1. INTRODUCTION

1.1. Origin of tea

The word tea is derived from t'ē of the Chinese Funkien dialect. In Cantonese, tea is known as Ch'a. In this form the name reached Japan, India, Russia, Iran and the Middle East.

Tea drinking originated in China, but the place of origin of tea is still a matter of speculation. According to Ukers (1935) the first authentic reference to tea is found in an ancient Chinese dictionary which was revised about the year A.D. 350 by Kuo P'o, a Chinese scholar. The information available from the Chinese sources do not throw much light on its place of origin. The origin and dispersal of the tea plant was examined in depth by Wight who made his views known to Kingdon Ward (1950). According to Wight, tea might have originated in the region around the part of intersection of latitude 29° N and longitude 98° E, near the source of the river Irrawaddy, which is the meeting ground of Assam, North Burma, South-West China and Tibet. Tea has its principal area of distribution in the South-East Asia ranging from Nepal to Formosa and Japan. The earliest historical records show only that tea was grown in South-Eastern China some 2000 years ago but the crop appears much older than this. It is possible that China and Assam teas had separate origins and the small leaved China tea was the older type from which the large leaved Assam forms originated with Southern Assam as the secondary centre of origin. The question of real discoverer of the Assam tea plant has not yet been settled. According to Ukers (1935), Robert Bruce is considered to be the real discoverer of the Assam tea plant and he is supposed to have seen the plant growing wild in some hills near Rongpur, then the capital of Assam which he
visited in 1823. But some sources (Baildon, 1877) reported that the tea plant of Assam was discovered by a local Assamese nobleman, Moniram Dewan, who latter worked in the Assam Company. It may so happen that Moniram Dewan brought the plant to the notice of Robert Bruce during his visit to Rongpur in 1823. The role of Singphow tribes of Assam in bringing the local plant to the notice of the outside world also cannot be ignored. Therefore, it is doubtful whether the controversies on the origin of Assam tea plant could ever be settled.

1.2. Classification of tea

Tea (Camellia Sinensis, Linn) belongs to the family Theaceae. Wight (1962) gave a concise description of the China and the Assam varieties of tea while proposing their specific ranks. Barua (1963) provided morphological and anatomical description (Table 1.1) of the three races of tea which was later elaborated by Bezboruah (1971).

Table 1.1 Distinguishing anatomical and chemical characters of the three races of tea (Barua, 1963).

<table>
<thead>
<tr>
<th>China</th>
<th>Assam</th>
<th>Southern Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sclereids absent or rare</td>
<td>Sclereids numerous</td>
<td>Sclereids numerous</td>
</tr>
<tr>
<td>Sclereids slender, almost without specules.</td>
<td>Sclereids stout with a few specules.</td>
<td>Sclereids stout with numerous specules</td>
</tr>
<tr>
<td>Lumen of sclereids almost completely closed.</td>
<td>Lumen of sclereids of irregular width, closed in places.</td>
<td>Lumen of sclereids of irregular width but not closed.</td>
</tr>
<tr>
<td>Triglycosides present in fairly large amount</td>
<td>Triglycosides absent.</td>
<td>Triglycosides absent.</td>
</tr>
<tr>
<td>IC absent</td>
<td>IC absent.</td>
<td>IC present in fairly large amount</td>
</tr>
</tbody>
</table>

IC = Unknown substance
It is clear that the sclereid features and chemical composition together with morphological differences make a strong situation in favour of the classification proposed by Wight (1962) for the three races of tea. According to Wight's nomenclature, *Camellia sinensis* L. or the China tea plant is a big shrub, 1-3 m tall with many virgate stems arising from the base of the plant near the ground. Leaf hard, thick and leathery; marginal veins indistinct and appear sunken in lamina. Petiole short, 3-7 mm long, stout, usually giving the leaf an errect pose.

*Camellia assamica* (Masters) or the Assam tea plant is a small tree, 10-15 m tall with a trunk sometimes upto one third of its height, possesses a robust branch system. In typical plants, leaf dependent, thin, glossy with more or less acuminate apex and distinct marginal veins. *Camellia assamica* sub sp. *lasiocalyx* (Planch, MS) or the *Cambodiensis* or Southern form of tea is a small fastigiate tree, 6-10 m tall with several upright, almost equally developed branches. Leaf more or less erect, glossy, yellow-green when young, light-green at maturity changing to coppery-yellow or pink-red from autumn till the end of the season. Petiole pinkish-red at the base. Leaf size intermediate between *sinensis* and *assamica*, broadly elliptic, marginal veins not very prominent.

