Chapter 1
Introduction

The Way of Tea states

"One cup does all disorders cure
With two, your troubles will be fewer
Thrice, to the bone more vigour give
With four, forever you will live
as young as on your day of birth
A true immemorial on the earth"

Old Chinese Poem

Many of us would agree with the ancient Chinese saying: *"Better to be deprived of food for three days, than of tea for one"* (Ody 1993).Tea, *Camellia sinensis* (L.) O. Kuntze belongs to Thea section of the *Camellia* genus in Theaceae. Chemical composition of *Camellia sinensis (*Ukers 1935) is given in Table 1.1. It is the oldest caffeine containing beverage which has been used for two to three hundred years in Southeast China and continues to be the most popular and widely consumed beverage in India and across the world.

Tea plant is a woody perennial shrub or tree (9 -15 meters in height) under natural conditions and 1.5 meters under cultivated conditions (Fig.1.1). Leaves are alternate, elliptical on short stalks, leathery and with toothed margins. The flowers are white in colour and born singly or pairs at the axils. The fruits are green in colour with 2–3 seeds and start bearing within 5–6 years after planting. The cultivated varieties separate into two main groups on the basis of foliar and growth characteristics. China teas, *Camellia sinensis* var.*sinensis*, are slow growing, dwarf trees, with small, erect, comparatively narrow, dark green leaves and are resistant to cold. In contrast, Assam tea, *C. sinensis* var. *assamica,* is quick- growing taller tree with large, drooping leaves and resistant to cold, while natural triploid (2n=45) and tetraploid (2n=60) varieties adapted to this environment have also been discovered. Various hybrids between China and Assam types are planted according to easy intercrosses. Hybrids are characterized by the intermediate charac-

Table 1.1 Chemical Composition of tea (Ukers 1935)

Constituent	Percent (%)
Water	5.00-8.0
Caffeine	2.50-5.0
Nitrogen	4.75-5.5
Soluble matter	38.0-45.0
Tannin	7.00-14.0
Mineral elements	5.00-5.75

teristics of leaves and growth of trees when compared between the two types.

1.1. Distribution of tea (worldwide and in India)

Tea is cultivated in 31 countries, scattered from 45°N to 33°S of the equator. From the main centers of its primary origin in South-East Asia, tea has spread far and wide into tropical and subtropical areas and adopted broad characteristics corresponding to regions of tropical rainforest, tropical Savannah and summer rain areas (Eden 1976; Greenway 1945); Kingdon ward 1950; Kulasegaram 1980).The earliest knowledge of the tea- the oldest caffeine-containing beverage has been derived from China for about 3000 years back. It is believed to have originated somewhere in South-East Asia (Kingdon-Ward, 1950) but the current distribution patterns of tea varieties suggest that the centre of origin of tea is probably near Irrawady (Burma) region from where it has been dispersed to South-Eastern China, Indonesia and Assam.

In India tea seeds from china were brought and sown at Botanical Garden, Calcutta in 1780 (Bezbaruah, 1999). Tea (china type) was introduced in north east India in 1836, although in 1823, Major Robert Bruce discovered tea plants growing wild in some hills near Ragpur (now Sibsagar) the then capital of Assam (Ukers 1935). In South India one Dr. Christy has experimented on growing tea in Nilgiris in 1832 (Muralidharan 1991). But the tea did not get under way until 1893 when the planting of tea increased rapidly (Harler 1963). On the basis of its distribution tea is classified into two main varieties: var. sinensis which originated from mainland China recognized by its small leaf, slower growing bush withstanding colder climates (Wight 1962; Banerjee 1992) and var. assamica discovered in the nineteenth century in the Assam region in India and later in other regions of south east Asia as well (Kaundan & Park 2002) which can be easily identified by its large leaf, tall and quick growing characters which is well suited to very warm tropical climates (Wight 1962; Banerjee 1992). Besides these two basic varieties there exists *Camellia assamica* ssp. lasiocalyx (Planchon ex Watt) with its intermediate leaf size also called cambod variety.

