ABSTRACT

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Tea, derived from the tender shoots of the tea plant *Camellia sinensis* (L.) O. Kuntze, family Theaceae, is termed as the queen of beverages due to its palatable acerbity and superlative aroma. The plant probably had it's origin in South-East Asia, but it is cultivated over a wide geographical range from Georgia (45^0 N) in South Russia in the north down to South Africa (30^0 S) and from New Guinea (150^0 E) to Argentina (60^0 W) and its cultivation is expanding day by day into new areas.

Tea is by far India's most valuable plantation crop producing about 28 percent of the World's supply. India also produces a large varieties of tea ranging from high quality teas such as fragrant Darjeeling, flavoury second flush Assam and highly grown Nilgiris. In Darjeeling, tea plants are cultivated from foot hills to more than 2000 meters above median sea level. The quality of tea greatly differs on the basis of their parentage, agrotype and ecology.

Following the recommendation of various committee to increase the annual growth rate of production, resorting to higher rates of replantation and extension of planting per annum, the tea industry has embarked on a large scale planting programme. However, extension and replantation programmes will prove beneficial only if the areas are planted with planting materials superior to the existing ones.

Historically tea was cultivated in India by seed from the very beginning of the tea industry. But seed populations are highly heterogeneous, as a result of free outcrossing among themselves from which superior clones have been selected. The basic difference between clones and seed population in tea is composed of a large number of genetically distinct characters. As a result the seed populations are elastic and can be fitted to a wide range of environmental and cultural conditions without much change in its overall performance. Contrary to the seed population, thousands of bushes of a clone separated widely in space and time behave in most ways as single bush. Consequently, a clone lacks elasticity and for which a clone is more selective of environment and cultural treatments.

In India the use of clones started in fifties after the release of Tocklai clones in 1949. Since then a large number of proven elite clones were selected and released by both Tocklai, Assam and other tea estates of North-East India are available as planting materials and a large number of tea estates have already been planted in a considerable area. During the last few decades, Clonal Proving Station (Ging) in Darjeeling district has also released tea clones but they have not been studied properly. It is expected that for the development of tea industry in Darjeeling district, high standard tea clones should be selected out of them, and for which comparative study of several clones should be grown at the same ecological condition and out of the study, clones

suitable for the region may be selected for their commercial utilisation.

With this background Clonal Proving Station (Ging) Darjeeling has been selected to conduct research activity. Five genetically different tea clones such as HV-39, T-78, B-157, TV-19 and Thurboo-9 have been selected and grown at the station to maintain the source of research materials required for different lines of investigation, the observations and results of which have been represented in six chapters.

Chapter-I deals with the review of literature related to various aspects of tea. Chapter II deals with the ecological investigation on the selected tea clones. But before starting any experiment the ecological condition of the place for conducting experiment should be surveyed properly and for this reason special emphasis on the application of Abney-Level survey of the Clonal Proving Station, which has not been done earlier has been ultimately initiated. Abney-Level is a kind of clinometer that records the inclination either of rock strata or of surface. It is one of the common instrument used for recording the elevation and depression of relief and the observation of which has been represented diagrammatically. Topographically the Clonal Proving Station under consideration is situated on slopping ground with South-West to North-East-North slope trend. The South-West-South section of the Clonal Proving Station occupies a higher ground and gradually declines towards North-East-North at an average slope of 19^0 36'. HV-39 and T-78 are proved towards the South-West-South

portion having a mean slope of 29^0. Thurboo-9 on the other hand is nurtured at a relatively lower elevation and on a comparatively steep slope, $34^0 30'$ than HV-39 and T-78.

The meteorological data has been collected from its own observatory situated inside the Clonal Proving Station, and for this reason understanding of accurate ecological status of Ging Station is possible. It has been observed that during May to August, higher air temperature has been observed. The highest mean maximum temperature 23.7^0C has been recorded in the month of August. The lowest temperature 7.5^0C has been recorded in December. Due to very high temperature during May to August, a drought situation prevailed. It has been observed that high rainfall during that period has not been able to bring down the temperature. The difference in mean maximum and minimum temperature has been observed to be the highest in April and lowest in September. It reflects that the month of April in the area generally has hot days but colder nights and all these factors are expected to be related to the quality of tea. High sunshine hours (6.3 hrs. day^{-1}) has been recorded in the month of November but the mean sunshine hours has a range between 1.0 to 5.1 hours day^{-1}. During rainy season from June to September the very low sunshine hours (1.0 to 1.9 hrs. day^{-1}) has been recorded and which interfere the yield of tea as long sunshine hours are essential for maximum yield of tea. During June to August high wind velocity (5.6 to 5.8 km. hr^{-1}) prevails, while the

rest of the year an average value of 4.2 - 5.4 km. hr^{-1} has been recorded. Highest evaporation rate (4.9 mm. day^{-1}) has been observed in the month of August while the lowest value (2.0 mm. day^{-1}) has been recorded in the month of January. The high evaporation rate in the month from May to August has been expected due to accumulated moisture in the soil during rainy season while the winter months evaporation rate show the average value. High soil temperature (21.1^{0}C to 21.5^{0}C) has been recorded during June to August and low soil temperature ranging 7.9^{0}C to 9.7^{0}C has been observed during December to February. It is also expected that low soil temperature affect growth and yield of tea and triggers winter dormancy in this hill region.

