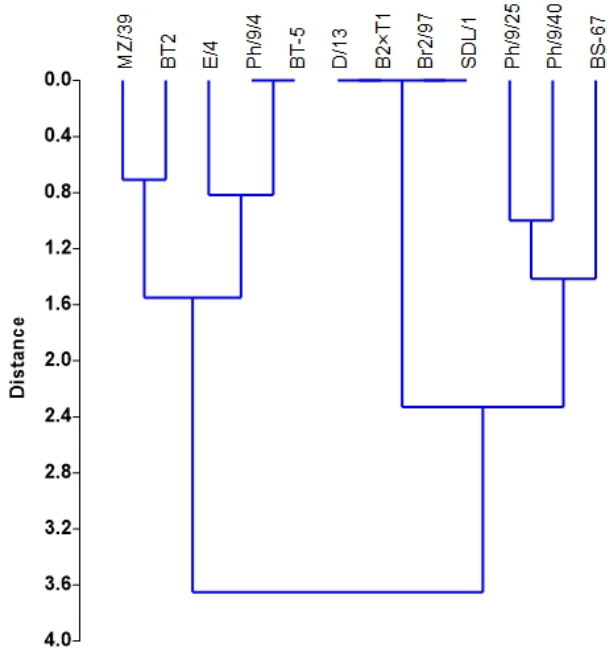


Figure 4.20 Phylogenetic dendogram generated from the banding pattern developed with primer OPD15 from the genomic DNA of 12 tea genotypes.

Table 4.12 Jaccard's similarity matrix of gel banding pattern generated by the primer OPD15 from the genomic DNA of 12 tea genotypes.

Genotypes	**MZ/39**	**E/4**	**D/13**	**B2×T1**	**Br2/97**	**SDL/1**	**BT2**	**Ph/9/4**	**Ph/9/25**	**Ph/9/40**	**BS-67**	**BT-5**
MZ/39	**1.00**											
E/4	0.50	**1.00**										
D/13	0.40	0.25	**1.00**									
B2×T1	0.40	0.25	1.00	**1.00**								
Br2/97	0.40	0.25	1.00	1.00	**1.00**							
SDL/1	0.40	0.25	1.00	1.00	1.00	**1.00**						
BT2	0.75	0.67	0.50	0.50	0.50	0.50	**1.00**					
Ph/9/4	0.75	0.67	0.20	0.20	0.20	0.20	0.50	**1.00**				
Ph/9/25	0.40	0.25	0.50	0.50	0.50	0.50	0.50	0.20	**1.00**			
Ph/9/40	0.75	0.25	0.50	0.50	0.50	0.50	0.50	0.50	0.50	**1.00**		
BS-67	0.50	0.17	0.33	0.33	0.33	0.33	0.33	0.33	0.60	0.60	**1.00**	
BT-5	0.75	0.67	0.20	0.20	0.20	0.20	0.50	1.00	0.20	0.50	0.33	**1.00**

4.3.1.11. Consensus phylogenetic dendogram

An overall consensus phylogenetic dendogram was constructed on the basis of the phylogenetic dendograms generated from the banding pattern generated with 10 different primers viz., OPA7, OPA9, OPA10, OPB10, OPB13, OPC9, OPC17, OPD3, OPD8 and OPD15, and the results are shown in **Figure 4.21**.

Phylogenetic dendogram (**Figure 4.21**) shows that the 12 tea genotypes could be categorized into three main groups when divided by the genetic distance of 6.4, the average genetic distance of all the 12 tea genotypes (**Figure 4.21**). Group A contained four genotypes, i.e., E/4, D/13, MZ/39 and SDL/1. Among these four genotypes MZ/39 and SDL/1 were genetically closed to each other. The genetical distance of these two genotypes was 1.4. Group B included three genotypes such as B2×T1, BT2 and BT5. Among these three genotypes BT2 and BT5 were very close to each other. The genetical distance of these two genotypes was 2.4. Group C comprised three genotypes such as Br2/97, Ph/9/4 and Ph/9/40. Among these three genotypes Ph/9/4 and Ph/9/40 were genetically close to each other. The genetical distance of these two genotypes was 1.8. The similarity index in group B and group C ranged from 1.8 to 4.0. So, the genotypes of these two groups were near to each other. However, BS-67 and Ph/9/25 were failed to be included in either A, B or C group. Although these two genotypes were different from the genotypes of other groups but BS-67 is near to B2×T1 of group B and Ph/9/25 is near to E/4 of group A.