1.2. Agro-Climatic conditions required for tea cultivation (Tea board of India)

A suitable climate for cultivation has a minimum annual rainfall of 45-50 inches (114.3-

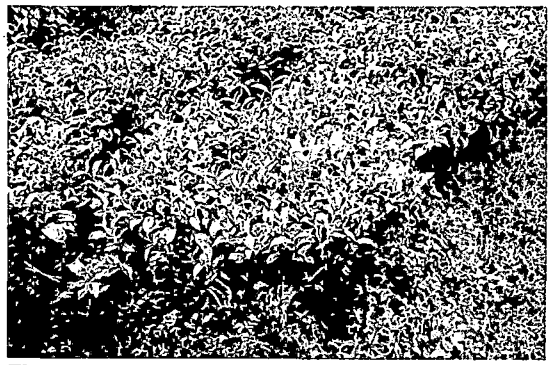

Fig 1.1 A cultivated shrub of tea plant.

127 centimeters). Tea soils must be acidic since tea plants will not grow in alkaline soils. A desirable pH value is 5.4-5.8 or less. Tea can be cultivated up to 7,218.2 feet (2,200 meters) above sea level and can grow between the equator and the forty-fifth latitude. The plants are reproduced through tile-laying or through seeds from trees that have grown freely.

1.2.1. Air Temperature

Tea is grown under a regime of air temperature that varies between 8° and 35°C. In Darjeeling, the extension growth stops at monthly mean maximum and minimum temperatures of 19.4°C and 12.4°C respectively in November and it start flushing during end of March when mean maximum and minimum temperatures exceed 21°C and 14°C respectively. The extension growth of the tea plant in general ceases below a minimum temperature of 13°C. In Darjeeling, highest yield can be achieved in June when mean maximum and minimum temperatures remains 23.5°C and 18.3°C respectively. The rapid decline in yield in Darjeeling during October and then stops during November until end of March indicates that low temperature is one of the major climatic variables, limiting yield. It has been reported that the higher yield in Darjeeling can be achieved during the period from June to September when the differences between maximum and minimum temperatures remains least in comparison with the rest of the year (Fig. 1.2).

1.2.2. Soil temperature

In many instances soil temperature is of greater importance to plant life than air temperature and soil temperature influences to growth and yields of tea. Soil temperature is an important variable, with a lower limit of