### 1.3. Clonal tea

Barua (1963) has observed that the basic difference between clone and seed progeny is one of adaptability. A seed population composed of a large number of genetically distinct units is elastic and can be fitted into a wide range of cultural and environmental conditions without much change in its overall performance. Consequently a clone lacks elasticity which makes it more selective of environment and cultural treatments. Against this drawback, clones have certain advantages over the seed progenies. As clones are selected for high
yield and good cup characters, selection from the existing fields of mature tea is necessary for meeting the immediate need of the tea industry for improved planting material. Cultural practices like pruning, plucking, manuring and control of pests and diseases can be standardised for a clone since it is morphologically alike. Clonal selection therefore offers opportunities of instant fixation of superior genotypes as well as exploitation of hybrid vigour provided the selection of mother bushes is accurate.

In 1918, Mrs. Tanstall, the Microbiologist, collected tea seeds from 54 different sources and laid out a field trial at Tocklai. For vegetative propagation cuttings from different bushes were taken. Bush number 13 of plot 29 attracted attention due to its better rooting, and, made tea obtained from this clone, was highly appreciated by the tasters of India and England for its liquor characters and quality. Thus the first vegetative clone was selected and released as clone 19/29/13 - latter named as TV1.

The first batch of three clones was released to the industry from Tocklai in 1949. Since then clones with diverse characters have been released to the industry at regular intervals the last clone being TV30 in 1993.

1.4. Cultural practices of tea

Any particular soil type where tea is grown requires rainfall and certain conditions for the plant to flourish and make its cultivation commercially viable. Normally suitable pH of the tea soil is between 4.0 to 6.0 i.e. acidic in reaction. Tea requires a soil which should not normally be less than 1.5 to 2.5m deep. The soil up to a depth of about 90 cm should be free from excess of water at all times of the year.

At present, commercial cultivation of tea extends from $44^\circ$ N to $34^\circ$ S
latitudes. In the tropical belt, it is usual to grow tea on slopes of mountains and high plateaus at altitudes varying from about 700 m to 2400 m above sea level. In temperature zone, low hills of less than 700 m elevation are generally chosen for its cultivation. In the plains of N. E. India, temperature during summer occasionally rises as high as 37°C. Plants use light energy in the visible part of the solar energy for carrying out photosynthesis which contains less than 50% of the total incident energy.

Tea is a rainfed crop. Rainfall in the tea areas of the world varies from less than 1000 mm to 6000 mm in a year. Irrespective of the total quantity, nowhere is rain distributed evenly throughout the year.

1.5. Chemical composition of tea

The fresh tea leaf contains all the essential chemical and biochemical constituents, viz. enzymes, intermediates, structural elements, flavour and colour precursors etc. that determine the final quality of manufactured tea. The levels of these constituents vary with geographic locations, agronomic practices, method of plucking etc. (Wood et al., 1964, Bhatia and Ullah, 1968). Tea leaf contains more than 20% polyphenols and they are the most important determinants of tea quality. Thus the biochemical potential of leaf to form grades of black tea can be predicted even at the plucking stage. The general chemical composition of tea shoot consisting of two leaves and a terminal bud is presented in table 1.2.

The chemical constituents of tea leaf can be broadly categorised into two groups viz. phenolic compounds and nonphenolic compounds.