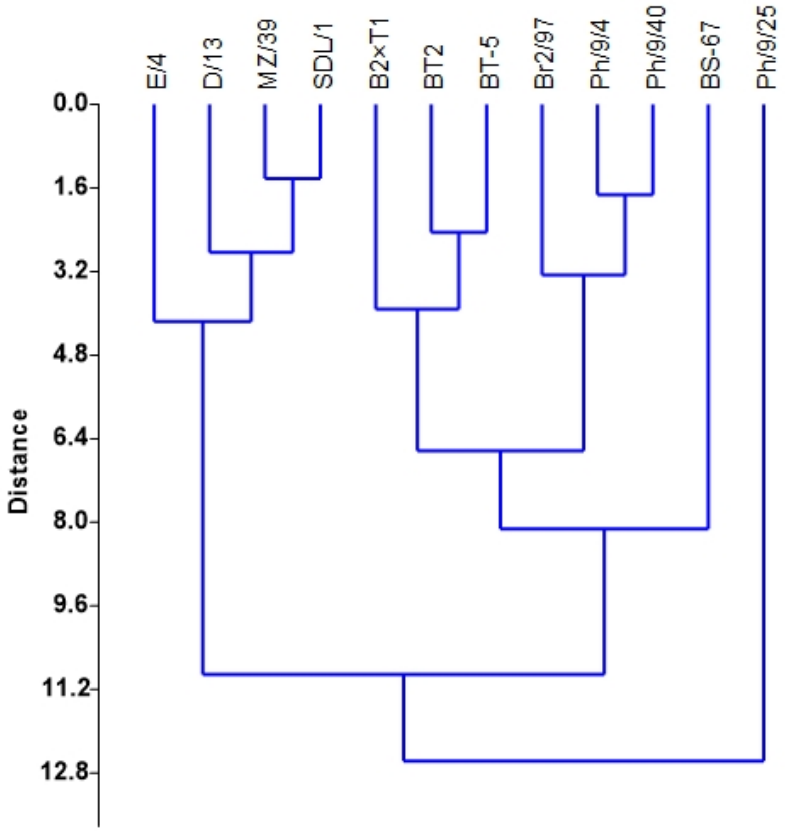


Figure 4.21 Consensus phylogenetic dendogram based on phylogentic tree generated from the banding pattern by different primers used in this study.

4. 4. DISCUSSION

The successes of tea breeding and improvement, genetic resource conservation and utilization programmes rely utterly on the full mastery and clear understanding of the quantity, distribution and relationship of genetic resources. In the hundreds of thousands of years of evolutionary history, continuous outcrossing of the tea plant with its allied species created tea genetic resources with overlapped morphological, physiological and chemical characteristics, as well as chiasmatic genetic background.

The analysis of tea cultivars through morphological features is inefficient and inaccurate. This problem is further compounded by perennial nature of crop. The use of biochemical and genetical markers for the identification of varieties offer a viable alternative method but now at present several tea cultivars have many synonyms or genetic analysis based on phenotype is a function of heritability of the trait. Factors like environment, traits of multigenic and quantitative inheritance, or partial and incomplete dominance often compound the expression of genetic traits. Many of this complication of a phenotype based assay can be overcome through direct identification of genotype with DNA-based genetic markers. PCR had led to the development of several novel genetic assay based on selective DNA amplification. RAPD is an amplification based assay experiment is performed with only nanogram of DNA.

