CHAPTER

REVIEW OF LITERATURE

1

Historical account of discovery and utilisation of tea

The tea plant, *Camellia sinensis* (L.) O. Kuntze, has been cultivated for so long time that its home as a wild plant is a matter of speculation. Nothing is known with any certainty of the early history of tea. Tradition indeed ascribes the discovery of tea bush to the Emperor Shin Nong of China about three thousand years B. C. However, according to Samuel Bell (1848), in the Standard English work on tea in China, it was used to be a common practice among empires, even at the present day, to ascribe the discovery of their several nostrums to Empire Shin Nong, though all such pretension is treated as fabulous and with respect to tea in particular. According to much later legend also quoted by Samuel Bell (1848), tea was discovered during the time of the Tsin dynasty in the third century A. D. The first authenticated references to tea in China were found in the 4th century A. D. and it is clear that its medicinal use was then well-known. It was well regarded at the Imperial Court suggested by Ukers (1935), whose book "All About Tea" has laid all students of the tea industry under a heavy debt, quotes from a fifth century chinese scholar who refers to a mountain on which grows the tea reserved for the Emperor as tribute tea. Tea soon ceased to be considered as a medicine and its popularity as a beverage increased so steadily during the seventh and eighth centuries that the revenue authorities in China levied a duty on it. In the eighth century, a Chinese tea scholar, LuYu, wrote a remarkable book the "*Ch'a Ching*"

or the tea book. A translation of this work was also mentioned by Ukers (1935). Tea was first used as a beverage in the reign of Wen Ti of the Sui dynasty (589-620 A. D.) (Bala Subramaniam 1995).

Introduction of tea cultivation of different countries in the world. (Fig. -1).

Tea is considered as the queen of beverages due to it's palatable acerbity and incomparable aroma. The word tea is derived from the China Fukien dialect *t'e*. In Cantonese, tea is known as *ch'a* (Barua, 1989). Legendary origin of tea plant can be dated back to around 2737 B. C., when tea was ascribed as medicinal plant. The then Chinese Emperor Shin Nong called it a devine healer. The people of China used to drink tea in some form or the other since the fourth century. *'Wutu'*, a mountain situated in Szechwan province of China, is celebrated as birth place of tea industry, where the tea plants are said to have been first cultivated around 350 A. D. and used as a medical decoction. In Japan, the first cultivation of tea plant began around 200 A. D., introducing tea seeds from Buddhist Missionaries from China. Since fifth century, tea had become a commodity of trade as it was introduced by the Dutch to European countries. In 1497 A. D., the Portuguese facilitated large scale tea trading between Europe and Orient. Accordinig to Barua (1989) and Bala Subramaniam (1995), tea cultivation was introduced in Java in 1825 A. D., Sri Lanka (1875 A. D.), Sumatra (1910 A. D.)., Turkey (1939), Malawi (1878),

Fig. –1. World map indicating the major Tea growing regions.

Schematic Diagram, not according to scale.

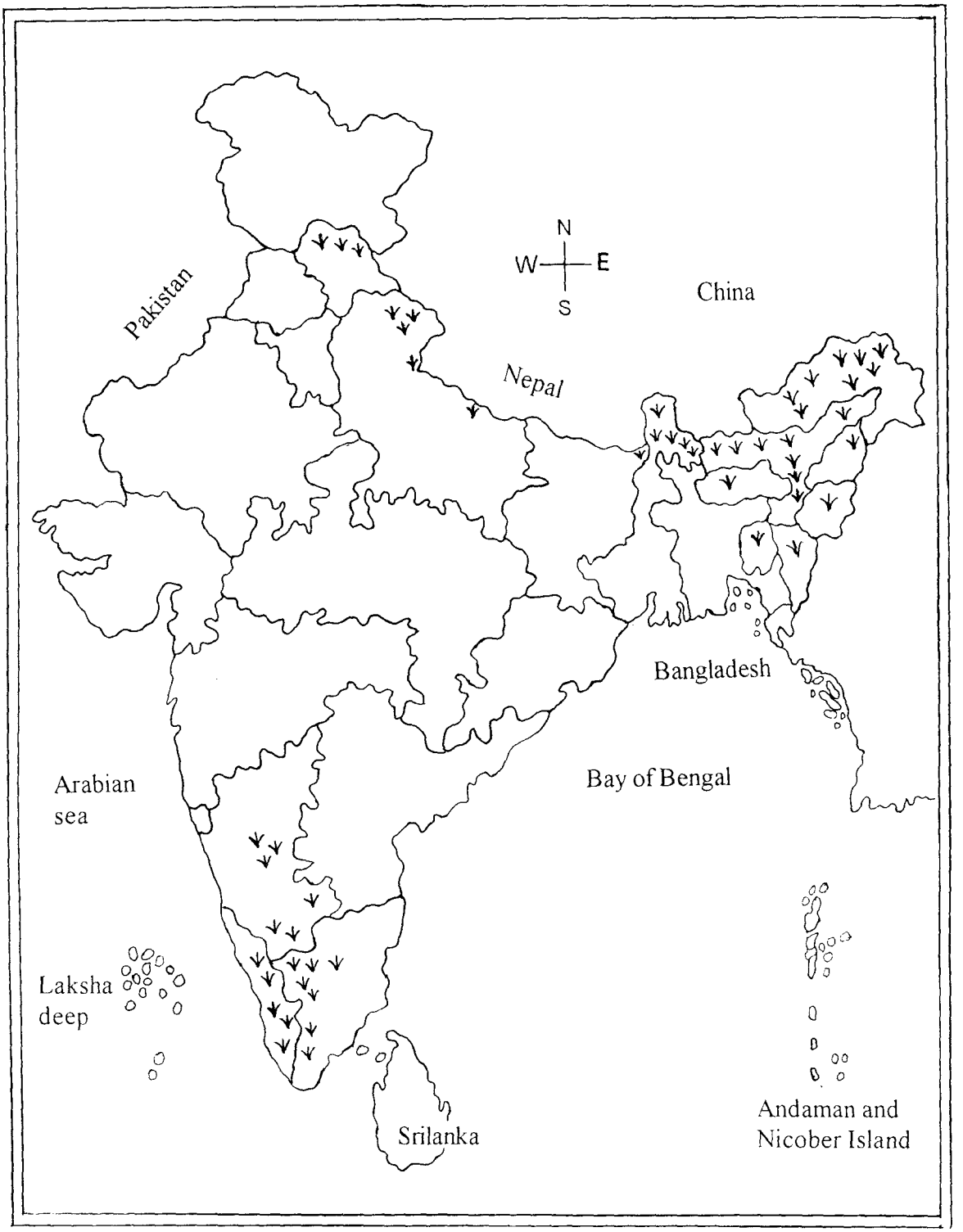
Zimbabwe (1925), Tanzania (1931), New Guinea (1954), Australia (1960), Argentina (1946), and Malaya (1935), South Africa (1964), Uganda (1909), Kenya (1960), Iran (1936), U. S. S. R. (1905), Taiwan (1868). Authentic information about introduction of tea cultivation to other tea growing countries are still lacking. Lu Yu (780 A. D.), a Chinese tea-scholar published first authentic book in Chinese language *"Ch'a Ching"*, which contains various kinds of tea, method of cultivation and distribution of tea growing places in China (Barua, 1989).

Tea cultivation in India. (Fig. -2).

Tea is one of the oldest industries in India and today it enjoys the status of one of the best organised industries in the country. Although tea has been known since 2737 B. C. and consumed as a beverage for over 1200 years, its cultivation in India commenced only in the middle of 19th century (Ukers 1935).

The weakening of trade relations between China and Britain during 1780 led to the initiative of the East Indian Company to raise the commodity in India. In 1778, Sir Joseph Banks was asked to prepare a series of notes for the Company. He recommended the cultivation of tea as an article of greatest national importance to Britain. The first recorded mention of tea in India was 1780, when Robert Kyd experimented tea cultivation with imported seeds. Warren Hastings had some interest in tea cultivation.

Fig. –2. Tea growing areas of India.



Schematic Diagram, not according to scale.

An indigenous Assam variety of tea was, however, known to the hill tribes of North-East India, that made use of it over long historical times unknown to the outside world. Robert Bruce, who was presented with a few indigenous tea plants by a Singpho chief in 1823, reported about this variety as a possible source of commercial tea. The discovery that the tea plants grow wild in the upper part of the Brahmaputra valley was made by Robert Bruce in 1823 and rise of industry in India owes its origin to the momentous discovery of this indigenous tea plant.

The inception of the tea industry in India can be associated with refusal of the Chinese government in 1833 to renew the agreement of granting the East India Company the rights of monopoly of British trade with China. This removal of the monopoly of the China trade in 1833 quickened their perceptions to the advantages likely to accrue to India by the establishment of new industry. Subsequently in 1834, Lord William Bentinck, the then Governor- General appointed a committee called "Tea Comittee" with Dr. N. Wallich, as the Head to study a plan for the accomplishment of the introduction of tea culture in India. The committee recommended that G. J. Gordon, should be directed to proceed China to obtain more knowledge about the tea cultivation. In 1835, the Secretary of the Committee despatched the tea seeds from China

which reached Calcutta later in the same year. An experimental area was opened at Sadiya (Assam) with the seeds obtained from China. Later, another experimental tea garden was opened at Chabua (Assam), planted with indigenous Assam seeds. The superiority of indigenous tea variety in growth, yield and quality over the introduced China variety was soon established and its use became widespread (Barua 1989).