1.5.1. Phenolic compounds:

The phenolics or polyphenolic compounds make up 25 -30% of the dry
Table 1.2: Approximate chemical composition of young shoots of tea (Assam variety, Roberts, 1962)

<table>
<thead>
<tr>
<th>Compound</th>
<th>% Dry Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Soluble in cold water</strong></td>
<td></td>
</tr>
<tr>
<td>Flavonols: (-) Epigallocatechingallate (EGCG)</td>
<td>9 - 13</td>
</tr>
<tr>
<td>(-) Epigallocatechin (EGC)</td>
<td>3 - 6</td>
</tr>
<tr>
<td>(-) Epicatechingallate (ECG)</td>
<td>3 - 6</td>
</tr>
<tr>
<td>(-) Epicatechin (EC)</td>
<td>1 - 3</td>
</tr>
<tr>
<td>Gallocatechin (GC)</td>
<td>1 - 2</td>
</tr>
<tr>
<td>(+) Catechin (C)</td>
<td>1 - 2</td>
</tr>
<tr>
<td>Flavonol and their glycosides</td>
<td>3 - 4</td>
</tr>
<tr>
<td>Leuco anthocyanins</td>
<td>2</td>
</tr>
<tr>
<td>Phenolic acids: Theogallin</td>
<td>2</td>
</tr>
<tr>
<td>Others</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total phenolics</strong></td>
<td>20 - 30</td>
</tr>
<tr>
<td>Caffeine</td>
<td>3 - 5</td>
</tr>
<tr>
<td>Amino acids</td>
<td>2 - 4</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>2 - 4</td>
</tr>
<tr>
<td>Organic acids</td>
<td>0.5</td>
</tr>
<tr>
<td>Volatile substances</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Partially soluble in hot water</strong></td>
<td></td>
</tr>
<tr>
<td>Polysaccharides: Starch &amp; Others</td>
<td>2 - 5</td>
</tr>
<tr>
<td>Protein</td>
<td>12</td>
</tr>
<tr>
<td>Ash (inorganic material)</td>
<td>5 - 6</td>
</tr>
<tr>
<td><strong>Insoluble in water</strong></td>
<td></td>
</tr>
<tr>
<td>Cellulose</td>
<td>7</td>
</tr>
<tr>
<td>Lignin</td>
<td>6</td>
</tr>
<tr>
<td>Lipids</td>
<td>3</td>
</tr>
</tbody>
</table>
weight of tea shoot and they predominantly form the precursors of the nonvolatile components of black tea which are essential for the liquor characters. Again the polyphenols are of four major categories namely the flavanols or catechins, the flavonols, the flavandiols and phenolic acids and their derivatives.

1.5.1(i). Flavanols or Catechins

Flavanols or catechins are by far, the most important components of the tea shoot because of their contribution to the colour, aroma and flavour of black tea. They are produced in the plant from simple reducing sugar such as glucose by either the acetic acid pathway or the shikimic acid pathway (Bokuchava and Skobeleva, 1969).

1.5.1(ii). Flavonols

The flavonols occur as a complex mixture in the fresh tea shoot and their characteristics are changed relatively during fermentation (Millin et al., 1969). Flavonol compounds though quantitatively important constituents of tea, seem to play a very minor role in the taste, contributing to astringency or a 'woody' nature. Flavonol glycosides have been reported to have 'vitamin P' activity which strengthens the walls of capillary vessels.

1.5.1(iii). Anthocyanins

Anthocyanins are flavonoid group of coloury pigments which are quite conspicuous in young shoots of the first and the autumn flush (Baruah, 1990). The anthocyanin pigments play a major role in the development of characteristic colour of the processed tea.

1.5.2. Nonpolyphenolic compounds

1.5.2(i). Caffeine

Caffeine forms 3 - 4 % of the tea shoot. The presence of caffeine, along
with other alkaloids present in tea viz. theobromine and theophylline, is an important factor in the popularity of tea as a beverage. Caffeine levels are known to increase appreciably during withering (Wood et al., 1964).

1.5.2(ii). Protein and Amino acids

Proteins play an important role in determining tea quality. Due to protein-flavanol interactions, water insoluble compounds are produced which lead to reduced flavanol levels, thus reducing the quality of black tea (Bokuchava and Skobeleva, 1969). The amino acid, theanine, constitutes 50% of the free amino acid content of tea. But its role in black tea quality is not known. Other amino acids of tea shoot include leucine, isoleucine, valine and phenylalanine.