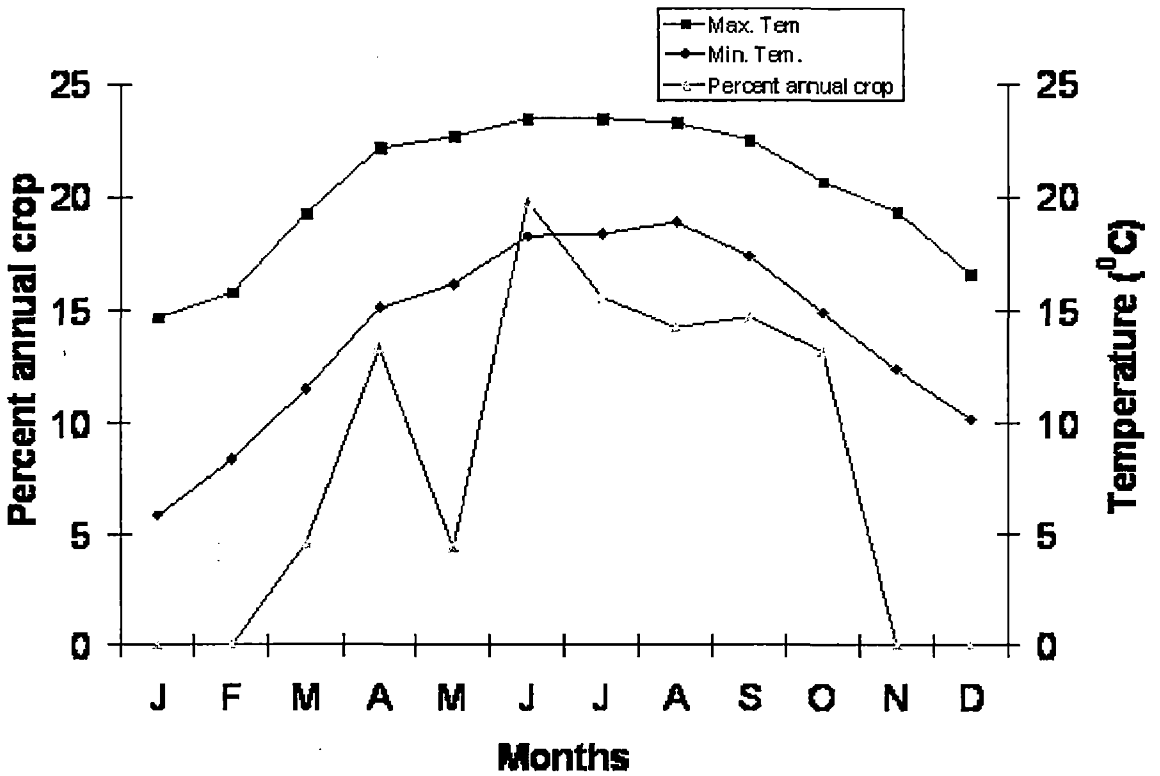

Fig. 1.2 The relationship between monthly yield and air temperature at Darjeeling. Source: Darjeeling Tea Research and Development Centre, Tea Board of India.

about 20°C, at 0.3 m under short grass surface (or 16°C beneath a canopy tea), below which shoot extension rates will be reduced. The corresponding upper levels are 29°C and 25°C respectively.

1.2.3. Rainfall

Tea is basically a rain-fed crop. It is grown well in areas where annual rainfall varies from 1150 to 6000 mm. Tea should not normally be grown in areas where the rainfall is below 1150 mm, unless irrigation is available. The effect of precipitation is perhaps more manifested by its influence on moisture status of the soil and in inducing vegetative growth. Therefore, distribution of rainfall over the year is as vital as the total annual rainfall. Annual rainfall varies from 2274 (Kalimpong sub-division) to 4082 mm (Kurseong sub-division) in Darjeeling where in May, the southerly winds reaches the hills and cause increased precipitation which is at times very high (Fig. 1.3). There is some residual effect of monsoon in November. But, there is almost no rain in November and December and the light showers which fall in January and February occur when shallow depressions are passing eastward over the plains. In October, northerly winds begins, cloud is much less than in previous months and rainfall occurs, mainly owing to cyclonic storms which generally recurve towards North Bengal at the end of the season

1.2.4. Humidity

It has influence in determining the loss of moisture by evapo-transpiration. The invisible water content of the air is expressed as relative humidity (RH), saturation vapour pressure deficit or dew point. RH of 80 - 90 % is favourable during the growth period of tea plants, below 50 %, shoot growth is inhibited

