Chapter 1

General Introduction
Tea [Camellia sinensis (L.) O. Kuntze] is one of the most important commercial crop generating employment as well as foreign exchange earnings for a number of developing countries. Its tender growing shoots are used for making different types of tea which are the popular non-alcoholic hot beverage commonly used all over the world. This plant was known to the Chinese people before A.D. 350 (Ukers, 1935) and the tea drinking was originated in China. The Dutch traders popularized tea as beverage among the peoples of different countries and dominated the tea trade for more than a century (Barua, 1989). Tea grows over a wide range of latitudes from the Black sea coast at 42°N to 35°S latitude in Argentina, China, India, Japan, Sri Lanka, Indonesia, Kenya, Malawi etc. which are the major tea growing countries between the two extreme latitudes.

1.1 Origin of Tea

Although the place of origin of tea is not yet known exactly, South East Asia is believed to be the original home of tea. According to Wight (1962), tea might have originated in the region around the point of intersection of latitude 29°N and longitude 98°E near the source of river Irrawady. This region, which is the meeting ground of North East India, North Myanmar, South West China and Tibet, was considered as the primary center of origin of tea (Wight, 1958). Kingdon-Ward (1950) on the other hand, advocated that the Altai or somewhere in the Mongolian plateau must be the primary centre of origin of tea. Sealy (1958) identified the origin of about 82 species of Camellia in the source of river Irrawady. However, according to Hasimoto and Simura (1978) tea was originated a wild plant in Yunnan and Sichuan provinces of China and north eastern part of Assam in India. Small-leaf varieties of China tea were originated in eastern and southeastern China where they were cultivated, while broad-leaf varieties originated independently in India and Yunnan (Cohen Stuart, 1919). Therefore, tea plants have been classified generally into two major varieties, var. sinensis from the temperate regions and var. assamica from the tropical regions. The original tea plantations were mostly with small-leaf China bushes. The Assam and Cambod types of broad-leaf tea were introduced later although these varieties/types were discovered earlier than the introduction of commercial tea cultivation using small leaf China type plants brought from China during 1834-35.
1.2 Tea Taxonomy

Tea belongs to the family Theaceae, which consist of three tribes according to the morphological study by Takhtajan (1997) as well as chloroplast sequence data of Prince and Parks (2001). Theaceae consists of the tribe Theeae and includes 7-21 genera depending on the system of classification employed. However, the only genus recognized as economically important, is the *Camellia* which includes 82 species, most of them are indigenous to the highlands of South East Asia (Sealy, 1958). These 82 species are further grouped into 12 sections. Tea that is known only in cultivation comes under the section *Thea*. A few species like *Camellia japonica*, *C. sasanqua* and *C. reticulata* are grown widely for ornamental purposes while *C. oleifera* is cultivated in China for the edible oils to be extracted from seeds. *C. sinensis* is the most important species of the family for its beverage value. Early researches in tea varieties revealed that extensive natural hybridization, not only between the geographical races but also involving related species, might have taken place during the large-scale dispersal of the tea plant in South East Asia (Bezbaruah and Dutta, 1977). Due to this free natural hybridization each of the variety resulted in a form of complex taxonomic group of related plants. The three main varieties are the Assam Variety (*Camellia assamica* (Masters) Wight) tall tree with broad leaf, the Cambod/Indo-China/Southern Variety (*Camellia assamica* sub. sp. Lasiocalyx (Planchon ex Watt) Wight), with pigment in leaf petiole and the China Variety (*Camellia sinensis* (L.) O. Kuntze), shrub with small leaf. However, a fourth type called Hybrid Variety with mixed taxonomic characters also exists (Wight, 1959), in the tea cultivation.

