Introduction

1.1 Tea in India

Tea (Camellia sinensis [L.] O. Kuntze) family- Theaceae is a popular beverage crop of the world. It is grown in India, Sri Lanka, China, Japan and Kenya. India is the largest producer and consumer of tea. India is also a major tea exporter and earned a foreign exchange equivalent to Rs 3200 crores during 2012 from tea exports which is 12% of the world share (IBEF, 2013). Tea is produced in over 1500 tea estates across the country and engages over 3.5 million employees.

Tea is indigenous to Southeast Asia covering the parts of North Eastern India, North Burma and South-West China. The Indo-Burma region along the course of Irrawaddy River reportedly harbors high levels of genetic diversity of tea and is rightly identified as the primary birthplace of tea (Wight, 1962; Singh and Bera, 1994). Tea cultivation in India began with the discovery of “Assam” tea in 1823 by Robert Bruce (Singh, 1979). Subsequently, in the year 1836, tea germplasm was introduced from China. Natural hybridization of China tea with indigenous tea germplasm has resulted in the present day gene pool of Indian tea. The resultant planting material was also donated to other countries such as Sri Lanka, Mauritius, Bangladesh, Indonesia, Malaysia and east and central African countries. Thus, more than 60% of tea across the world has received its basic planting material directly or indirectly from India (Singh, 1979).

The major tea producing states in the country are Assam, Manipur, West Bengal, Tamil Nadu and Kerala. Three-fourth of the total tea produced in India is accounted by Assam and West Bengal together. While Assam teas (produced in plains of Assam and foothills of Manipur) are famous for their strong, brisk and full bodied liquor, Nilgiri teas (produced in Tamil Nadu and Kerela) are well known for their delicate flavor, strength and brightness. On the other hand, the...
Darjeeling tea is famous for its premium quality in international market which is due to the low temperature in hills of Darjeeling. With their own diverse agro-climatic conditions, other areas produce a number of varieties of teas which suit different tastes. The distinct characteristics of each region set them apart from one another in many different ways.

1.2 Classification of tea

Of over 80 species described under the genus *C.*, only three are under commercial cultivation for tea production (Sealy, 1958). These are a) the China type, represented by *C. sinensis*; b) the Assam type, represented by *C. assamica*; and c) the Southern or Cambod type, represented by *C. assamica* ssp. *lasiocalyx* (Wight, 1959, 1962). Wight (1962) considered the first two namely, *C. sinensis* L. and *C. assamica* Masters, as distinct species, while the third type known as the ‘Southern’ (or Cambod) type, was regarded as a sub-species of the Assam type naming it as *C. assamica* ssp. *lasiocalyx*. These three types have been traditionally classified based on morphology and overall plant type and loosely referred to as “Assam”, “Cambod” or “China” types depending on their morphological proximity to the main taxa (Banerjee, 1992; Konwar, 1999). Other species such as *C. irrawadiensis* and *C. taliensis*, have also morphological proximity to the cultivated teas (Banerjee, 1992). All these species have contributed to the tea gene pool. Therefore, the cultivated tea is highly heterogeneous, having several intergrades, introgressants, and putative hybrids which are categorized from extreme China type through intermediate to extreme Assam type, and hence the existence of true (Pure) China type, Assam type, or Cambod tea is doubtful (Visser, 1969).
The China type plants have numerous virgate stems arising from the base. Leaves are erect, small, 1.5-14 cm long wide, leathery dark, and green in colour (Figure 1.1). The Assam type plants have a distinct trunk, which grow up to a height of 10 to 15 meter if left unpruned (Figure 1.2a). Leaves are big, 8-20 cm long, 3.5-7.5 cm wide and glossy with distinct marginal veins (Figure 1.2b). The Cambod type is more or less similar to the Assam type. Leaves are more or less erect, yellow or light green in color, often turning into coppery-yellow or pink-red during autumn (Figure 1.3).

Figure 1.1. A China type tea bush

Figure 1.2. a) Stem and b) leaves of an Assam type tea plant
1.3 **Major problems faced by tea industry**

In India, tea industry is one of the oldest agro-based well organized industries. More than a million workers get direct employment from this industry of which a sizeable number are women. A large number of temporary workers are also engaged during the plucking season.

The labor cost is the largest cost overhead accounting for about 60% of the total cost of production. On the other hand, the tea plantations are not just economic production units, but rather social institutions, which control the lives of their resident work force to a large extent. Apart from employment, the plantations are also responsible for providing house, water, fuel wood, welfare and many other facilities that affect the daily lives of the workers.

The other major problem faced by tea industry is that more than 21.2 million hectares of Indian tea gardens are at the end of their economic life at present. As this is a substantial chunk of the total area of tea, the productivity of industry is running down gradually which is leading to a high cost of production. There is large number of ‘vacancies’ in these plantations. This situation can endanger the prospects of this industry if proper steps are not taken for its solution.
Therefore, a strategy to replace the existing plantations with new material needs to be developed. The strategy should take into consideration the fact that most of the current plantations were raised through seeds originally brought from China and different regions of Assam and adjoining areas (Singh and Bera, 1994). Thus, the current plantations harbor a major part of genetic diversity of Indian tea germplasm. The replacement strategy should ensure that this diversity is conserved within the new plantations.