1.5.2(iii). Carbohydrates

Tea shoot is known to contain various carbohydrates, that includes simple sugars like glucose, fructose, sucrose, rhamnose etc. and complex polysaccharides such as cellulose and hemicellulose (Bokuchava and Skobeleva, 1969). The most important role of sugar is the biosynthesis of polyphenols. Nonenzymic browning reactions involving sugars and amino acids contribute to tea colour. Pyrazine, furan and furfural types of compounds produced during processing may add to flavour of made tea (Sanderson and Graham, 1973, Takeo, 1975).

1.5.2(iv). Chlorophylls

The role of chlorophyll pigments in tea leaf is not confined only to photosynthetic activities of the plant, but is also reported to be primarily responsible for the appearance of manufactured tea. The blackness or brownness of tea largely depend on the content of chlorophylls and their transformation as shown below. A low level of chlorophyll in tea leaf is reported to be a major
factor for aroma development in tea (Wickremasinghe, 1974):

![Pathway of Chlorophyll degradation in green tissue.]

**Fig.1: Pathway of Chlorophyll degradation in green tissue.**

### 1.6. Manufacture of black tea

The manufacture of black tea is a complex process in which plucked fresh tea shoots are subjected to processing conditions that are aimed to maximising the revenue from the finish product. Black tea processing involves several biochemical reactions that are known to contribute to three broad categories of quality viz. colour, taste and aroma. There are five significant steps in black tea manufacturing namely withering, rolling, CTC, fermentation and drying or firing.

**1.6.1. Withering**

Withering is the first stage in the processing of leaf and consists principally of reduction in moisture content from the 75 - 81% characteristic of fresh leaf to 55 - 70% according to the process to be used subsequently. The time taken is generally 12 - 15 hours.

During withering the leaf becomes flaccid and certain changes take place in its chemical composition. As soon as the shoot is plucked, the metabolic activities within the leaf become imbalanced. While the supply of nutrients from other parts to the leaf is cut off, the transportation of the products manufactured within the leaf can no longer be possible to other parts of plant. Photosynthesis ceases quite rapidly, respiration continues for much of withering period and is typically responsible for a loss of around 4.0% of the dry matter.
in the form of carbondioxide. Other metabolic activities continue at decreasing rates, some breakdown of proteins and polysaccharides occur and is naturally accompanied by increase in the levels of free amino acids and simple sugars. Synthesis of caffeine also continues (Bhatia, 1962; Bhatia and Deb, 1965; Devchoudhury and Bajaj, 1980a; Roberts and Wood, 1951; Roberts and Sanderson, 1963). It is desirable that withering should be continued for a minimum of 10 -12 hours at the lowest practical temperature which will be consistent with moisture removal. Generally alternate circulation of hot (upto 40°C) and ambient air is carried out in practice. Temperature above 43°C during withering is reported to result in reddish colouration of the leaves (Choudhury, 1964).

1.6.2. Rolling

The next step to follow withering is the rolling in which maceration and distortion of leaf are done either in a three crank single or double action roller or in a continuous roller. Due to cell distortion complete protoplasmic disorganization of the leaves takes place facilitating inter mixing of cell components and oxidation reactions. The polyphenols of the cell are released from the vacuoles into the cytoplasm due to rupturing the vacuolar membrane and in the cytoplasm the enzyme polyphenol oxidase react with the polyphenols to form some coloury compounds (Roberts, 1949).

1.6.3. Crushing, Tearing and Curling (CTC)

In the manufacturing of CTC teas, the plant cells are further ruptured vigorously by subjecting the rolled leaves to dual action of compression and cutting in a CTC machine. The process enhances the accessibility of the leaf cells to atmospheric oxygen due to increase in their surface area.
The CTC machine consists of two contrarotating metal rollers usually 8 inches in diameter. The surface of each roller is engraved with a matching pattern of teeth and the roller is mounted in such a way that the teeth mesh together and the gap between the rollers remain adjustable. One roller is run at 700-750 rpm and the other usually at 1/10th of this speed. Leaf passing between the roller is said to experience a crushing, tearing and curling action, hence the name CTC is so adopted.