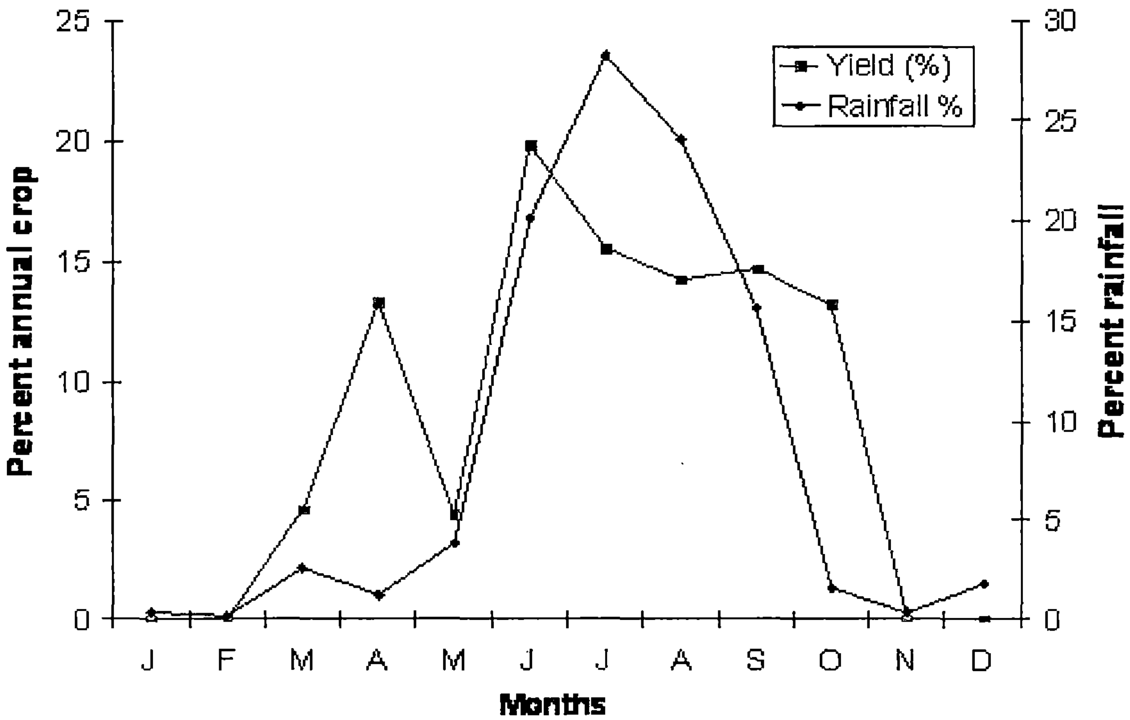

Fig. 1.3 The relationship between monthly yield of processed tea and rainfall at Darjeeling gardens. Source: Darjeeling Tea Research and Development Centre, Tea Board of India.

and below 40 % growth is adversely affected. In Darjeeling, even during the driest part of the year in March and April, mean RH never drops below 60 % in this region.

1.2.5. Solar radiation

It is the source of energy which sustains organic life on the earth surface. Crop production is in fact an exploitation of solar radiation. The intensity and duration of sunshine has also an important influence on the growth of tea plant. The hours of bright sunshine are fewer in the rainy season of Darjeeling hills due to the overcast clouds.

1.2.6. Day length

There are remarkable variations in day length between 9 and 15 h prevailing in Darjeeling. In Darjeeling, highest yield was recorded during June when average day length was 13 h 49 m. In fact, 50 % annual crop is produced in Darjeeling during June to August when day length obtained in between 12-13.5 h is a factor contributing to high productivity during this period.

1.2.7. Altitude

The China variety in particular and the hybrids in general produce very fine, flavoury tea when they are grown above an altitude of about 1100 m as in the case of Darjeeling. China hybrid bushes from Darjeeling fails to reproduce the full hill muscatel flavour if grown in the plains of Assam and Dooars. It may thus be stated that the Darjeeling teas owe their unique flavour partly to the type of bush and partly to the climate.

1.2.8. Hail

It is unpredictable and of a localized nature. Hail in Darjeeling is almost a regular phenomenon every year during spring and cause severe losses. Hailstorms occur in one valley or other during March to May, but end of March to early April is the period when it occurs most. The severity of damage ranges from shattering of leaves to peeling of bark in trunks, young stems breaks and bushes are defoliated. It can be particularly harmful when the bushes are recovering from the effect of a severe drought and are re-foliating after pruning. In Darjeeling hills, loss of higher priced quality crop during first and second flush due to hail damage is actually the real damage in terms of income. Despite destruction of current crop it damage stems which lead to disease and pest problems (Fig. 1.4)