Previously morphological, cytological and biochemical markers were used for estimation of genetic diversity in commercially important crop species. However, with the recent developments in DNA technology, molecular markers based on the variation in DNA base sequences have been widely used in cop improvement (Paterson *et al.*, 1991; Sumikova and Kernlova, 2010; Todorovska *et al.*, 2009; Wang et al., 2007). Various DNA based markers commonly used to estimate genetic diversity in crops of agronomic importance include restriction fragment length polymorphism (RLFP), simple sequence repeat (SSR), amplified fragment length polymorphism (AFLP) and single nucleotide polymorphism (SNP) (Chen *et al.*, 2006). These procedures though highly reliable, are expensive, time consuming and sometime require working with hazardous chemicals (example, use of radioactive

P32). In contrast, RAPD analysis is not only easier, quicker, cheaper and more user's friendly assay procedure but has an added advantage that RAPD primers do not require any prior sequence information on the target genome (Williams *et al.*, 1990). Hence, RAPD analysis is more suitable for handling larger germplasm accessions/segregating populations of commercially important crops like tea in the developing countries where technical expertise and financial support for scientific research are limiting factors.

Genetic diversity in tea has been studied by various workers using DNA based markers (Kaundun and Park, 2002; Chen *et al.*, 1998, 2005b; Chen and Yamaguchi, 2002; Paul *et al.*, 1997; Shao *et al.*, 2003; Kaundun and Matsumoto, 2003; Huang *et al.*, 2004, 2006; Yao *et al.*, 2007; Chen *et al.*, 2007; Gul *et al.*, 2007; Afridi *et al.*, 2011). In the present study, there were total 88 RAPD loci were generated in 12 tea genotypes with 10 primers among which 64 loci were found to be polymorphic. The average number of amplified fragments during the present study were 8.6 per primer per genotype and the average numbers of polymorphic bands were 6.4 which was higher than reported in some earlier studies (Chen *et al.*, 2005a) who reported approximately 3.5 alleles per primer per genotype. It may be because in earlier study (*Afridi et al.*, (2011); Chen *et al.*, 2005a) selected elite genotypes were used where most favored alleles are retained as compared to the rare ones. Relatively, higher genetic distances estimated during the present study could result from the fact that material used belonged to various geographical regions in contrast to previous study example Chen *et al.* (2005b) who used Chinese genotypes which were established from a limited gene stock.

The results of the present investigation showed that the DNA genetic diversity of Bangladeshi elite tea genetic resources was 77.18%. Chen *et al.* (2005) reported that the DNA genetic diversity of Chinese elite tea genetic resources was as high as 94.2%. An investigation on 38 Kenyan tea clonal genetic resources belonging to *C. sinensis*, *C. assamica* and *C. assamica* ssp. *lasiocalyx* showed that the diversity was 62% (Wachira *et al.*, 1995). The diversity of 35 native Korean tea genetic resources was reported as 84.5% (Oh, 1994), 81.0% for 14 seed selections (Kaundun *et al.*, 2000), 57.8% for 20 abandoned plantations (Park *et al.*, 2002), 73.3% for 10 Indian

tea genetic resources (Mirshra and Sen-Mandi, 2001), and 56.9% for eight Japanese clones (Kaundun *et al.*, 2000).

Phylogenetic dendrogram of all 10 primers was generated using the method of majority rule consensus (Nei and Kumar, 2000). The genetic distance of 12 tea genotypes under the present investigation was ranged from 1.4 to 4.2, and averaged at 7.0. Chen *et al.* (2005) investigated that the genetic distance of 15 Chinese elite tea genetic resources ranged from 0.16 to 0.62, and averaged at 0.37. That of 35 Korean genetic resources ranged from 0.07 to 0.37, averaging at 0.16 (Oh, 1994). The diversity would increase with the increasing quantity of genetic resources studied and primers used (Wang *et al*., 1996). Although only a small number of Bangladeshi tea genetic resources were investigated in the current study, very high proportion of DNA diversity and high genetic distance were observed. These results support the presence of the high degree morphogenetic diversity among Bangladeshi tea genetic resources as we observed in the results presented in Chapter 2. This is not unexpected in view of the fact that a complex genetic background of tea plant may have been resulted from long-term outcrossing among the tea genotypes in Bangladesh. The data presented here will help in establishing maximization strategies for the improvement of tea in Bangladesh. The range of diversity exhibited by the genotypes studied shows potential for selection. In this respect, priority should be given to the marginal genotypes (belonging to group A and group C, **Figure 4.21**) which appeared to be most distantly related.