1.6.4. Fermentation

After disruption in rolling, the leaf is allowed to 'ferment' for 1-3 hours before passing on to the dryer. The term fermentation is a misnomer since no microorganism is involved in the process of tea fermentation. The process is principally an enzymic as well as chemical oxidation/reduction process, that starts right from the time of plucking. However, this oxidative process is accelerated just after rolling and attain its peak during the so-called fermentation process. The enzymic oxidation of the leaf polyphenols by polyphenol oxidase leads to formation of a series of orthoquinones which further undergo a series of inter and intra molecular nonenzymic oxidation and condensation to form two classes of compounds known as theaflavins (TF) and thearubigins (TR) (Roberts, 1962a). Degradation of chlorophylls also takes place during fermentation forming brown and black pigments. These pigments contribute towards the appearance of made tea but have little influence on the character of tea liquor.

1.6.5. Drying / Firing

The fermented leaf is then passed on to the dryer where it is subjected to a blast of hot air to reduce the moisture content to 2.5 to 3.0% and to cut down
the fermentation process, as continuation of fermentation beyond certain limit is detrimental to the quality of tea brew. In the earlier part of the drying the leaf experiences a period of accelerated fermentation during which important flavour developments take place. The enzymes polyphenol oxidase and peroxidase remain active until the moisture content comes down to around 20 % and at this point final product is virtually fixed. It is desirable for the leaf to reach a temperature of around 100 °C otherwise the product may deteriorate quickly due to inadequate inactivation of the enzymes. Both faster as well as slower drying produces tea of undesirable character (Bokucheva & Skobeleva, 1969).

1.7. The enzyme RUBISCO

Ribulose-1,5-bisphosphate carboxylase / oxygenase (RUBISCO) (E.C. 4.1.1.39) was discovered by Wildman and Bonner (1947). The enzyme is composed of two subunits, one is known as small subunit (SSU) coded for nuclear genetic material and the other large subunit (LSU) coded for chloroplastic DNA. Baker, Eisenberg and Eiserling (1977) suggested that the enzyme has a bilayered structure, each layer containing four large and four small subunits. Active sites of the enzyme are located in the large subunits.

One of the striking feature of RUBISCO is its sheer abundance. Upto 65% of the soluble protein in extracts of photosynthetic cell can be accounted for this single enzyme.

The major biochemical process in the photosynthesis is considered to be the fixation of CO₂ by RUBP carboxylase and its further reduction by the Calvin cycle which results carbohydrate formation (Lorimer & Andrews, 1981). The biochemical processes are mainly affected by temperature and CO₂ concentration and slightly by light intensity (Enoch et al., 1986). Temperature
effects mainly Michaelis constant and maximum velocity of the enzymic reactions. Since RUBISCO fixes CO₂ and O₂ competitively, increasing ambient CO₂ concentration results increased gross photosynthesis due to advantage of CO₂ over O₂ in their competitive uptake. Reduction of O₂ uptake results in supressed photorespiration and consequently CO₂ evolution results in increase in net assimilation.

RUBISCO is by far the best studied plant enzyme and its properties have been extensively reviewed (Andrews & Lorimer, 1987; Gatteridge, 1990). Because of its bifunctional nature, the ratio between the competing reactions determines the efficiency of CO₂ and the importance of this in determining plant productivity has made it a target for genetic manipulation (Ellis & Gatenby, 1984).

1.8. Background of the current study

Extensive work has been done concerning ribulose-1,5-bisphosphate carboxylase / oxygenase (RUBISCO) in various plants than about any other plant protein. There are two aspects of this chloroplast enzyme that are responsible for attracting such singular attention: its properties which determine the relative rates of photosynthesis and photorespiration and its synthesis, which involves an interaction of light with two distinct genetic systems. Under normal atmospheric conditions, the rate of photorespiration is about 15 - 20 % of the rate of apparent photosynthesis (Farquaher et al., 1980), while the ratio of the maximum rates of the carboxylase and oxygenase reactions catalysed by isolated RUBISCO is between three and four to one in most cases (Badger and Andrews, 1974; Christeller and Liang, 1979; Jordan and Ogren, 1981). It has been reported that oxygen shows linear competitiveness for carboxylation
and so does carbon dioxide for oxygenation (Laing et al., 1974). These observations suggest that carbon dioxide and oxygen compete with each other for ribulose bisphosphate bound to the corresponding enzyme. If it was possible to improve the efficiency with which carbon dioxide competes with oxygen at the active site of RUBISCO, the rate of photosynthesis would be increased relative to the rate of photorespiration. The increase rate of carboxylation can be effected by increasing (i) the amount of RUBISCO accumulated within the cell (ii) the affinity of the enzyme for CO₂ and (iii) the ratio of carboxylation to oxygenation.