1.3 Tea Production

Tea, currently manufactured by the tea growing countries can be classified into three types namely- black, green and oolong tea. However, the principal types of tea produced and consumed in the world are black and green tea, with small amounts of oolong and other types like white, yellow and reprocessed tea (International Tea Committee, 2003). Tea is mainly produced by most of the tropical countries in Asia, South America and South Africa. In Asia, India, Sri Lanka, China, Vietnam, Japan and Indonesia are some of the leading countries
producing tea. India and Sri Lanka produce most of the black tea while the other countries produce green tea and other varieties. India is the largest tea producing country in the world followed by Kenya and Sri Lanka in 2007 while Kenya is also the largest exporter of tea followed by China and Sri Lanka (www. Indiatea.org). According to the latest Tea Board data (www.teaboard.com), the volume and value of teas crossing the Indian shores are on the decline as it is getting out-priced in international markets by Kenyan and Sri Lankan brews because of the high prices of Indian tea compared to other countries. So, due to stiff competition in the global tea market, the cost of production of Indian tea has increased and India no longer enjoys the reputation of leading position in the world market. World black tea production has been projected to increase upto 2.4 million tonnes in 2010. This expected growth would result largely from the improvement in yield. Improved classical breeding technology and the advances in plant biotechnological research have opened new vistas in the propagation of tea plants with higher productivity and improved resistance to the biotic and abiotic stress factors. Thus, renewed research efforts on breeding and genetic improvement through biotechnological tools will help in attaining the new objectives which are otherwise difficult in conventional methods. The improvements in the yield potentials and cup quality is urgently required to meet the requirement of the ever increasing population as well as for changes in consumer’s preferences, with a stronger market prospect for the tea industry.

1.4 Tea Cultivation in India

Tea was first introduced in India during the year 1834 with seeds and plants imported from China (Barua, 1989). But Robert Bruce discovered the indigenous Assam tea plants in 1823 which were used by the hill tribes in making traditional beverage for a long time. These tea plants were growing wild in the Upper Brahmaputra valley. However, it took long time for getting recognition as Tea. In May 1838, the first Indian tea from Assam was sent to England for public sale (Tea Board of India, 2007).

Generally low hills, below 700 m elevation of temperate zone are preferred for tea cultivation but the large tea areas of N.E. India and Bangladesh are located on flat valleys of less than 200 m above sea level (Barua, 1989). India has three
distinctly different tea-growing regions viz. Darjeeling (North-Eastern India), Assam (far North-East India) and Nilgiri (South India). These regions are geographically separated, thereby producing three different teas both in style and in taste/flavor. At present about 432,000 hectares of land are under tea cultivation in India. Northeast India produces a wider variety of tea than any other tea growing areas of the world.

Tea grows up to 2,400 m (8,000 feet) height above sea level in the tropical belt. It prefers a warm, humid climate, with plenty of well-distributed rainfall and long sunlit days. Therefore, it flourishes nearer the equator. Because of specific climatic requirements, tea plantations are confined to certain regions, and there is a limit to the areas of suitable land available for future plantations. There is, thus, a need to increase the productivity of existing plantations through the rapid introduction of improved technology for cultural practice and genetically improved superior planting materials. Due to the increasing popularity of tea throughout the world, the demand for high quality is also rising which could be met with the development of high yielding varieties having very high cup quality. The improved planting materials will be required to multiply fast for quick percolation to the industry with the help of a faster propagation method. Therefore, a technique of convenient micropropagation and genetic improvement of tea through biotechnological tools have tremendous necessity.

1.5 Background and purpose of the study in Tea

So far, selection of tea germplasms having required traits and the traditional breeding has been the only means for improvement of the tea plant. However, tea being a self incompatible and cross pollinated plant, the indiscriminate early introduction of genetic materials from various sources and free hybridization among them created great diversities in its morphological, physiological and genetical characteristics. Furthermore, tea plant being perennial and out crossing in nature, certain limitations make breeding a time consuming and labour intensive process. Seeds were the only means of propagation of tea until the development of vegetative propagation technique from single node cutting by Tunstall in 1931 at Tocklai. This encouraged the tea breeders to develop clonal varieties in North East India. But tea improvement by conventional
breeding is a prolonged process. Although the conventional vegetative propagation (VP) of tea, using single node cuttings is comparatively easy to perform, only one plant can be developed from one cutting and it takes 10-12 months to get one plantable plant. For clonal plantation industry needs 11,000-15,000 plants for planting in one hectare of land and a huge number of clonal plants are required every year. Virtually, it becomes difficult to produce such a large number of clonal plants within a short period of time. To meet the industries demand of clonal plantable plant particularly of the newly developed clones, the existing commercially used VP technique becomes insufficient. Under this circumstances, micropropagation for rapid and mass multiplication of elite tea clones has assumed importance.