1.4 Molecular Markers

Molecular markers are defined as landmarks on the genome since they refer to specific locations that can be appropriately mapped on the genome. A wide array of molecular genetic markers have found applications in genome analysis in plants. The major criteria of a good molecular marker are mentioned below.

- It should not be affected by environmental factors and developmental stages of the plant
- It should be distributed uniformly across the total genome to ensure wide genome coverage
- It should be efficient, fast, reproducible and economical
- It should display polymorphism to allow discrimination of genotypes
- A marker showing codominance is preferred over those showing dominance as a codominant marker permits discrimination between homozygous and heterozygous genotypes
1.5 Potential of molecular markers in germplasm characterization

Information on genetic diversity is a prerequisite for conservation and sustainable utilization of important genetic resources. Knowledge on genetic variation provides useful insights into taxonomy and phylogeny of plant species.

Tea is an open-pollinated species which is expected to harbour high levels of heterozygosity in its populations. In practice, tea genotypes display morphological features resembling from *C. sinensis* to *C. assamica* (Wight, 1959). Natural inter-specific hybridization among these species has been extensive and it is often conjectured whether at all pure genotypes of the above-mentioned three species still exist (Visser, 1969). The resulting hybrids have been exploited for developing many of the cultivated Indian tea varieties through selection from natural populations. Interestingly, these hybrids continue to be classified as China, Assam and Cambod types by tea breeders and planters solely on the basis of their morphological proximity to these prototypes (Konwar, 1999). Since the detectable diversity in morphological characters within each type is limited and difficult to score unambiguously, true estimation of genetic diversity using morphological characters is difficult. Moreover, these morphological traits are largely modified by environmental factors and, therefore, do not permit a realistic quantification of genetic diversity.

In recent years, several molecular studies have been carried out in tea aiming at a reliable assessment of genetic diversity using PCR based markers such as Randomly Amplified Polymorphic DNAs (RAPD), Inter-Simple Sequence Repeats (ISSR) and Amplified Fragment Length Polymorphism (AFLP) (Wachira et al., 1995; Paul et al., 1997; Lai et al., 2001; Balasaravanan et al., 2003; Yao et al., 2008; Sharma et al., 2010; Raina et al., 2011). A preliminary linkage map has been developed in tea using RAPD and AFLP markers (Hackett et al., 2000). Besides these, Cleaved Amplified Polymorphic Sites (CAPS) and Sequence Tagged Microsatellite Sites (STMS) have also been developed in tea (Ueno et al., 1999; Kaundun and
Matsumoto, 2003a; Freeman et al., 2004). Most of the studies have, however, utilized RAPD and AFLP markers for estimation of the genetic diversity. The application of RAPDs for assessment of genetic diversity has been widely criticised since it suffers from problems related to non-reproducibility and low multiplex ratio (Powell et al., 1996). In contrast, AFLP markers have been slated as highly reliable since they overcome several drawbacks associated with RAPD technique.

The AFLP techniques can be used as an ideal tool for genetic diversity studies owing to the following criterions

a) when there is no a priori sequence information to develop sequence based markers such as SSRs, SSAP, CAPS and SCARs
b) for intraspecific studies
c) and for rapid generation of large data

Considering these factors, AFLP markers seem to be a suitable technique in tea for analysis of genetic diversity.

The number and quality of bands in AFLP profiles is influenced by genome complexity of the species under investigation (Vos et al., 1995; Han et al., 1999). Use of inappropriate AFLP primer combinations can result in bad quality banding profiles which may be difficult to score. AFLP profiles can be modulated by changing the number of selective nucleotides in the AFLP primers (Vos et al., 1995). AFLP fingerprints of tea accessions obtained using EcoRI+3 / MseI+3 primers are characterised by high backgrounds resulting in low clarity due to sheer number of bands. The genome of tea is moderately large (2n= 3.8 x 10^6 Kb) (Hanson et al., 2001) and therefore requires refinement of conventional AFLP in order to generate good quality AFLP data. In this study, an attempt was made to evaluate two modifications of AFLP methods for their banding attributes and ease of scoring in tea accessions. The better of the two methods was
used to study the genetic diversity in selected tea accessions from different research institutes in India.

Microsatellites have higher information content than dominant markers such as AFLP and RAPD. These are co-dominant in nature and their assay methods are easier. However, their major limitation is that special efforts are required for their development in the species under investigation. When this work was initiated, less than a dozen of microsatellite markers were available in tea. Therefore, efforts were made to develop a larger number of novel microsatellite markers in tea for future genotyping applications.

Another category of useful markers are sequence specific amplified polymorphisms (SSAPs). In this study, SSAP markers in tea were developed through isolation of long terminal repeats (LTRs) of retrotransposons. So far, this is the only document describing the development of SSAP markers in tea.

1.6 Objectives

1. Standardization of an efficient AFLP based method in tea and analysis of genetic diversity in Indian tea accessions from different tea research institutes

2. Development of novel microsatellite markers in tea

3. Development of SSAP markers in tea through isolation of LTR sequences
1.7 Thesis structure

The thesis has been divided into following chapters-

1. Introduction
2. Review of literature
3. Materials and methods
4. Genetic diversity in Indian tea accessions
5. Development of microsatellite markers
6. Development of SSAP markers
7. Summary and Conclusions
8. Bibliography
9. Appendices