1.3. Methods of propagation

Tea is traditionally propagated through either seeds or stem cuttings, with a life span of more than 100 years. The plants are obtained from seeds because the flower has high cross pollinating ability. Biclonal seeds i.e., trees planted in two clones in the seed garden are used for propagation. Cross fertilized seed bushes are selected for yield and quality or other characteristics. Seeds are grown in special seed gardens and seedlings show high heterogeneity. Moreover, since seeds become recalcitrant and unable to retain their viability through long term storage, seeds need to be sown immediately. Vegetative propagation by using cuttings has become one of the most frequently used

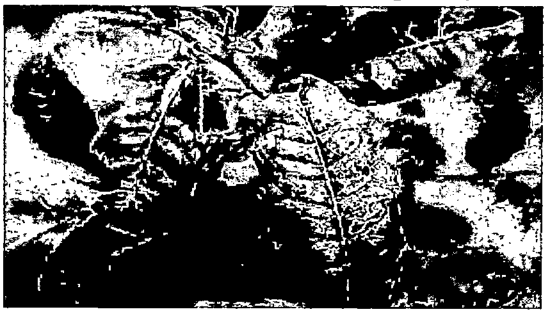

Fig. 1.4 Hail damaged tea plant in Darjeeling

methods. Usually single internode cuttings, taken immediately above a leaf and axillary bud, are planted into the rooting bed. New growing shoots with five to six leaves are also used for cutting materials. The cuttings from selected trees are planted in shaded propagating beds and given moderate amounts of water. Rooting ability varies among cultivars. Methods of propagation with seeds or cuttings require a longer period of time and space for production of nurslings. Shaping and pruning are done to maintain a convenient height for plucking, to induce vigorous vegetative growth and ensure a continuous supply of flushes. China types, being dwarf and slow growing, require relatively little pruning, where as Assam types and hybrids have to be kept within bounds. Pruning should be done during a dormant period (Purseglove 1974). Newly grown terminal buds with two or three leaves are plucked and brought to the factory.

1.4. Processing and marketing of Tea

The young leaves are processed into different types of tea, such as black, green, and oolong. The *assamica* variety contains large amounts of tannin and Catechin and is particularly used for black tea, where as *sinensis* tea accounts for most of the green tea production (Kaundan & Matsumoto 2003). Health benefits attributed to tea consumption are well proven. The processing of black tea comprises four main steps: withering or drying, rolling, fermentation and firing. During firing, the fermentation is stopped by destroying the enzyme with heat. This is done either by steaming or roasting. The process takes about 40 minutes at 71°C and 15 minutes at 99°C (Kihlman 1977). The fermented tea leaf enters the heating room, where it immediately undergoes heating at a high temperature of 90 to 95°C. The firing process is performed in special dryers. The hot air from the heater reduces tea moisture content to 3% to 4%. The firing stage is followed by a sorting process to make the half finished product marketable (Bokuchava and Skobeleva 1980).

Tea (*Camellia sinensis* L.; family Theaceae) is the oldest non-alcoholic caffeine-containing beverage crop in the world, and India is currently the foremost producer, consumer, and exporter of commercial tea. India with 5.11 lakh hectares of tea under cultivation produces an average of 850 million Kg of tea per year. It accounts for a fifth of the global tea area and nearly a third of the total world production. At present tea is grown in 16 states. Major tea growing states are Assam (50.7%), West Bengal (22%), Tamil Nadu (15.9%) and Kerala (8.3%) (Boriah, 2004).

Darjeeling produces the world's finest quality tea in the steep hill slopes of Eastern Himalayas. It is regarded as the most important beverage consumed in India and worldwide after water. This minor crop contributes significantly to the economy of many asian countries including India (Kaundan & Park 2002). Despite India's historical success with the tea industry, in recent years, Indian teas have faced serious competition in the international market. The UK now imports tea cheaper from Kenya and Malawi. To combat some of these challenges advertisers have made efforts toward building marketing campaigns in order to boost the tea market. Some efforts have even been made to promote tea like Coca Cola. Yet despite these efforts, tea producers in India continue to face serious challenges: how to keep quality, production, and exports up without driving prices down.