Production and productivity of tea in the major tea producing countries are declining and many of these plantations are suffering from mortality due to stresses caused by biotic and abiotic factors (Sivapalan, 1988). The replanting of older plantations, as well as an increasing demand for high quality tea has increased the necessity of clonal planting material (Akula and Dodd, 1998). In this context, use of tissue culture methodology offers a potential adjunct for mass propagation of selected elite clones of tea having very high yield and quality potential with resistance towards biotic and abiotic stresses. All these factors are governed genetically and therefore, a detailed and systematic cytogenetic and molecular investigation is inevitable for planning any genetic manipulation. In nature, there are variations from genotype to genotype and, morphological and physiological characteristics of phenotype serve as proxy measurements of genotypic variation. Therefore, screening of the micropropagated plants that are obtained by axillary and adventitious propagation methods is necessary before reintroduction, and justification on their fidelity is necessary for any commercial exploitation. An alternative method for multiplication and production of genetically uniform plants in a very short period of time could be through micropropagation of organized meristems. However, it is important to assess the genetic constitution and stability of both conventionally propagated and micropropagated plants before planning strategies for further propagation and also after consideration of economics. Detection of subtle somaclonal variations at phenotypic, cytological, biochemical and molecular levels among
micropropagated plants in many taxa including tea have brought into question on the validity of the concept that complete genetic stability is retained in plants derived from organized meristems (Devarumath et al., 2002).

Molecular markers have come up as the most desirable tool for establishing genetic uniformity of the micropropagated plantlets. PCR based markers such as RAPD (Random Amplified Polymorphic DNA), ISSR (Inter Simple Sequence Repeats) and SSRs (Simple Sequence Repeats) can characterize as well as compare the genomic instability within the micropropagated population. The present study for establishment of genetic fidelity between micropropagated plantlets derived from axillary buds of nodal explant taken from a field mother plant and explants from *in vitro* germinated seedling explants not only provides an alternative viable system of propagation but also explores the possibility of use of seedling genotypes as propagules.

Clonal propagation by conventional vegetative propagation method using single node cutting of selected elites has, over the years, resulted in substantive increase in overall quality and yield. *In vitro* culture methods along with advances in the field of genetic engineering are likely to make a major impact on the tea industry. Although impressive progress has been made in this field, the full impact of this technology on the industry has been somewhat restricted on account of (i) lack of a reproducible protocol applicable on a wide range of germplasms (ii) difficulty faced in rooting of regenerated microshoots of some germplasms (iii) economics of the whole process for commercial viability and (iv) mortality during the process of hardening and lack of a full-proof transfer and establishment system. The need is to explore alternative systems of propagations such as direct embryogenesis to overcome some of the associated problems and to go for further genetic improvement.

Agricultural crops offer potentially very high volume demand. However, high production cost of tissue culture plantlets limits this application for commercialization. This problem has been addressed to by evolving a reliable Cost Effective tissue Culture Production Technology. This is entirely a new perspective in commercial micropropagation industry. Since it combines cost effectiveness, process efficiency, flexibility in scale-up and uncompromising quality considerations, this is an advance over "State-of Art" technology and is set
to redefine strategic considerations and potential tissue culture applications which will have long-term effect on global micropropagation industry.

1.6 Objectives of Research

The present study was taken up to find out the best explant for tea micropropagation through multiple shoot proliferation from axillary bud of nodal explant of field grown mother plant as well as *in vitro* germinated seedling. For the development of an alternative method of propagation, studies on direct somatic embryogenesis have also been planned to explore the possibility of using it as an alternative method of clonal propagation. Towards this goal, the work would envisage simplifying the steps of tea micropropagation by reduction in the number of intermediate steps, increasing or optimizing the number of plantlets obtained per propagule so that the cost is reduced and verifying the genetic fidelity of the plantlets as a tool for authentication of uniformity. Moreover the somatic embryos produced from the germplasms will be screened for their transformation efficiency for planning any genetic manipulation for molecular breeding of the crop. The present research was based on the following objectives:

1. Establishment of optimized *in vitro* propagation systems for large-scale multiplication of elite clones of tea.

2. Standardization of a technique for cytological studies of obtained plantlets.

3. Study the genetic fidelity of the obtained plantlets in order to investigate any genetic variation from the mother plant using molecular markers.

4. Study the feasibility of using explants from seeds as an alternative source of propagules, i.e. propagation of individual seed genotypes and their genetic assessment.

5. Screen the germplasms for their transformation efficiency and explore the possibility of using alternative systems of propagation such as direct somatic embryogenesis and their commercial feasibility.