In 1838, the first commercial sample of Assam tea of eight chests, weighing 488 lbs., was sent to London and sold on January 10, 1839 at a fancy price. In 1851, a private tea garden was established and then the number of tea gardens began to increase. Thus the foundation of present tea industry was laid between 1856 to 1859 (Edgar 1873).

Historical events in connection with the development of tea industry in India

In India, merchants of East India Company were mainly responsible for plantation and trading of tea. The Company built factories at Surat in 1608 A. D., Madras (Chennai) in 1639 A. D., Bombay (Mumbai) in 1668 A. D. and in Calcutta in 1690 A. D. The first private owned tea company 'The Assam Company' was formed in 1839 after amalgamation of the Bengal Tea Company and the East India Company's plantation. In 1858, the East India Company handed

over its ruling function in India to the British Government. The first recorded mention of tea plant in India was in 1793, when imported tea seeds were brought from China (Canton) to Botanical Garden at Shibpur (Howrah) near Calcutta by the East India Company. Lieutenant Colonel Robert Kyd experimented possibilites of tea cultivation in Botanical Garden, near Calcutta. The then Governor-General, Warren Hastings had also some interest in tea cultivation in India. Tea plantation on a large scale was introduced in India by Sir Joseph Banks – the English Naturalist, in 1788. First experimental tea plantations were established on the Garho Hills in Assam in 1835 (Bala Subramaniam 1995).

Short account of development of Indian Tea industry is highlighted as follows

Year	*Historical events*
1823	Tea plant was discovered first in India in wild condition by Major Robert Bruce from 'Sadiya', Brahmaputra Valley, Sibsagar District, Assam (India) (Ukers, 1935). However, the real discoverer of tea plant in India, still controversial (Barua 1989).
1834	Lord William Bentinck, the then Governor-General of India, appointed a 'Tea Committee' to advise on the

Year	Historical events
	possibility of commercial cultivation of tea in India (Barua 1989).
1835	Mr. G. J. Gordon, the secretery of 'Tea Committee' despatched the seeds from China for commercial cultivation in India (Barua 1989).
1836	The British Scientific Commission was constituted in India (1935) with the following Botanists : Dr. N. Wallich (the then superintendent, Botanical Garden, Shibpur, Howrah, near Calcutta); Dr. W. Griffith and Dr. J. McLelland. They visited Assam under guidance of Mr. C. A. Bruce and collected first experimental samples from the indigenous tea plant and finally sent to Botanical Garden, Shibpur (Howrah) for necessary investigation (Barua 1989).
1838	Assam tea was forwarded to London in 1838 and auctioned in London on the 10th January, 1839 (Barua 1989).
1851	First privately owned Tea Garden was established by Colonel Hannay in Assam (Source - International Tea Festival, Darjeeling, 1991).
1853	Tea planting on a commercial scale was started in the Nilgiris (Barua 1989; Bala Subramaniam 1995).

Year	Historical events
1856	Tea had been planted first time in Darjeeling hills (Tukvar Tea Estate) and Cachar (Assam) after introduction of tea seeds from China (Rai 1978; Barua 1989; Bala Subramaniam 1995).
1862	First Terai (foot hills of Darjeeling) Tea Garden was established (Champta Tea Estate) (Barua 1989).
1874	Tea planting commenced in Dooars region, extending on the east of the river Tista (Barua 1989).
1876	First Indian owned Tea Garden was established (Source-International Tea Festival, Darjeeling, 1991).
1881	Indian Tea Association (ITA) was established (Ferguson 1961).
1893	United Planters Association of Southern India (UPASI) was established (Bala Subramaniam 1995).
1900	First Scientific Research Centre on tea was established at 'HEELIKAH', at present Mariani, Sibsagar district, Upper Assam (India). Later the Centre was shifted to Tocklai, Jorhat, Assam; Dr. H. H. Mann was the first Scientific Officer at Tocklai (Ferguson 1961).
1949	The Central Tea Board of India was constituted (Source-International Tea Festival, Darjeeling, 1991).

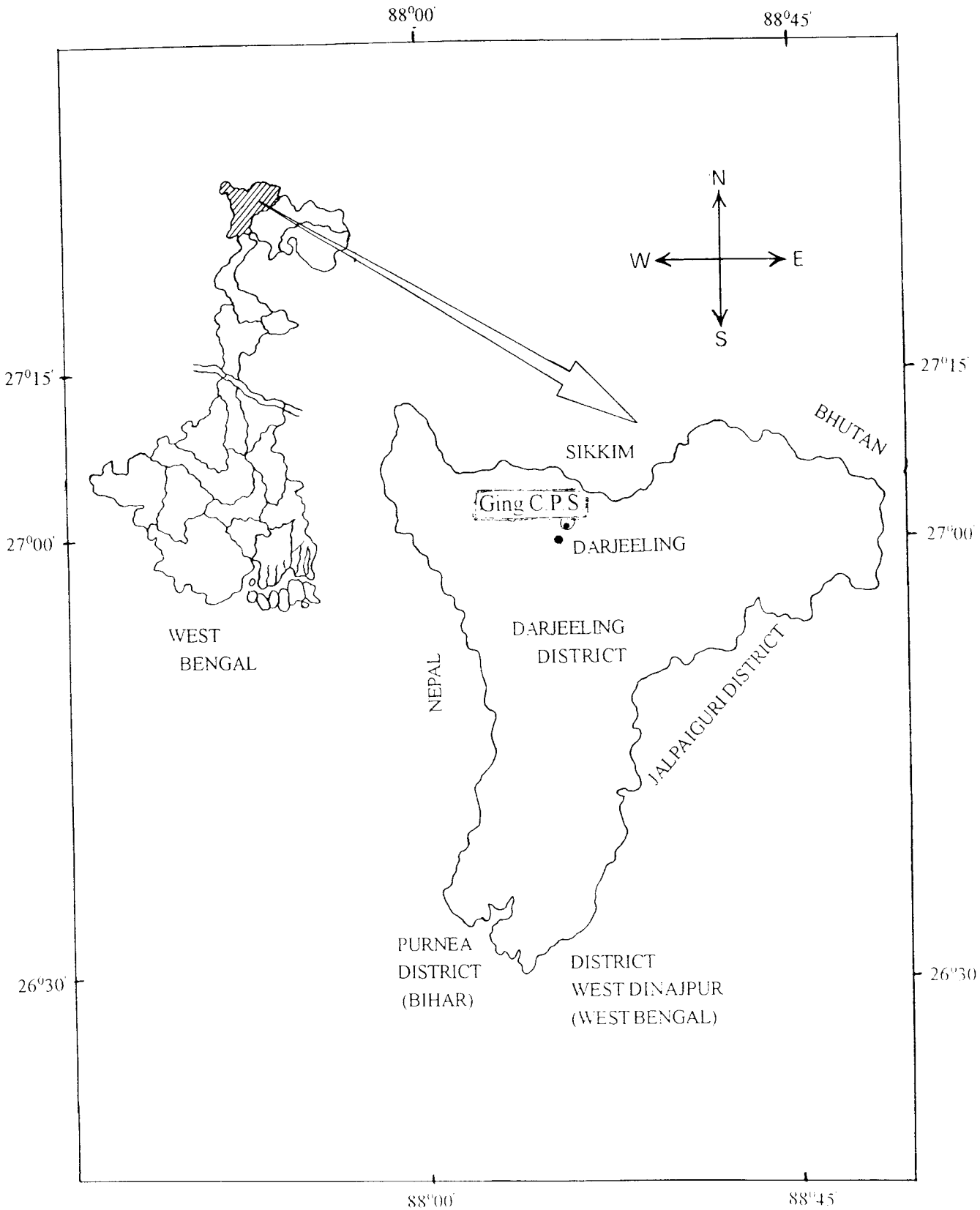
Year	Historical events
1953	Tea Board of India was established.
1978	Govt. of India declared tea as an essential commodity in India.

Tea cultivation in Darjeeling. (Fig. -3).

Tea growing in Darjeeling is more than 150 years old. Joseph Dalton Hooker, eminent plant systematist visited Darjeeling in the year of 1842 with a view to exploit Darjeeling flora (Bengal Dist. Gazette, Darjeeling, 1907). He successfully planted some imported China tea seeds near Lebong (about eight km. away from Darjeeling Town) and recommended in favour of suitability of tea cultivation in Darjeeling hills (Rai, 1978). Dr. Arthur D. Campbell, the then Medical Superintendent of Darjeeling, planted some tea seeds in his own bungalow compound on experimental basis in 1841. In 1847, some tea plants from his bunglow compound garden were selected for tea nursery in that area. Such tea plants were most probably of China variety (Dutta 1992).