Previously, morphological characters, pollen morphology (Shu *et al.*, 1998), chemical components (Takeo *et al.*, 1992; Borse *et al.*, 2002) and isozymes (Lu *et al.*, 1992) were used to identify tea genetic resources. However, they found that the results were Not to be reproducible because results can vary with deferent growing environmet, developmental stage, seasons, and even experimental conditions. RAPD markers are independent of environmental conditions and provide a quick and simple methodology for identification of plants at any developmental stage (Hu and Quiros, 1991; Khasa and Dancik, 1996; Sedra *et al.*, 1998; Kaundun *et al.*, 2000; Belaj *et al.*, 2001; Conner and Wood, 2001; Kadve *et al.*, 2012). Hu and Quiros (1991) successfully identified 14 broccoli and 12 cauliflower cultivars by RAPD markers

using 4 decamer primers. RAPD markers provided easy and rapid identification of the specific genetic entries of spruce species and their reported putative hybrids (Khasa and Dancik, 1996). Belaj *et al.* (2001) successfully identified 51 olive genetic resources using the RAPD band pattern combination of 4 primers. Conner and Wood (2001) used 42 RAPD markers to identify 43 pecan cultivars. Present study reveals all the twelve tea genotypes used in this study could distinctly be identified by the presence and absence of unique RAPD bands. Therefore, RAPD markers provided a Practical and effective method not only to evaluate the genetic diversity and relationships, but also for the identification of tea genetic resources in Bangladesh.'

In conclusion, the information generated from this study showed that there are significant genetic differences among the 12 tea cultivars studied and that they could be used to strengthen the future tea cultivar improvement programme effectively by incorporating potential progenitors from the available tea germplasm.

4.5. SUMMARY

In order to assess genetic diversity ten primers used viz., OPA-7, OPA-9, OPA-10, OPB-10, OPB-13, OPC-9, OPC-17, OPD-3, OPD8 and OPD-15 for the amplification of genomic DNA of 12 different tea genotype. The scored on total no. of bands, no. of monomorphic bands, no. of polymorphic bands, % of polymorphism and the range of band size.

In the present study, there were total 88 RAPD loci were generated in 12 tea genotypes with 10 primers among which 64 loci were found to be polymorphic. The average number of amplified fragments during the present study were 8.6 per primer per genotype and the average numbers of polymorphic bands were 6.4. The results of the present investigation showed that the DNA genetic diversity of Bangladeshi elite tea genetic resources was 77.18%.

Phylogenetic dendrogram of all 10 primers was generated using the method of majority rule consensus (Nei and Kumar, 2000). The genetic distance of 12 tea genotypes under the present investigation was ranged from 1.4 to 4.2, and averaged at 7.0. Although only a small number of Bangladeshi tea genetic resources were investigated in the current study, very high proportion of DNA diversity and high genetic distance were observed. These results support the presence of the high degree morphogenetic diversity among Bangladeshi tea genetic resources as we observed in the results presented in Chapter 2. This is not unexpected in view of the fact that a complex genetic background of tea plant may have been resulted from long-term outcrossing among the tea genotypes in Bangladesh. The data presented here will help in establishing maximization strategies for the improvement of tea in Bangladesh. The range of diversity exhibited by the genotypes studied shows potential for selection. In this respect, priority should be given to the marginal genotypes (belonging to group A and group C, **Figure 4.21**) which appeared to be most distantly related.

Present study reveals all the twelve tea genotypes used in this study could distinctly be identified by the presence and absence of unique RAPD bands. Therefore, RAPD markers provided a practical and effcctive method not only to evaluate the genetic diversity and relationships, but also for the identification of tea genetic resources in Bangladesh.

In conclusion, the information generated from this study showed that there are significant genetic differences among the 12 tea cultivars studied and that they could be used to strengthen the future tea cultivar improvement programme effectively by incorporating potential progenitors from the available tea germplasm.

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APPENDICES

Appendix I Analysis of variance (ANOVA) of genotypes for angle of first branch (AFB).