To improve the production of tea among all the constraints is to understand the basic biochemical factors which are involved in productivity and ultimate total production of plant is dependent on the main biochemical process photosynthesis. The main enzyme responsible for this photosynthesis is RUBISCO which catalyzes the initial step in Calvin's reductive pentose phosphate cycle, the major soluble leaf protein in plants. A decade has elapsed since it was discovered that this enzyme is also a monooxygenase, which catalyzes the primary event in photorespiration. Improving the efficiency, at which CO₂ competes with O₂ for reaction, with ribulose bisphosphate, would improve the rate of photosynthesis relative to that of photorespiration and could substantially increase productivity in a large variety of commercially important crops.

In addition to the conventional biochemical approaches to improve photosynthetic yield, the techniques of molecular biology have recently been applied to this problem. Recombinant DNA technology promises to facilitate the genetic manipulation that may lead to improved crop productivity. There are encouraging indications that continuing investigations in this area will result in the elucidation of the mechanisms involved in processing, transporting and
assembling the newly synthesized peptides to produce a functional oligomeric enzyme. It has become axiomatic that control of flux through a metabolic pathway is usually exerted at an irreversible enzymatic step that occurs early in that pathway. Ribulose bisphosphate carboxylase fulfills these criteria, thus it is not surprising that enzyme activity can be modulated. Formation of a ternary enzyme-co-divalent cation complex is required to convert the protein into a catalytically functional species.

Various factors of both morphological and physiological aspects relating to the yield of tea have been extensively explored. Roberts and Keys (1978) evidently showed that tea followed the C₃ or photorespiratory pathway. The light or photorespiration proceeds simultaneously with metabolic or dark respiration, causing considerable loss of photo-assimilated carbon. This makes the C₃ species less efficient producers of dry matter than the small number of C₄ species. Evidence in recent years support that some plants within a C₃ species may also follow the C₃-C₄ pathway. Considering the existence of an extremely wide range of genotype in tea, including the polyploids, the chances of getting a few C₃-C₄ plants among the various strains look quite promising. Examination of morphological characters would help in their tentative identification, which could then subsequently be confirmed by measuring the relative activities of the two carboxylating enzymes - RUBISCO and PEP carboxylase (Barua, 1989). The above information warrant a closer study of the enzyme involved in the process of photosynthesis.

1.9. PRESENT INVESTIGATION

It is believed that *Camellia sinensis* L. is a C₃ plant. Based on the earlier work on C₃ plants it should be possible to reduce the rate of respiration and
enhance net photosynthesis with ultimate objective of improving yield. If one can correlate that the productivity depends on the pattern of the enzyme RUBISCO and can select those particular 'polypeptides' responsible for the product, isolate them and manipulate to other variety by genetic engineering, then higher yield will be possible from the same number of bushes i.e. to select high yielding cultivars. The efficiency of photosynthesis biomass productivity may be increased either by changing the ratio of CO₂ to O₂ at the catalytic site or by changing the kinetic properties of the enzyme. The knowledge of activity of the enzyme RUBISCO and understanding of kinetic behaviour in different cultivars may help not only in selecting plant materials with high photosynthetic ability but may also give clue to Genetic Engineers for increasing the efficiency of photosynthesis and search for a more efficient RUBISCO. As a part of modern approach to understand photosynthesis for the better utilization of CO₂ a systematic study of tea RUBISCO is essential. Therefore, the following areas of investigation have been included under the present study.

(i) Isolation and purification of RUBISCO
(ii) Standardisation of methodology for the assay of carboxylase and oxygenase activity of RUBISCO in tea.
(iii) Studies of some of the kinetic properties of RUBISCO.
(iv) Studies on the variation of RUBISCO in high, average and low yielding tea cultivars.
(v) Studies on effect of shade on RUBISCO.
(vi) Electrophoretic studies of RUBISCO in tea cultivars.
(vii) Study of isozyme pattern of subunits of RUBISCO in tea cultivars.