Tea cultivation in the hill district of Darjeeling started by the end of 1856 (Barua 1989, Bala Subramaniam 1995). Tukvar Tea garden was first established (1856) in Darjeeling hills (Barua 1989). Later, Makaibari T. E. (1857) was also established in Kurseong sub-division (about five km. away from Kurseong Town) under

Fig. –3. Geographical position of Darjeeling district in West Bengal, India.



Darjeeling Hill district along with other tea gardens (Bengal Dist. Gazette, Darjeeling, 1907). It was also reported that tea seeds were imported to Darjeeling hills directly from China (particularly Sinkiang, Amoy district) from unknown seed stock. The then British India Government in collaboration with East India Company deputed Dr. Gordon and Dr. Gutyleff to procure tea seeds from China (Rai 1978). Sir Precival Griffiths, the then ICS Officer reported the expansion of tea plantation in Darjeeling hills as follows :

Table –1. *Expansion of tea plantation and production of tea in earlier days in Darjeeling hills, West Bengal, India.*

Year	*No. of tea Gardens*	*Area under tea Cultivation*	*Total turnover of Processed tea (in lb.)*
1870	56	11,000 acres	17,00,000
1874	113	18,888 acres	39,28,000

(Bengal Dist. Gazette, Darjeeling, 1907).

The figures for area and production of tea are given in the table –2, to show the further growth of the tea industry in West Bengal (Darjeeling hills, Terai and Dooars area).

Table –2. *Growth of tea industry at different times in West Bengal, India (Barua 1989).*

Year	Area in thousand hectare	Production in million kg.
1885-89*	30.2	8.3
1924*	73.5	39.5
1980**	93.5	133.2

* Including Sylhet until 1947. ** Now Sylhet is included in Bangladesh.

A noteworthy event was the establishment of Tea Research Station in 1900 for the overall development of tea cultivation and management for all the tea growing regions of North-East India (Ferguson 1961).

In 1914, first breeding of tea done for Darjeeling by using Chinese tea seeds and pollen grains was successfully carried out by Tocklai Experimental Station, Assam. Out of 325 seedlings of tea raised from these seeds, 25 seedlings were selected and finally 14/9 clonal progeny was selected and released as TV-7 for cultivation in Darjeeling in 1959 (Singh 1980). The first biclonal seed variety bred specially for the hill-district of Darjeeling was released in 1967. The seed progeny produces flavoury tea characteristic of Darjeeling district. Two biclonal stocks were released for the plains district of Darjeeling in 1970 followed by the release of three more stocks, one in 1975 and the other two in 1981 (Ann, Sci. Rep. TES 1969-70,

1975-76, 1981-82). Uprooting, replnting and bringing up young tea in Darjeeling was successfully done at that time and salient features of the certified clones from Tea Research Association for Darjeeling was also highlighted (Tailor 1978).

In 1967, one of the glorious contributions of Tea Research Association, Tocklai, Assam, was the establishment of the Clonal Proving Station, Ging, about 13 km. North-East from Darjeeling Town. This Clonal Proving Station has been established for the betterment of tea cultivation, management and advisory requirements from the member of tea gardens in Darjeeling hill areas. The main objectives of this Station are as follows :

(a) To develop superior clonal/seed cultivars of tea for cultivation in Darjeeling hills.

(b) To establish nucleus block of certified clones for distribution of cuttings to the member garden of Tea Research Associations.

(c) To help Tea gardens in selecting their own clone/seed stocks in miniature manufacture through clonal selection scheme.

(d) To study the field and factory performance of various planting materials of tea developed by Tea Research Association and member garden for their possible release to the tea industry of Darjeeling hills.

(e) To conserve tea genetic resources of Darjeeling (conservation of germplasm) started in 1984 (Rep. Sem. Darj. Tea, TRA, 1978; Rep. Jt. Area Sci., Com., TRA, 1989). Besides these, TRA also developed a Clonal Selection Scheme (CSS) in Darjeeling (established in 1975) with the objective of helping tea estates to develop the clones of their own preference. The scheme, Valleywise Clonal Proving Station (VCPS) in Darjeeling was also initiated in 1981 by TRA (Rep. Sem. Darj. Tea., TRA, 1978; Rep. Jt. Area Sci. Com. TRA, 1989). Side by side, Tea Board of India had established Darjeeling Tea Research Centre (DTRC) at Kurseong in 1977, including an experimental farm of 21.6 hectares. This Research Centre, besides catering to the advisory requirement of Darjeeling tea gardens, has developed technical know-how under four main divisions of tea research, viz. Farm Management (Botany and Agronomy), Soil Science, Biochemistry and Plant Protection. This Centre has *inter alia* a library, miniature manufacturing unit and an agro-meteorologicl observatory (Ann. Sci. Rep. 1990-91 DTRC, Kurseong [Darjeeling], Tea Board).

Taxonomic status of tea plant

Tea plant belongs to the family Theaceae representing Magnoliopsida (Dicots) of Magnoliophyta (Angiosperms). However, it was accommodated under the family Ternstroemiaceae by some earlier authors like Bentham and Hooker (1862-1883). Cronquist (1981) sub-divided Theaceae into 4 subfamilies, viz. Theoideae (Camellioideae), Ternstroemioideae, Bonnetioideae and Asteropeioideae. Some authors have merged additional families with Theaceae including the genus *Camellia* (Keng 1962). Keng included 12 genera including *Camellia* in the sub-family Camellioideae and divided this sub-family into 3 tribes and 6 sub-tribes.

Previously, taxonomic status of the tea plant was highly controversial. The China tea plant was for the first time scientifically described by Kaempfer (1712) with a few hand drawing without any voucher specimen. Linnaeus (1753) used Kaempfers illustration of tea plant to typify the China tea plant under the name *Thea sinensis* in Volume I of his *Species Plantarum*, while two related ornamental species, viz. *Camellia sasanqua* and *Camellia japonica* in Vol. II.

George Joseph Kamel, a German missionary first described about tea plant in Asia during the latter half of the 17th Century. The generic name of tea plant *Camellia* was derived from his name (Bauua, 1989). In 1762, Linnaeus, again distinguished two kinds of tea and named them *Thea viridis* and *Thea bohea*. The former was

suppoed to produce green tea and the later, black tea. The specific name *sinensis* was discarded. Later, it was found that green and black tea could be prepared from the same plant, the name *Thea viridis* was dropped, retaining the name *Thea bohea* for the tea plant. In the International Botanical Congress in Amsterdam (1935), it has been resolved to unite the two genera viz. *Thea* and *Camellia* into a single genus *Camellia* and appointed a special Committee to consider the nomenclature of tea and number of other economic plants. The Committee decided *Camellia sinensis* (L.) O. Kuntze to be correct name of the tea plant. Technically, *Camellia sinensis* (L.) O. Kuntze is the full name of the tea plant, since it gives recognition to the authority responsible for the union of the old name *sinensis* with the new genus *Camellia* (Barua 1989).

The varieties of tea plant and their nomenclature is another riddle. Sealy (1958) listed 82 recognised species within the genus *Camellia* and 16 other imperfectly known species whose taxonomic status was not decided. Wight (1962) emphasized to give specific rank to the Assam variety of tea and proposed the name *Camellia sinensis* L. for China tea plant only. The tea plant came under the *Thea* section along with four other species viz. *Camellia irrawadiensis*, *C. taliensis*, *C. gracilipes* and *C. pubicosta* which were merged under one species, *Camellia sinensis*. The large-leaved Assam tea plants placed under the species *sinensis* were included in var. *assamica*.

The 'Southern form' of tea plant was referred to as *Cambodiensis* by Kingdon-Ward (1950) has long been recognised as a distinct type. Earlier worker, Watt (1908) also pointed out that it was different from the species *Thea sinensis* named by Linnaeus (1753). However, the difference between 'Southern form' and *assamica* was much less than that of *assamica* and *sinensis*.

So Wight (1962) did not consider this race of tea plant to merit a separate specific rank and treated it as a sub-species of *Camellia assamica*. This variety resembled Planchon's *Thea lasiocalyx* and thus the 'Southern form' was also named as *Camellia assamica* sub-species *lasiocalyx* (Planch. MS). Masters (1844) being the first author to describe the Assam plant as a separate type. At present three varieties of tea, China variety, Assam variety and Cambod variety are usually named as *Camellia sinensis L.*, for China variety, *Camellia assamica* (Masters) for Assam variety and *Camellia* sub-species *lasiocalyx (Planch. MS)* proposed for Cambod variety respectively. These three varieties are distinguished from each other by the nature of their floral structure, floral morphology, leaf form, colouration of the leaf, growth habit, leaf structures, sclereid morphology, leaf anatomy and chemical constituents (Barua 1965; Bezbaruah 1976; Wight 1959, 1962).