Item	df	SS	MS	VR_1	VR_2
Genotype (G)	11	21826.4333	1984.2212	32.702**	34.2378**
Replication (R)	2	124.516667	62.258333	1.02608 NS	1.07427 NS
G × R	22	393.15	17.870455	0.29452 NS	0.30835 NS
Within Error	324	19659	60.675926		
Pooled Error	346	20052.15	57.954191		

** indicates significant at 1% level of probability.
NS = None significant

Appendix II Analysis of variance (ANOVA) of genotypes for height of the plucking surface (HPS).

Item	df	SS	MS	VR_1	VR_2
Genotype (G)	11	3122.85556	283.89596	27.5611**	24.7967**
Replication (R)	2	304.605556	152.30278	14.7858**	13.3028**
G × R	22	623.927778	28.360354	2.75327**	2.47712**
Within Error	324	3337.4	10.300617		
Pooled Error	346	3961.32778	11.448924		

** indicates significant at 1% level of probability.
NS = None significant

Appendix III Analysis of variance (ANOVA) of genotypes for length of the plucking surface (LPS).

Item	df	SS	MS	VR_1	VR_2
Genotype (G)	11	10488.2333	953.47576	9.18221**	8.96425**
Replication (R)	2	559.616667	279.80833	2.69462 NS	2.63066 NS
G × R	22	3158.05	143.54773	1.3824 NS	1.34959 NS
Within Error	324	33644	103.83951		
Pooled Error	346	36802.05	106.36431		

** indicates significant at 1% level of probability.
NS = None significant

Appendix IV Analysis of variance (ANOVA) of genotypes for breadth of the plucking surface (BPS).

Item	df	SS	MS	VR_1	VR_2
Genotype (G)	11	5945.33333	540.48485	5.217**	5.35659**
Replication (R)	2	1431.31667	715.65833	6.90786**	7.09268**
G × R	22	1345.15	61.143182	0.59018 NS	0.60597 NS
Within Error	324	33566.6	103.60062		
Pooled Error	346	34911.75	100.90101		

** indicates significant at 1% level of probability.
NS = None significant

Appendix V Analysis of variance (ANOVA) of genotypes for leaf length of the fifth leaf (LLFL).

Item	df	SS	MS	VR_1	VR_2
Genotype (G)	11	1845.85456	167.80496	76.2690**	75.8077**
Replication (R)	2	2.41438889	1.2071944	0.5487 NS	0.5454 NS
G × R	22	53.0362778	2.4107399	1.0957 NS	1.0891 NS
Within Error	324	712.856	2.2001728		
Pooled Error	346	765.892278	2.2135615		

** indicates significant at 1% level of probability.
NS = None significant

Appendix VI Analysis of variance (ANOVA) of genotypes for leaf breadth of the fifth leaf (LBFL).

Item	df	SS	MS	VR_1	VR_2
Genotype (G)	11	652.679639	59.334513	217.803**	215.47**
Replication (R)	2	0.26005556	0.1300278	0.4773 NS	0.47219 NS
G × R	22	7.01394444	0.3188157	1.1703 NS	1.15776 NS
Within Error	324	88.265	0.2724228		
Pooled Error	346	95.2789444	0.2753727		

** indicates significant at 1% level of probability.
NS = None significant

Appendix VII Analysis of variance (ANOVA) of genotypes for leaf length and breadth ration of the fifth leaf (LL/LBFL).

Item	df	SS	MS	VR_1	VR_2
Genotype (G)	11	65.3291398	5.9390127	110.72**	111.857**
Replication (R)	2	0.23840484	0.1192024	2.22227 NS	2.24508 NS
G × R	22	0.99153073	0.0450696	0.84023 NS	0.84885 NS
Within Error	324	17.3793033	0.0536398		
Pooled Error	346	18.3708341	0.0530949		

** indicates significant at 1% level of probability.
NS = None significant

Appendix VIII Analysis of variance (ANOVA) of genotypes for leaf angle of the fifth leaf (LAFL).