1.5. Diseases of tea

Diseases of tea plants differ within the types of plant and planting areas. In areas where Assam teas (var. *assamica*) are grown, as in India, Srilanka and many other countries, blister blight caused by *Exobasidium vexans Massee* remains the most dangerous disease. Air borne basidiospores of *Exobasidium vexans* spread and form white blisters on young leaves and stems. Buds can be attacked and whole shoots may die. Anthracnose caused by *Gloeosporium theae-sinensis* Miyake is the most serious disease in Japan and China. It spreads over the area where the China tea (var. *sinensis*) is produced. It has been found very recently that fungus invades the plant only through the tricomes of three young leaves from the top of the growing shoots (Hamaya 1981, 1982). The fungus, however, does not attack the stem. Variety *assamica* and its hybrids are highly resistant to this disease. Grey blight caused by *Pestalottia theae* Sawada, *Pestalotia longiseta* Spegazzini and brown blight or copper blight by *Glomerella cingulata* (Stomen) Spalauding et Schrenck, white scab by *Elsinoe leuospila* Bitancourt et Jenkins (*Sphaceloma theae* Kurosawa) infect the leaves or growing shoots. Root disease, red root disease caused by *Poria hypolateritia* Berk. is very serious in Srilanka, India and Indonesia, but not observed in Japan. In its mode of development it resembles *Armillaria*, by causing the sudden death of the tree. *Armillaria mella* (Vahl: Fr.) Kummer is common in Africa but remains rare in Srilanka, India and Japan. Charcoal stump rot caused by *Ustilina deusta* (Fr.) Petrak and black root disease by *Rosellinia arculata* Petch are serious diseases in India, Srilanka and Indonesia, while charcoal stump rot is sporadically found in Africa. In Japanese *Pythium* cuttings, root rot by *Pythium spp*. is becoming a prevalent disease with the extention of vegetative propagation. The affected roots become watery, whitish brown then turn a brown colour and often flatten without inner substance. White root rot caused by *Rosellia necatrix* (Hartig) Berlese infests the plant and is also a very common disease in ornamental fruit trees. Stem disease, brunch canker caused by *Phomopsis theae* Hara, *Nacrophoma theicola* Petch, Nectria spp. and *Poria hypobrunnea* Petch are common in Srilanka and India. It is considered that a viral disease causes necrosis of phloem which can be present in root, stem or leaf bud. However, the vector has not been discovered and the diagnostic symptoms are spreading in tea producing areas of Srilanka and India. According to variety, tea plants differ in tolerance to some diseases such as anthracnose. It is suggested that there is scope for breeding of resistant varieties. Since some diseases are common in one area or country but not in others, the prevention of epidemics in international trade still represents a very important goal.

1.6. Uses of Tea (*Camellia sinensis*)

Tea has been consumed socially and habitu-

Table 1.2 Nutritive value of tea/tea infusion

Nutrients	Amount from 1 cup of tea	Actual requirement
Riboflavin	1mg	1.5mg per day
Nicotinic Acid	7.5mg	16mg per day
Pantothenic	2.5mg	6-10mg per day

ally by people for so long (since ±3000 BC), that aside from the astringent taste and boost it provides its medicinal properties are often over-looked. However, traditional healers have long believed that drinking tea is a means of prolonging life (Chopra 2000). It helps cure digestive complaints, infections and pain relief. Tea is one of the most popular beverages and is consumed by nearly half of the world's population. Though it is not a favoured drink for children, it is consumed by almost all adult population. Nutritive value of tea/tea infusion is given in (Table 1.2). However, a cup of tea with one teaspoon of sugar and two tablespoon of milk gives 42Kcals (1 teaspoon sugar=20 Kcalorie, 2 tablespoon milk=22 Kcalorie).