Tea Research in connection with the use of planting materials

In India the industry has set a production target of about 1200

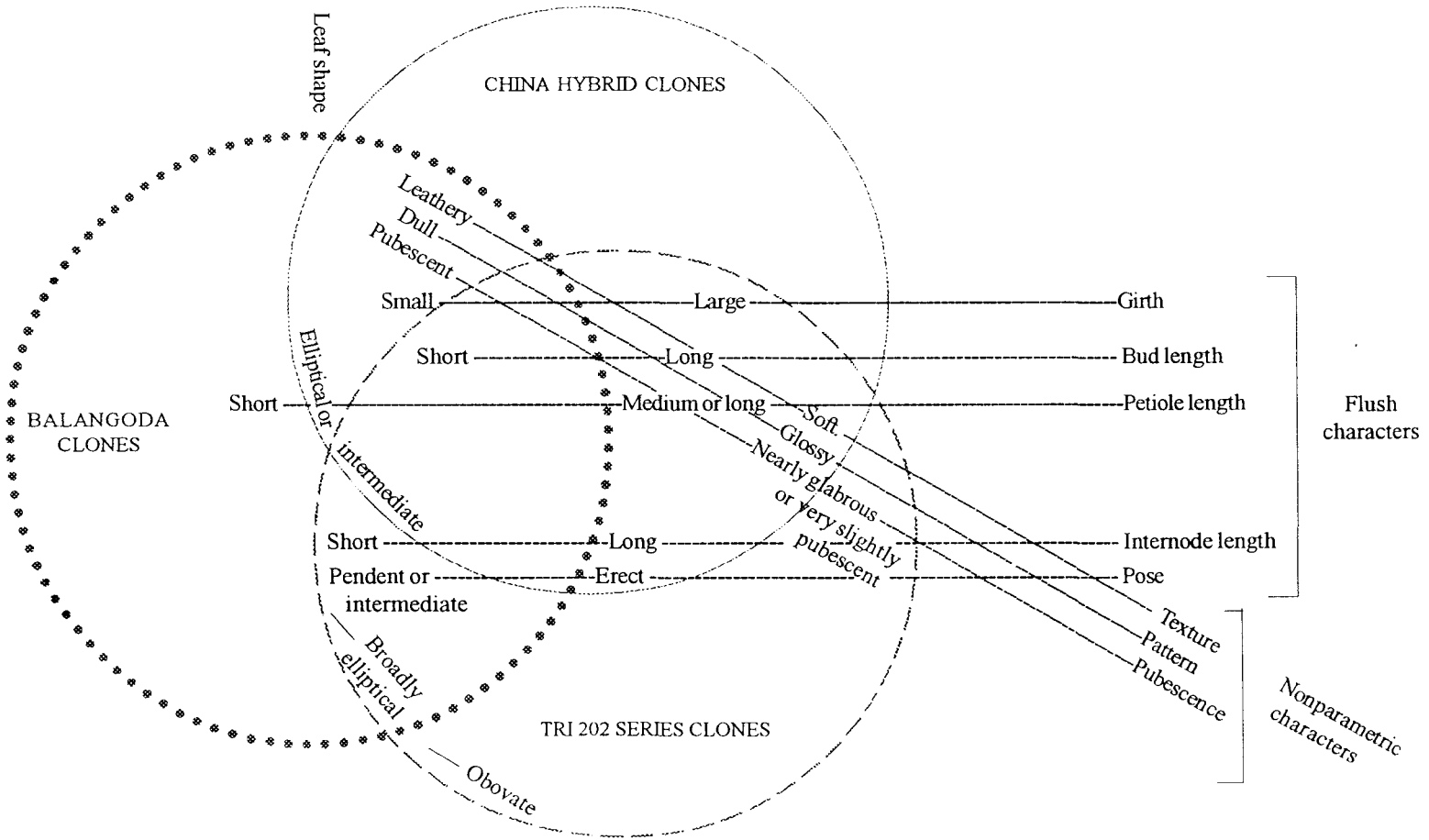
million kilogram of made tea by 2000 A. D. This target has to be met through increasing productivity of the existing tea areas as well as through developmental plant of rejuvenation, infilling, replanting and extension plantings. Among the various factors of production, soil and plant play major role. Since the improvement of soil is a slow process, major increase in productivity could be achieved by use of proper and improved planting materials. Historically tea was cultivated in India by seed from the very beginning of tea industry. It remained in use for more than hundred years. The use of clones started in fifties after the release of Tocklai clones in 1949 (Green 1960). Seed populations are highly heterogeneous as a result of free crossing among themselves from which superior clones have been selected. Barua (1963a) mentioned the basic difference between clones and seed population with special emphasis on their field performance (Table –3). With the help of ideograph, Wickramaratne (1981) has shown grouping of clones according to their common features (Fig. –4) like girth, leaf angle, the length of bud, petiole and internode length. Non-parametric characters of clones viz. texture, pattern and pubescence of leaf also considered.

Plants propagated by cuttings are clones which originate from a single individual by asexual reproduction. Therefore, all the members of clone are not only morphologically similar but also genetically identical. Hundreds of tea clones are now cultivated throughout the

Table –3. *Characteristics of tea clones and seed varieties (Barua, 1963a).*

Seed	*Clone*
A. *Nursery*	
1. Easy to grow; no special technique needed.	Needs special nursery techniques.
2. Soil heterogeneity is not reflected in germination.	Soil characteristics (pH, organic matter) affect rooting success.
3. Seed contains reserve food for initial growth: root and shoot tissues exist in primordial stage.	Root tissues require differentiation - had to be self-sufficient for food.
4. High genetic variability.	Genetically almost uniform.
B. *Field performance*	
1. Fits into a wide range of environment because of genetic heterogeneity.	Mostly specific to an environment, though there are some exceptions.
2. Soil and environmental heterogeneity has little effect.	Effects of environmental heterogeneity are often pronounced.
3. Yield fluctuations are marked.	Yield fluctuations less pronounced.
4. Strong tap root provides several physiological advantages like resistance to drought and better utilisation of nutrients.	Mostly fibrous root - hence initial disadvantage.
5. Many seed jats have long life span - some well over 50 year.	Life span would vary depending on source of genotypes.

Fig. –4. *A typical ideograph showing grouping of clones according to their common features (Wickramaratne 1981).*



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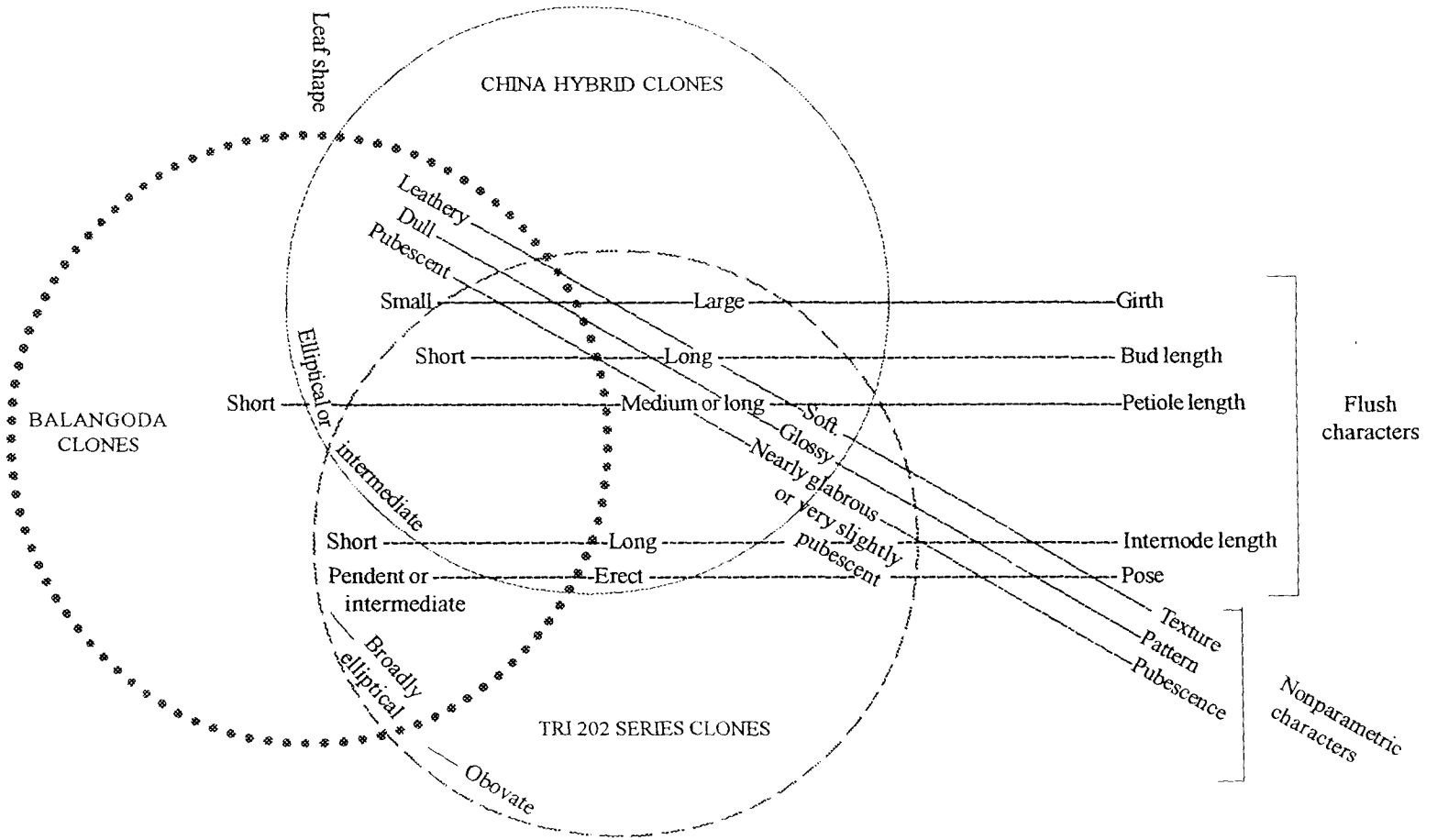