Item	df	SS	MS	VR_1	VR_2
Genotype (G)	11	72662.6083	6605.6917	125.59**	127.325**
Replication (R)	2	308.466667	154.23333	2.93235 NS	2.97285 NS
G × R	22	909.2	41.327273	0.78573 NS	0.79658 NS
Within Error	324	17041.5	52.597222		
Pooled Error	346	17950.7	51.880636		

** indicates significant at 1% level of probability.
NS = None significant

Appendix IX Analysis of variance (ANOVA) of genotypes for pluckable shoot length (PSL).

Item	df	SS	MS	VR_1	VR_2
Genotype (G)	11	436.372083	39.670189	12.727**	11.9557**
Replication (R)	2	2.3195	1.15975	0.37207 NS	0.34952 NS
G × R	22	138.153167	6.2796894	2.01466**	1.89256**
Within Error	324	1009.909	3.1170031		
Pooled Error	346	1148.06217	3.3180987		

** indicates significant at 1% level of probability.
NS = None significant

Appendix X Analysis of variance (ANOVA) of genotypes for fresh weight of pluckable shoot (FWPS).

Item	df	SS	MS	VR_1	VR_2
Genotype (G)	11	25880.0408	2352.731	62.143**	53.8994**
Replication (R)	2	1163.0743	581.53715	15.3602**	13.3226**
G × R	22	2836.43407	128.92882	3.40542**	2.95367**
Within Error	324	12266.6169	37.859929		
Pooled Error	346	15103.051	43.650436		

** indicates significant at 1% level of probability.

Appendix XI Analysis of variance (ANOVA) of genotypes for dry weight of pluckable shoot (DWPS).

Item	df	SS	MS	VR_1	VR_2
Genotype (G)	11	3191.72843	290.15713	100.908**	81.8431**
Replication (R)	2	80.9795217	40.489761	14.0811**	11.4207**
G × R	22	295.015592	13.4098	4.66351**	3.78243**
Within Error	324	931.65353	2.8754739		
Pooled Error	346	1226.66912	3.5452865		

** indicates significant at 1% level of probability.

Appendix XII Analysis of variance (ANOVA) of genotypes for fresh and dry weight ratio of pluckable shoot (FW/DWPS).

Item	df	SS	MS	VR_1	VR_2
Genotype (G)	11	122.81307	11.164825	23.9423**	18.9914**
Replication (R)	2	0.81783771	0.4089189	0.8769 NS	0.69557 NS
G × R	22	52.321074	2.3782306	5.09997**	4.04538**
Within Error	324	151.088376	0.4663221		
Pooled Error	346	203.40945	0.5878886		

** indicates significant at 1% level of probability.
NS = None significant

Appendix XIII Analysis of variance (ANOVA) of genotypes for leaf area of pluckable shoot (LA).

Item	df	SS	MS	VR_1	VR_2
Genotype (G)	11	172419.176	15674.471	47.155**	50.0106**
Replication (R)	2	289.396517	144.69826	0.43531 NS	0.46167 NS
G × R	22	745.733689	33.896986	0.10198 NS	0.10815 NS
Within Error	324	107698.687	332.40335		
Pooled Error	346	108444.421	313.42318		

** indicates significant at 1% level of probability.
NS = None significant

Appendix XIV Analysis of variance (ANOVA) of genotypes for pluckable shoot density (SD).

Item	df	SS	MS	VR_1	VR_2
Genotype (G)	11	68699.4972	6245.4088	37.8832**	22.5162**
Replication (R)	2	3721.75556	1860.8778	11.2877**	6.7089**
G × R	22	42557.1111	1934.4141	11.7337**	6.97401**
Within Error	324	53414.5	164.85957		
Pooled Error	346	95971.6111	277.3746		

** indicates significant at 1% level of probability.

Appendix XV Analysis of variance (ANOVA) of genotypes for pluckable shoot yield/plant (Y).

Item	df	SS	MS	VR_1	VR_2
Genotype (G)	11	153975667	13997788	14.1791**	14.239**
Replication (R)	2	3700666.67	1850333.3	1.8743 NS	1.88221 NS
G × R	22	20282666.7	921939.39	0.93388 NS	0.93782 NS
Within Error	324	319857000	987212.96		
Pooled Error	346	340139667	983062.62		

** indicates significant at 1% level of probability.
NS = None significant