1.7. Tea Genetic Resources, conservation and its yield worldwide

1.7.1. Tea Genetic resources and conservation

A total of 2532 accessions have been collected and preserved in the field gene bank at Tocklai Experimental Station (Singh 1999). This collection has undoubtedly helped India as well as other tea growing countries in evolving superior plant materials (Bezbaruah, 1974; Singh, 1979). In South India, a majority of the selections available in the germplasm at present have been selected from some of the commercial tea estates of that region (Satyanarayana and Sharma, 1986). East Africa's tea germplasm is predominantly of the Assam type (*C.sinensis* var. *assamica*), and although appreciably diverse, the variability is not sufficiently broad since many of the clones are genealogically related (Wachira *et al.*, 1995). The preservation of tea germplasm has great importance primarily because the seed-grown sections of tea are being massively uprooted, the seed sources of which no longer exist and are lost forever. The wide variability in the commercial tea populations offers scope for the selection of elite mother bushes with desirable attributes in a practical plant improvement programme (Richards 1960; Bezbaruah 1975). Therefore, it needs to be preserved and utilized judiciously.

1.7.2. Tea Yield

Tea yield not only varies seasonally (Ghosh Hajra and Kumar 1999) but also with genetype, altitudes, climatic and edaphic factors (Carrr 1972) The exact contribution of each factor to total variability of yield is not yet known but the effect of environment seems to be quite profound. Since in selection and breeding, genetype environment interactions assume greater relevance (Wickramaratne 1981), therefore, a plant selected solely on the basis of yield in a particular environment should invariably be evaluated under different agroclimatic conditions. Harvest index is one of the determinants of yield in any crop. Tea is the one commercial crop with very low harvest index (HI) of 16 percent observed for clone UPASI 3 in South India (Murty and Sharma 1986), 12-13% in Assam tea in North East India (Hadfield 1974), 24 percent for clone S15/10 for the period two to four years after field planting in the Mufindi district of Southern Tanzania (Burgess and Carr 1996) and 8 percent reported for clone 6/8 at high altitude in Kenya (Magambo and Cannell 1981). In tea, yield potential realization is limited by inefficient conversion of intercepted radiation to dry matter and low HI (Singh 1999). Therefore, breeding more efficient plant type and to increase the partitioning of more photosynthates towards the harvestable dry matter (so that Hi can be raised to 20-25%) is the solution (Jain and Tamang 1988).

1.8. Problem areas in Tea

It is a well known fact that tea is highly outcrossing and clinal introgressants arising from these two extremes are frequently observed. Thus, a large variation in several important and desirable characters occurs from bush to bush in the existing tea populations (Ghosh 2001). Selection and breeding for the production of desirable traits in this perennial crop have resulted in severe erosion of its genetic base over the time. Lack of proper conservation programmes have caused major reduction in its gene pool, as most of the breeding and conservation programs are still based on conventional morphological and agronomical descriptors, which are dependent on environmental and developmental factors thus reflecting the base of the gene pool (Green 1971; Wilkremaratne 1981) with no true genetic relatedness. The breeding of experimental planting material of a perennial, heterozygous crop like tea is difficult. However, like any other crop, the main objective of tea breeding is to improve the quantity and quality of the end product. The methods of introduction, selection, and hybridization have been used with success for tea improvement. The different varieties have been developed to suit the requirements of the various agroclimatic regions. However, tea genetics is still poorly understood. Proper selection criteria have not yet been established. This apart, the prediction of the performance of mature tea based on their evaluation in the early years has not been perfected. Application of modern techniques, as has been done in other crops, is a greater challenge to tea breeders and tea biotechnologists.