world and clones differ in their growth habit, branching pattern, size, shape, texture, colouration, leaf pose as well as in their inherent quality and yield (Bezbaruah 1975, 1976). Difference between the clones are also evident in physiological and genetical characters (Pool 1982). Wight (1953, 1958) pointed out the difference between the leaf character of the clone and also suggested that this might be used as 'agrotype index' of tea clones. Several publications released by Tea Experimental Station, Tocklai, Assam, also reported morphological variations in tea leaf at clonal level, particularly regarding their size, shape, colour, texture, leaf apex, venation pattern, nature of pubescence, leaf angle, petiole length, stomatal nature etc. (Richards and Sebastiampilli 1964, Pethiyagoda and Rejendram 1965; Krishnapillai and Pethiyagoda 1978; Barua 1959; Barua 1961; Hadfield 1968 and Barua and sharma 1982). Dry matter contents as well as the yield per unit area of the surface also vary significantly from clone to clone (Wight 1956, Bezbaruah and Hussain 1975a; Magambo and Waithaka 1986). Magambo (1977), Murthy and Sharma (1986) confirmed that canopy characters of tea clones have significant difference. Naturally, it is really interesting to study the morphological variations at the clonal level. In agronomic practice, qualitative and quantitative variations among the clones should also be recorded.

The first clonal release from Tocklai Experimental Station took place in 1949 and three standard clones were released, which are

1/7/1, 19/29/13 and 20/23/1 (Green, 1960). Three categories of clone are differentiated on the basis of their yield and quality (cup-character). These are :-

(1) Standard Clones :- It is be expected to produce an appreciable but not extra-ordinarily increase in yield with no loss of cup-character.

(2) Quality Clones :- Those are likely to give much more pronounced improvement in cup-character without any change of yield.

(3) Yield Clones :- These might have unusual high yield with a probable loss in cup-character.

About 160 tea clones have been selected and released for commercial cultivation in different agro-climatic conditions of North-East India and South India. Tea Research Association, Jorehat, Assam (India) has tested and released 76 clones for North-East India (Subramaniam 1995). Singh (1989) reported that 30 clones and 2 seed cultivars were released from Clonal Proving Station, Ging (Darjeeling) for cultivation in Darjeeling and adjoining hill areas. Some noteworthy clones of India are :-

(1) High potential yield :- UPASI-17 (Swarna), TV-18, TV-20 etc.

(2) Excellent flavours :- B-688, T-383 etc.

(3) Drought resistance :- UPASI-8 (Gloconda), UPASI-20, TS-379, etc.

(4) Red spider and Blister blight resistant :- TRA/TIV-1 etc.

(5) Good strength/liquor :- UPASI-8 (Golconda) etc.

The following major characters should be taken into consideration in selection programme for developing elite clones of tea.

(a) Vigour means high yielding characters.

(b) Adaptibility to local environment, such as 'Drought resistance', 'Frost resistance' quality etc.

(c) Resistance to pest and diseases.

(d) High quality, such as liquor and flavour character of black tea.

(e) Flexible leaves–which are easier to roll and ferment during manufacture.

Microscopical characters of tea leaf

Eames and MacDaniels (1947) observed differences in upper and lower epidermal cells in tea leaves and other ornamental garden varieties of *Camellia* leaves and expressed that epidermal cells in those leaves may provide confirmatory evidence on taxonomic uses. Matzke (1947) suggested the importance of epidermal cells of plant leaves so that diagnostic value may be drawn on leaf anatomy in relation to taxonomy. Terashima and Saeki (1983) carried out the comparative studies of leaf anatomy in some genus of *Camellia*, including detailed studies on epidermal cells and found non-significant variations among them.

Stomata are minor openings present on the leaf surface through which gaseous exchange takes place and execss amount of plant metabolic water is transpired out in the form of vapour. Stomatal opening and closing is regulated by two specialised cells called guard cells. The guard cells, together with the opening between them actually constitute the stoma. The stoma and subsidiary cells (if present) collectively is known as stomatal complex or stomatal apparatus (Fahn 1985).

Tea stomata on the basis of arrangement of epidermal cells neighbouring the guard cell is defined as Anomocytic type or Ranunculaceous type (Metcalfe and Chalk 1950). However, Nakayama (1980), on the basis of developmental process, reported that tea

stomata is anisocytic type. In different clones of tea, even in the same clone, frequency and size of stomata varies (Saikia and Dey 1987) and showed that two clones of tea, viz. TV-1 and TV-7 showed higher frequency and larger size of the stomata in upper leaves as compared to those in lower leaves. Stomatal behaviour was considered as physiological index of moisture stress. Saikia and Dey (1987) further observed that stomatal size of tea leaf was between 905-1025 sq. mm. According to some authors there was no correlation between leaf area, size and density of stomata as observed from some clones released by Tea Experimental Station, Tocklai, Jorhat (Assam) India (Mazumdar and Bezbaruah 1978; Basu 1975). Mazumdar and Bezbaruah (1978) reported significant differences in size and number of stomata per unit area of leaf surface among the clones of tea.

All unicellular and multicellular appendages of epidermal and sub-epidermal origin are known as trichomes which are also called hairs or pubescence. Some families can be easily identified by the presence of a particular type or types of trichome. In other case, the trichomes are important in the classification of genera and species and in analysing inter-specific hybrid (Metcalfe and Chalk 1950; Carlquist 1961; Metcalfe and Chalk 1963).

Tea leaf trichomes are categorised as unicellular, non-glandular, non-flattened structure with conspicuous stalk and basal cells (Payne 1978). Density of hair was found to fluctuate during growing season.

Thus, density on young shoot of tea had been observed maximum in the second flush period from April to June, minimum in the rains from July to September, and raised again after September. This seasonal variation of the pubescence was associated with the quality of tea (Wight and Gilchrist 1959, Wu *et al.* 1958 and Ahmed and Bezbaruah 1982). Wight (1961b) also reported that the pubescence character was preferred in comparison to glabrous or hairless condition during selection of hybrid tea simply because pubescence had the direct relation with quality of tea. Singh (1989) also evaluated pubescence as a criterion to determine the quality, when he discussed about the characters of different tea cultivars of Darjeeling, released from Clonal proving Station, Ging (Darjeeling). Wight (1961a) mentioned that three factors, viz. 'pubescence', 'phloem-index' and 'vascular index' have direct influence on quality and strength of tea. Recently, trichome of a Chinese tea, 'Maofeng' was studied in details by Scanning Electron Microscope (Liang *et al.* 1993), such trichomes are cylindrical in appearance with expanded joints by which they are attached to the lower epidermis.

The term 'sclereid' was first introduced by Tschirich (1885). Sclereids may be defined as special type of thick-walled lignified sclerenchymatous cells with little lumen, often contains many pits and no functional protoplast, regarded as the mechanical tissue by nature. Foster (1944) first studied sclereidology in details with a taxonomic

approach. In tea leaf, sclereids were first discovered and illustrated by Mirabel and Payen (1849, 1850). Some authors proved sclereid to represent one of the definite histological characters of the majority of genera in the Theaceae (Beauvisage 1920; Melchior 1925). Cavera (1897) adopting a term originated by Sachs (1882) designated the isolated branched cells in Camellia as 'idioblasts'. The nomenclature of scelreids includes many of the term as 'Fibres', 'Sclerenchyma cells', 'Bast cells', 'Idioblasts', 'Trichoblasts', 'Astro-sclereids', 'Bast fibres', 'Stone cells', 'Sclerocytes', 'Sclerites' by different authors in the past while describing the sclereids of tea leaf (Foster 1944). Detailed investigations of leaf sclereids were carried out by Barua and Dutta (1959) in China and Assam varieties and a third intra-specific taxon described as a Southern form of tea. They came to the conclusion that sclereids have great taxonomic importance (Barua and Dutta 1959). Choudhury and Bezbaruah (1985) studied leaf sclereids morphology and distribution both in aneuploid and polyploid teas. Foard (1959) and Foard and Lewis (1961) used sclereid pattern as a possible key to the identification of *Camellia* hybrids. They concluded that various pattern of sclereids could be used as a reliable diagnostic character at the specific as well as intra-specific level (Foard 1959, Foard and Lewis 1961). Keng (1962) had categorised the leaf sclereids into 5 types and found that the genus *Camellia* essentially had similar pattern of sclereids with few execptions.

Importance of sclereids in the taxonomy of the family Theaceae and identification of the species was also reported by Wight (1962). Extensive studies of mesophyll sclereids of tea leaf was made by Yan (1981).

Plant anatomists have amply described the various forms of crystal idioblasts in a variety of plant parts in a number of species (Scott 1941; Price 1970; Horner and Whitemoyer 1972; Arnott 1973; Arnott and Pantard 1970). Wight (1958) observed calcium oxalate crystal containing specialised cells appeared mostly in the phloem parenchyma cells in the transverse section of the petiole of tea leaf, termed these idioblast cells as 'phloem index'. These idioblasts, containing calcium oxalate crystals varied significantly between clones and progenies. In this context, Wight (1958, 1959) proposed a term called 'agrotype index', based on idioblast frequency, determined by him from tea bushes and suggested that this 'agrotype index' would fix the position of *jats* (varieties) of tea cultivars. Wight (1958, 1959) also observed that this 'index' was variable among the tea clones and other tea cultivars. However, proposed 'agrotype index' was proved to be impractical, because idioblast frequency in tea leaves and petiole found to be affected by cultural practices, environmental stress and also altered by the use of fertilizers (Wight and Barua, 1954; Barua 1956 and Green 1971). Wight and Barua (1956) also proposed certain methods for microscopic studies of calcium oxalate containing

idioblasts present in tea leaves and petioles. They had correlated such idioblasts with the chemical substances present in tea leaves and which ultimately determine the quality of processed tea.

Chemical constituents in tea

Upto date a large number of chemical constituents in tea have been reported. The distribution of different chemical compounds in the tea bush has been worked out by Wickremasinghe (1974) and has been represented in the Table –4. Polyphenols are major components of tea as they produce the characteristic liquor of tea. The following classes of phenolic substances were detected from tea, flavan-3-ols, depsides, flavonols, leuco-anthocyanine and uncharacterised substance (Roberts 1958b). Young leaves (apical bud and two youngest leaves) were found to be richer (2-7 folds) in polyphenols than older leaves. Again polyphenols found to be higher (1 to 4 fold) in summer than spring. Unfermented, green tea contains highest concentration of polyphenols whereas fermented Assam black tea contains lowest amount of these compound (Lin *et al.* 1996). 'Catechin' is considered as major constituents of phenol. (-) Epigallocatechin-3-gallate (EGCG) is the main polyphenolic constituent present in this product (Katiyar and Bhatia 1992). Green tea contains about 30% polyphenols and the most important are flavonols or tea catechins. Such compound, by fermentation or oxidation by polyphenol oxidase (PPO) gives the

Table –4. *Distribution of chemical compounds in the tea bush (Wickremasinghe, 1974).*

Compound	*Flash*	*Mature leaf*	*Green stem*	*Mature stem*	*Root*	*Seed*
Polyphenols	++	+	+	+	+	–
Amino-acids	++	+	+	+	++	+
Nucleotides	+	+	+	+	+	+
Phosphate esters	+	+	ND	ND	ND	ND
Caffeine, theobromine	++	+	+	+	+	–
Carbohydrates	+	+	+	+	+	+
Lipids	+	+	+	ND	ND	+
Organic acids	+	+	+	+	+	+
Chlorophyll	+	+	+	+	–	–
Carotenoids	+	++	+	+	–	ND
Unsaponifiables	+	+	+	+	+	+
Saponin	+	+	+	+	+++	+++
Minerals	+	+	+	+	+	+
Volatile compounds	+	+	+	+	+	+

characteristic liquors (Lunder 1992).

Strekova *et al.* (1989) reported that there was correlation between extent of chloroplast development and cell capacity for the synthesis of phenolic compounds in callus tissue of tea leaf.

Tea polyphenols have the following functions and properties :

(i) It gives characteristic liquor of the tea (Roberts 1962).

(ii) It has antioxidative effect as reported by Lunder (1992), Okuda *et al.* (1994).

(iii) Polyphenols inhibits the production of histamine and leucotriene (Matsuo *et al.* 1996).

(iv) Polyphenol have antimutagenic and anticarcinogenic action (Stoner and Mukhtar 1995) which is mainly due to green tea polyphenols (GTPS).

(v) Catechin analogue and its derivatives have also antimutagenic properties (Apostolides and Weisburge 1995).

(vi) Polyphenolic compounds act as antioxidative agent (Haslam 1985).

(vii) As there is a direct correlation between the extent of chloroplast development and cell capacity for the synthesis of phenolic compound in callus tissue of tea leaf, it may be presumed that polyphenols may have some role on chloroplast synthesis (Strekova *et al.* 1989).

The term phenolic compound embraces a wide range of naturally

occurring substances. They are aromatic compounds and may be simply represented by having the -OH group attached directly to an aromatic ring. They are located at the vacuoles of the palisade cells of the leaves (Selvendran and King 1976).

Phenolic compounds are widely classified into two major groups (Haslam 1985):-

(a) A simple phenols as catechol, phenolic acid like caffeic acid etc. and (b) Flavonoids –which comprises water soluble plant pigments like anthocyanin and flavones and their related substances like catechin, tannin etc.

Different kinds of phenolic compounds worked out by Sanderson (1972) in the fresh tea flush have been represented in the Table –5.

Tannins and related compounds were isolated and purified by Hashimoto *et al.* (1987, 1989a, 1989b), from commercial Oolong tea and fresh leaves of Assam tea, evaluated their chemical properties. Tannins give astringency and bitterness of tea. The relation between the tea tannin content and the astringency test revealed the interesting results. Georgian teas were the lowest both in tannin content and in the astringency followed by Vietnamese teas, while Chinese and Indian teas were most astringent. In tea infusions, it has been observed that addition of tannic acid linearly increased astringency (Pokorny *et al.* 1988).

Caffeine (1, 3, 7-trimethyle-xanthine) along with two isomeric

Table –5. *Phenolic compounds found in fresh tea flush (Sanderson, 1972).*

	Amount in flush (% dry wt.)
Flavanols	
(–) Epicatechin	1-3
(–) Epicatechin gallate	3-6
(–) Epicatechin digallate	–
(–) Epigallocatechin	3-6
(–) Epigallocatechin	9-13
(–) Epigallocatechin digallate	–
(+) Catechin	1-2
(+) Gallocatechin	3-4
Flavonols and flavonol glycosides	
Quercetin	–
Kaempferol	–
Quercetin-3-rhamnoglucoside (rutin)	–
Kaempferol-3-rhamnoglucoside	–
Quercetin-3-rhamnodiglucoside	–
Myricetin-3-glucoside	–
Flavones	
Vitexin	–
6, 8-di-C-glucosyl apigenin	–
Leucoanthocyanins	2-2
Acids and depsides	5
Gallic acid	–
Chlorogenic acids (4 isomers)	–
p-Coumarylquinic acids (4 isomers)	–
Theogallin	–
Ellagic acid	–
Total polyphenols	25-35

dimethylxanthin, theobromine and theophylline are present in minor amount in tea (Stagg and Millin 1975). Dry tea leaf contains about 5% of Caffeine (Bala Subramaniam 1995). Table –6 indicates that orthodox tea in general shows high concentration of caffeine level than that of CTC (Crush, Tear and Curl) tea (Goswami 1990). It was reported that in *Camellia irrawadiensis*, caffeine content was below 0.02 per cent (Nagata and Sakai 1985). Tereda *et al.* (1987) while comparing manufactured Green tea, Oolong tea and Black tea, did not found any difference in caffeine content. The caffeine content in small leaf species (var. *sinensis*) and large leaf (var. *assamica*) were quantitatively analysed in HPLC method. The correlation co-efficient between dried caffeine and dried total nitrogen was 0.93 in small species (var. *sinensis*) and 0.92 in large leaf species (var. *assamica*) has been observed by Kawakami *et al.* (1987). Caffeine content is found to differ with a change of climate as well as altitude (Owuor *et al.* 1990). Caffeine levels were lowest during the cold winter months (from October to November) when low night temperature and short-day length severely limited the growth rate and crop production (Squire 1974, 1979).

Effect of altitude on the caffeine content in black tea manufactured from clonal teas was determined by Owuor and Chavanji (1986). It had been observed that caffeine additions increased the bitterness of tea infusions proportionately to the amount

Table –6. *The concentration levels of caffeine (both Orthodox & C. T. C.) of different TV clones under the different seasons (Goswami 1990).*

	Orthodox			C. T. C.		
Clone	Early	Main	Back	Early	Main	Back
TV 7	3.97	4.15	3.92	3.68	3.96	3.61
TV 9	3.92	3.98	3.91	3.51	3.75	3.48
TV 10	3.68	3.87	3.65	3.53	3.71	3.31
TV 12	3.52	3.72	3.50	3.18	3.51	3.16
TV 18	3.76	3.87	3.75	3.46	3.70	3.43
TV 20	3.17	3.52	3.16	3.12	3.43	3.04
TV 22	3.91	3.99	3.81	3.77	4.00	3.71
TV 24	3.77	3.97	3.72	3.66	3.96	3.63
TV 25	3.68	3.84	3.62	3.51	3.81	3.47

(% Dry weight)

added, but caffeine content had not influenced on the astringency, colour and acidity of tea infusion (Kim *et al.* 1988). During withering, caffeine content found to be increased (Bhatia 1962).