Since the chemical composition of calli in the tea plant was analyzed (Ogutuga and Northcote 1970a, b), callus induction and organogenesis have been reported in several papers from the year 1980 to 2001. In tea research the previous era held major emphasis on standardizing parameters of the *in vitro* protocol, such as using a suitable explant, overcoming microbial contamination, and optimizing media composition combined with growth regulation for better proliferation (Kato1989). Following this era the efforts turned towards hardening micro-shoots to achieve a higher survival percentage. Accordingly, several non-conventional approaches, such as a CO_2-enriched hardening chamber, biological hardening, and micrografting, were developed till 2001 (Mondal *et al.*, 2001). Presently, attention is increasingly focused on evaluating field performance of the transformed *in vitro* grown whole plantlets. Although there is no stable technique developed so far to produce transformed invitro grown whole plantlets of tea except the one made by Mondal *et al.*, in 2001 by micrografting seedling grown roots on the *in vitro* grown shoots of tea. Besides this the other major concern for loss in productivity of an important crop like tea in India is the damage caused by various diseases e.g., foliar fungal disease as it grows mainly under prescribed moisture conditions. Some of the most important foliar fungal diseases of tea such as blister blight, black rot, brown blight and grey blight caused by *Exobasidium vexans*, *Corticium invisum*, *Glomerella cingulata* and *Pestalotiopsis theae* respectively are responsible for considerable economic losses. Drought is another major constraint over the years on production of tea in India. In order to stop further reduction in its gene pool and to breed for new tea types with more productiveness; less prone to natural calamities, diseases, as well as new flavors, a thorough knowledge of the existing genetic diversity, *in vitro* culture studies and improvement of the existing varieties through various Molecular biology, tissue culture and biotechnological techniques is a pre-requisite in tea research.

Therefore the research needs

- The choice of disparate parents is critical for the realization of genetic advance, and to avoid inbreeding and narrowing of the genetic base in advanced generations. Although the prospect of production of high yielding tea plants during last two decades have improved, it did not help in widening the genetic variability of tea, as attempts were mostly restricted to selecting elite mother bushes or progenitors from within the natural hybrid populations of tea (Banerjee 1992). The danger of narrowing the genetic base in most tea growing countries due to popularity of a few clones used for infilling/replanting and extension. In particular it has recently been established through the use of DNA markers that close genetic relatedness exists within certain clones developed by the African Highlands Produce and Brooke Bond companies (Wachira 1997). A narrow genetic base can pose risk to natural hazards like pests, diseases and drought. Appropriate strategies should be designed to broaden and maintain a sufficiently large genetic base.
- The danger of loosing many valuable tea germplasm is increasing due to fast uprooting of older sections of tea estates and clearing of jungles practically all over the world. Reexamination of the wealth of this diverse material should be continued.
- For a better yield, breeding for more efficient plant type with efficient interception, absorption and photosynthetic utilization of light energy is required.
- Development of polyploid plants by gene cloning which can provide avenue through which genetic base can be broadened and vigor and greater variability can be introduced into the genetic pool of the tea germplasm (Wachira 1994).
- Search for new biochemical markers associated with quality parameters and genes. Once such genes are identified using such markers and their segregation

pattern would be possible to breed specifically for quality (Wachira 1990).

- Standardization of a protocol for production of completely *in vitro* grown plantlets from cotyledon cultured embryos.
- Thus, standardization of the gene transfer in tea plants using various methods like *Agrobacterium* and Biolistics is a prerequisite.

Objectives

1. DNA finger printing study of tea

- Collection and maintenance of various tea cultivars available in different regions of North Bengal.
- Genomic DNA isolation from fresh & tender leaf samples of various cultivars.
- Detection of genetic variability and the phylogenetic relationship among these tea cultivars by different PCR based fingerprinting methods like Random Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP) and Microsatellite markers.
- Sequencing of the tea varieties exhibiting a diverse genetic base.

2. *In vitro* culture studies in tea

- Induction of embryogenic lines from cotyledonary explant.
- Maintenance and multiplication of embryogenic lines.
- Maturation of somatic embryos and conversion into viable plantlets.
- Histological study of the early developmental stages and structural organization of the resulting embryos from cotyledons.

3. Genetic transformation study of tea

- Trial of different gene transfer methods to evaluate the suitability of their use in tea.
- Induction and multiplication of callus tissue from the genetically transformed explants on the antibiotic selective medium.
- Differentiation of genetically transformed tissue by subjecting it to various hormone combination & concentrations.
- Confirmation for the integration of transgene into the tea nuclear genome using *GUS* assay and PCR analysis using *npt*-II specific primers.