Clonal variations of tea corresponding to seasonal variation of caffeine content in Western Kenya highlands had been observed by Owuor (1994). Caffeine content was recorded maximum during main flush and gradual decline with progress in season, showing a minimum during main flush and slight improvement through backened flush has been observed in infusion quality of orthodox Kangra tea cultivated in Himachal Pradesh, India (Gulati and Rabindranath 1996). ~~Detailed analysis of individual caffeine in green tea was also undertaken recently (Goto *et al.* 1996).~~ Rates of infusion of caffeine from the green tea and its temperature dependence were also investigated by Price and Spitzer (1994).

The liquor characteristics (colour, brightness, strength and flavour) of tea are developed as a result of complex metabolic changes during processing (Sud and Bhattacharya 1992). Wood and Roberts (1964) held that theaflavin content was responsible for quality and briskness of tea. Roberts and Smith (1963) found that the theaflavins and thearubigins formed by enzymatic oxidation of polyphenols during fermentation of tea manufacture which ultimately determine the colour, strength and brightness of tea liquor. O-quinone was reported to be transformed into theaflavins, thearubigins and

theaflavic acids, which gave black tea its characteristic colour and also contributed to the taste of brewed tea. Soboleva *et al.* (1966) found two stages of enzymatic oxidation of catechins, that is formation of O-quinones, accompanied by the development of free radicals of the semiquinone type.

Various classes of organic compounds including hydrocarbons, alcohols, carbonyl compounds, phenols, acids and esters constitute resinous substances which was regarded as aroma bearers and aroma fixers of tea (Voronotsov 1946). Seventy-nine compounds were positively identified and ten compounds were tentatively identified in the oil obtained from a methyl chloride extracts of the steam distillate of green tea leaf. The compounds were reported include 17 hydrocarbons, 17 alcohol, 16 aldehydes, 13 ketones, 8 easters, one acid and seven others. Major constituent of this oil were identified as 2, 5, 6, trymethyle-2-hydroxycyclohexanone, linalool, geraniol, Cis-vasmone, beta-ionone, cyclohexanone, 5, 6 epoxy-beta-ionone, (Yamaguchi and Shibamoto 1981).

Volatile Flavoury Components (VFC) of Assam and Darjeeling teas were investigated by GC-MS. Grassy flavours were attributed to aldehydes such as hexenal that were predominant in CTC teas while the alcohols such as linalool oxides and linalool having fruiting aroma were predominant flavour components of orthodox teas. It has been suggested that the difference in flavour, attributed to the genetic

variation in tea cultivars, including fineness of plucking and method of processing. It has been estimated that the orthodox teas of Darjeeling that are withered and rolled to higher degree, contain three times higher amount of volatiles as compared to orthodox teas of Assam. Furthermore, the content of monoterpene alcohols are about five times higher in Darjeeling hill teas as compared to the plain Assam teas. A typical difference in the content of linalool and geraniol was observed in volatile oils of black teas made from cultivars of var. *assamica*, hybrid *assamica* and *sinensis* grown in Darjeeling hills (Mahanta and Singh 1990). The impact of various cultural and manufacturing techniques on VFC was studied in order to optimize the conditions for production and retention of aroma in relation to tea quality (Ravichandran and Parthiban 1998).

The aroma pattern of Darjeeling black tea shows that the concentration of geraniol, benzyl alcohol, 2-phenylethanol, linalool and its oxides are between var. *assamica* and *sinensis*. The ratio between the terpenoid to non-terpenoid was greater value in Assam teas in comparison to Chinese teas. Furthermore, the amount of linalool and geraniol occurs in similar proportion in Darjeeling teas, whereas in Assam teas linalool always higher than geraniol (Mahanta and Singh 1990).

Climatic conditions greatly influence the production of flavours. Tea grows under artificial shade produced black tea with higher

Table –7. *Volatile compounds in orthodox black teas manufactured with different fermentation times during July (Hazarika et al., 1984).*

Compounds	*Under fermented*	*Normal fermented*	*Over fermented*
1-Penten-3-ol	0.29	0.27	0.28
Z-2-Penten-1-ol	0.42	0.39	0.47
n-hexenal	0.14	0.12	0.15
Z-3-Hexenal	0.44	0.29	0.34
E-2-Hexenyl formate	0.46	0.32	0.41
Linalool oxide (furanoid-Z)	0.32	0.27	0.29
Linalool oxide (furanoid-E)	0.89	0.82	0.85
Benzaldehyde	0.11	0.16	0.17
Linalool	2.76	1.78	1.98
Methyl salicylate	0.84	0.66	0.78

theaflavins (TF) and reduced thearubigins (TR) concentration and with a better flavour index (Owuor *et al.* 1988). Changes in the quality parameters of CTC clonal teas are also due to variation in altitude within a radius of 10 km. were determined. The Group I VFC decreases with the decrease in altitude and Group II VFC, flavour index (FI), theaflavins (TF), thearubigins (TR) and caffeine increases with the increase of altitude. The tasters evaluation also increased with the increase of altitude (Owuor *et al.* 1990). Wickremasinghe *et al.* (1967), Wickremasinghe (1978) reported that cool weather (20^0C) with colder nights (6-10^0C) and dry cloudless days are good for aroma,while warm and wet weather favours tea growth. The same phenomenon had been observed in Darjeeling hills. He also demonstrated that relative contents of chlorophyll, leucine, alpha alanine, 2-keto-4-methyle pentanoic acid, carotenes, beta-ionone, dehydroactiniolide, theaspirone, beta-amyrin acetate, trans-hex-2-enol and phenylacetaldehyde change during black tea manufacture, depending on climatic conditions (Wickremasinghe, 1974). The difference in the content of teaflavins (TF) and thearubigins (TR) of tea infusion varies with country of origin and type of manufacture (Smith and White 1965). Seasonal variations of theaflavins (TF) and thearubigins (TR) was observed by Sud and Bhattacharya (1992). The first flush (April-May) yielded the best quality of tea, with low ratio of thearubigins: theaflavins, while liquors of tea manufactured

Table –8. *Ratios of main volatile compounds to total volatiles in black tea (Takeo and Mahanta, 1983).*

Compound	*Sri Lanka*			*India*				*Japan*
		Var. assamica				*Hybrid of assamica × sinensis*		
				Assam		*Darjeeling*		
	Rf	*Uva*	*Dimbula*	*(1)*	*(2)*	*(1)*	*(2)*	*Benihomare*
t-2-Hexenal	0.40	3.1	2.6	4.9	3.1	0.7	0.3	1.5
cis-2-Penten-1-ol	0.53	2.8	4.3	0.2	3.8	1.4	0.1	6.1
cis-3-Hexenal	0.65	9.5	11.8	11.9	5.0	5.7	3.1	5.2
Linalool oxide (furanoid-cis)	0.77	3.4	3.2	3.5	3.6	8.2	4.7	3.8
Linalool oxide (furanoid-trans)	0.83	10.3	8.8	8.0	12.0	16.7	12.0	12.0
Linalool	1.00	24.0	15.5	18.3	32.8	15.6	13.7	9.3
Phenylacetaldehyde	1.20	0.2	0.5	4.0	5.0	1.1	1.8	1.0
Linalool oxide, pyranoid-cis	1.40	0.3	0.4	trace	trace	1.0	6.0	0.3
Linalool oxide, pyranoid-trans	1.46	0.2	0.3	trace	trace	1.8	trace	0.5
Methylsalcylate	1.50	18.6	18.8	9.0	13.2	9.8	5.3	4.9
Geraniol	1.67	1.3	2.2	3.3	1.6	7.3	15.9	21.7
Benzylalcohol	1.71	1.0	1.9	4.3	1.0	1.7	2.0	2.6
2-phenylethanol	1.78	0.2	0.9	3.4	1.0	2.0	6.7	7.5
cis-Jasmone+ β-Ionone	1.83	0.2	0.1	7.4	1.5	0.5	4.4	0.3
Nerolidol	2.09	0.2	0.1	0.1	0.5	0.5	2.5	0.6

during June-October, showed an increased thearubigins: theaflavin ratio. During the rainy season, thearubigins increased to a maximum level and theaflavins reduced significantly, compared with the rest of the seasons. Various kinds of volatile compounds as reported earlier with special reference to their formation and distribution in different kinds of tea have been represented in Table –7 and Table –8 respectively.

Biochemical observation of tea

The tea pigments comprises of total anthocyanin and total flavone glycosides together with chlorophyll and carotenoid were estimated quantitatively in green leaf of tea during different flushes from the samples of Tea Experimental Station, Tocklai, Assam. Seasonal variation of the above mentioned tea pigments have been observed to be much significant. It has been suggested that climatic conditions of North-East India appeared to have influence on the biosynthesis of the pigments like chlorophyll and carotenoid, along with anthocyanin and flavanol glycosidase to reflect on the quality of made tea (Ann. Sci. Rep., 1985-86;.TRA, TES). Taylor and McDowell (1991) classified and tentatively identified 28 pigments from tea leaf on the basis of spectral data. Isolated chlorophylls of immature tea leaf cells showed higher photosynthesis than that of cells isolated from matured tea leaves. Kenyan tea clones distinguished from one another on the basis of their green leaf plant pigments, chlorophyll

and carotenoid composition (Taylor *et al.* 1992).

It is also reported that chlorophyll in the processed black tea was much lower in amount than that in the green unprocessed tea leaf of the same clone (Van Lelyveld *et al.* 1990). Kato (1992) suggested that there was correlation of the internal leaf architecture with photosynthetic rate and light intensity. Experiments of Zapromotov and Zagoskina (1987) showed that the chloroplasts were responsible for synthesis of flavonol in callus tissue of tea. Growth hormones like IAA and GA enhanced photosynthetic activity while ABA reduced the same activity at 20^0c (Satoshi 1987b).

Ohtsuki *et al.* (1987) described method for rapid and useful determination of free amino acids. Bokuchava and Popov (1954) claimed that amino acids by simple polyphenols (pyrocatechin, pyrogallol) or by catechin and o-diphenol oxidase, results in the formation of corresponding aldehydes, NH_3 and CO_2. Kretovich and Tokareva (1948) showed at high temperature, amino acids reacts with sugars resulting in the formation of aldehydes. Amino acids and amides in fresh as well as withered tea shoots vary significantly at clonal level (Bhatia 1962). From 15 kinds of green tea, 16 free amino acids were detected where in theanine, glutamic acid, aspartic acid and arginine content give 'brothy' test of green tea (Oh *et al.* 1988). Effect of nutrients and amino acid accumulation in tea leaves has

been investigated recently. It has been observed that the increased free-amino acid content in tea leaves was accompanied by enhanced nitrate reductase activity. This phenomenon indicated that potassium and magnesium application improved the nitrogen metabolism, leading to an increased synthesis of amino acids (Ruan *et al.* 1998).

Total available carbohydrates (TAC) content seemed correlated with growth of the buds of tea stored at 2^0C by water culture under dark or light condition (50-120 Ix). The tea shoots stored in light survived longer and healthier than those in the dark. The TAC content gradually declined with the duration of storage (Takeda, 1979). Sugars obtained by hydrolysis of the eleven flavonols, among them all contained one molecules of glucose in the sugar moity (Tsushida *et al.* 1986). New carbohydrate compound quercetin and kaemferol triglucoside were isolated from tea and their structures were determined (Finger *et al.* 1991).

Kurasanov and Shubert (1936) developed a technique for determining in the aqueous distillate the acid number, ester number and saponification number, as well as a technique for determining the high volatile fraction in tea leaf processing.

Biosynthesis and chemical nature of phenolic compounds are well determined but our knowledge regarding their physiological role is rather limited. Phenolic acids like P-coumaric acid, ferrulic acid and sinapic acid are activated as their Co-A esters and reduced to primary

alcohols which play a direct role in the synthesis of lignin — the cell-wall material (Haslam 1985).

During processing, at the stage of rolling of tea, polyphenols particularly catechins are oxidized by polyphenol oxidase to yield two flavours such as, theaflavins and thearubigins which give taste, briskness, colour and strength of the tea (Ullah *et al.* 1984). Due to presence of caffeic acid and alkaloid like caffeine, the leaves produce a stimulatory nature of tea which is the characteristics of beverage (Stagg and Millin 1975).

The problem to investigate tea aroma constituents had attracted the attention of many scientists in the past. Kozai (1890) proposed the theory of tea aroma formation by the effect of an enzyme on glucoside but he did not indicate the enzyme actually used. Mann (1914) considered that tea aroma formed from enzymatic oxidation of tannin and also have a role in aroma formation from tea leaf chlorophyll, during the formation of black tea manufacture. He also concluded that maximum accumulation of volatile compounds occurred during rolling and fermentation process of tea leaf manufacture. One of the earliest definitions of tea essential oil was given by Mulder (1838). He described the oil as liquid of a lemon-yellow colour with a strong tea-like odour, astringent taste, and which could readily solidify.

Potapov (1940) described that tea essential oil was protein in

nature, and some amino acids which are accumulated during withering took part in its synthesis. His theory is of special interest in the light of present reaction data on the formation of aldehydes by oxidative deamination of amino acids under the effect of tea quinones. Takei *et al.* (1934 to 1938) had analysed the essential oil of the fresh leaves of green and black teas and provided a basis for further research in this field.

Enzyme activity in tea

Peroxidase in tea leaf homogenates (Roberts 1952) was also isolated in soluble form (Takeo and Kato 1971). This enzyme also exists in the form of several isoenzymes of some significance in tea processing. Ethylene causes activation of the different isoenzymes (Saijo and Takeo 1974). It has been observed that peroxidase activity in clonal tea leaves was higher than the seedling tea leaves eventually determined the quality of black tea. It has been suggested that clonal tea has a better quality of black tea than seedling tea and should be taken into consideration as a parameter during selection work in breeding programme of clonal teas (Van Lelyveld and Oe Rooster 1986). The specific activity of one of the principal enzyme peroxidase being responsible for the formation of brown compounds known as theaflavins was studied in fermented or black teas. Peroxidase activity increased during rolling (mechanical injury of withered tea leaves) leading to the oxidative polymerization (enzymatic browning) of

different clonal cultivars in black tea manufacturing under different conditions of withering and fermentation was also reported (Mahanta *et al.* 1993). Flavonols (catechin and gallocatechin) isolated from green tea leaves were treated with polyphenol oxidase, peroxidase and a combination of both. It has been observed that peroxidase is produced in higher amount of choromatographically unresolved thearubigins of higher molecular weight and it has been also observed that in the presence of peroxidase, a significant decrease was quite apparent in the levels of all flavonols glycosides (Finger 1994). Role of peroxidase in the melanoidin formation in tea leaf processing (fermentation) was reported by Chachua (1990). The result obtained by him suggested that peroxidase determines the rate of quinoid structure formation and the rate of melanoidin reaction. Simple methods for separate assays of ascorbate (ASA),peroxidase and guaiacol peroxidase and of the three isoenzymes of ASA peroxidase in tea leaves were also proposed (Amako *et al.* 1994). Recently a novel, basic, heme peroxidase isoenzyme that can account for a significant part of the ascorbate peroxidase activity in tea leaves has been purified and characterised (Kvaratskhelia *et al.* 1997).

Polyphenol oxidase (PPO) oxidises polyphenols and this enzyme activity steers the processing of tea leaves. PPO is involved in the manufacture of black tea from fresh green tea leaves and it is responsible for the formation of aromatic compounds by oxidising the

substrates (Srivastava 1986). PPO by oxidative condensation of flavonols yield theaflavins and thearubigins which render the positive quality to tea (Ullah *et al.* 1984). Overall progress regarding polyphenol oxidase activity in plants have been critically reviewed by Mayer (1987). Partially purified PPO from fresh tea leaves was found to form aldehyde from amino acid in the presence of (+) catechins or other diphenols, these aldehydes contribute to develop aroma (Srivastava 1986). Leucoanthocyanidins and querectin were showed to be non-competitive reversible inhibitors of phenolic oxidase (Pruidze 1985). It is also reported that PPO activity varies on the following factors :-

(a) depending on the flush and plucking seasons of tea shoots (Ann. Sci. Rep.; TRA, 1985-86).

(b) during tea processing, as fermentation gives maximum PPO activity, while during withering, PPO activities were found to be reduced in black tea manufacture (Ullah 1988).

(c) bud and first leaf content show lowest PPO activity while leaves showed rise of PPO activities with the age of the leaves. It was also noted that polyphenol content shows reverse pattern, as their amount is maximum in younger leaves and with aging of the leaves, the content gradually decreases (Thanaraj and Seshadri 1990).

Tolbert (1973) reported that PPO was predominantly located at the thylakoid membrane. Diethyl-dithiocarbamate inhibited both PPO activities and aldehyde formation (Srivastava 1986). In the presence of peroxidase, a significant decrease in the levels of all flavonol glycosides was observed, while in the presence of polyphenol oxidase, only myricetin glycosides levels were decreased (Finger 1994). Recently, analysis of tea polyphenol in details was presented at the Second International Scientific Symposium on Tea and Human Health held September 14, 1998 in Washington D. C. (Beecher *et al.* 1999). Besides, many useful and worthy papers were also presented at that Symposium.

Catalase is an iron porphyrin enzyme which catalyzes very rapid decomposition of hydrogen peroxide into water and oxygen. Catalase removes hydrogen peroxide, which is highly toxic to the cell and by breaking hydrogen peroxide, it supplies oxygen which is essential for cellular oxidation. From tea plant, catalase activity was determined by Tsagareli *et al.* (1988), Tsagareli and Pruidze (1990a and 1990b). This enzyme had maximum activity within the pH range 5.6 to 6.5 and temperature range 40-45^{0}C.

N-methylnucleosidase which hydrolyzes 7-methylxanthosine to produce 7-methylxanthin was detected in tea leaves. It also catalyzes the hydrolytic reaction of 7-methylxanthosine in the pathway of caffeine biosynthesis (Osamu *et al.* 1988).

Chlorophyllase activity was found to be high in tea leaves but it was also suggested that the colour of fresh tea leaves on blanching (immersing in boiling water) cannot be ascribed to chlorophyllase activity (Nagao *et al.* 1987).

3 neutral ribonuclease activities were detected in tea leaf extracts. One of these ribonuclease designated RNase C_1 was purified. It had been suggested that RNase C_1 preferentially attacks the phosphodiester bond (Osamu *et al.* 1985).