Comparative expression of eight vegetative characters of five selected clones, under the same ecological condition has been studied and presented by polygraph method. Accordingly, a key has been prepared in the shape of a wheel with radiating spokes, each spoke representing a particular character. From polygraphic representation it appears that Thurboo-9 shows very high rate of pubescent character though it shows good quality of tea instead of showing very high quality. On the other hand B-157 shows low pubescent character but considering quality, it shows the category of very high type. It is known for a long time that high pubescent character is a marker of high quality. Further works are needed for its verification in Darjeeling hills. But so far as the yield is concerned, T-78 may be considered as very high yielding

type out of all the five clones taken into consideration. As regards shoot type HV-39, T-78 and B-157 are medium in nature though TV-19 shows large and Thurboo-9 is of fairly (medium) large size shoot type. As it has been observed that HV-39, T-78 and B-157 are of China hybrid types as against TV-19, an Assam - Cambod hybrid and Thurboo-9, a China - Cambod hybrid, there is a possibility of retention by the clones of parentage character. In the ecological condition of Ging Clonal Proving Station, Darjeeling, all the five clones have been observed to be more or less susceptible against blister blight (*Exobasidium vexans*), Grey blight (*Pestalotia theae*) and Red rust (*Cephaleuros parasiticus*) of tea. Though T-78, TV-19, B-157 and Thurboo-9 show comparatively mild susceptibillty, HV-39 has been observed to be very much resistant to pests like Red spider (*Oligonychus coffeae*) and also like Scarlet mite (*Brevipalpus phoenicis*), TV-19 on the other hand is observed to become mildly susceptible to Red spider but highly susceptible to Scarlet mite. B-157 found to be resistant to Scarlet mite and Helopeltis (*Helopeltis theivora*).

Chapter III deals with the pharmacognostic study of all the tea clones under consideration. Pharmacognosy is mainly concerned with the identification of plant. It includes a number of methodologies i.e. organoleptic evaluation which includes the study of macroscopic characters with the help of different sense organs, microscopic

evaluation to study microscopic characters and quantitative microscopy involving quantitative estimations of some microscopic features. The methods and results in connection with all the three types of evaluation have been represented in three different sections under this chapter. In connection with the pharmacognostic study for identification of clonal tea, some organoleptic features such as colour, shape, vein markings, texture, fracture and odour of leaf of five different clonal tea leaves have been studied. The variation in colour of pluckable apical bud of different clones have also been noted. From morphological point of view the length, breadth of first to seventh leaf with special emphasis on length:breath ratio, the length of apical buds, petiole of leaves have been worked out. No difference in floral characters excepting stamen character has been observed. Different clones have been observed to differ in the stamen number, the highest (155) being observed in TV-19 as against the lowest (127) in B-157. No marked difference in fibonacci phyllotaxy, ptyxis, leaf margin and leaf apex in different clones have been observed. But all the clones have been observed to show marked variation in axillary angle of leaf such as HV-39 (49.5^0), T-78(43^0), B-157 (52.2^0), TV-19 (25^0) and Thurboo-9 (38.5^0). This criterion may be considered as very important identifying character for the clones of tea under study. Some of the characters present in the foliage leaves of all the five selected clones appear to be genetical attribute. Variable characters have been exploited in the

criteria for preparation of a tentative key for the rapid identification of five selected clones. This is the first attempt adopted in Darjeeling hills at clonal level which may be utilised for identification of all cultivated tea clones in Darjeeling hills and in the other tea growing areas of the world.

Pollen morphology has proved a great value in the field of tea taxonomy. Pollen morphology, size and shape of five selected clones are observed not having sharp difference. Pollen load or pollen productivity has been found highest in T-78 (678) indicating high reproductive potential, can be utilised effectively in tea-breeding programme. B-157 has lowest number (127) of stamens per flower count. TV-19 indicated lowest number of flowers per bush (37). It is suggested that ultra-structural study of tea pollen in Darjeeling hills is necessary, particularly at the clonal level for understanding more detailed information.

Stomatal architecture including guard-cells of five selected clonal leaves have been investigated in details for the first time in Darjeeling hills. Wide range of variations among the clones have been observed. Highest stomatal frequency is noted in Thurboo-9 (112.2 per sq. mm.) and lowest frequency in HV-39 (86.8 per sq. mm.). Area of stomata has been occuplied the highest at the middle fractions of the leaf and lowest area has been noted in the upper fractions of the same foliage leaf. Size of the stomata including guard-cell is seen not to be constant

considering the position of the stomatal complex on the foliage leaves. Considering the result of guard-cell, highest average value (13.54) in breadth has been recorded in HV-39 and lowest value (10.33) found in Thurboo-9, whereas highest length (42.57) has been observed in HV-39 and lowest (38.51) in T-78. The average area of the guard-cell also recorded lowest value (315.4 sq. μm) in Thurboo-9, whereas highest guard-cell area value has been recorded in HV-39 (453.08 sq. μm). Stomatal frequency and sizes including guard-cell frequency and sizes may not have a direct correlation with leaf area, but they depend mostly on environmental factors, particularly observed in hill areas. However, these differences in stomatal architecture in five selected clones may be possibly due to genetical make-up or certain affinities with one or the other physiological process affected by altitude.

Lower and upper epidermal cells are seen to be variable in their length and breadth. Palisade ratio has been found to be more or less constant, vein-islet frequency also indicating less variation, but vein-endings (vein-terminations) showing significant difference in their internal morphology, hence a rapid description has been proposed for the first time to provide an additional criterion to be used for tea taxonomy in relation to leaf anatomy. Trichomes (epidermal leaf-hairs) are confined to lower epidermal cells of tea leaf showing significant variation in density (frequency) per unit area, shape and size, follicles which is the base of the trichomes where they organically connected to

the lower epidermis of leaf. These characters are also to be exploited in tea taxonomy and may be concerned for breeding programme. Highest frequency of trichome has been observed in T-78 (103.90) and lowest in TV-19 (68.03), a significant variation also observed in different fractions of leaves, no uniformity, as to occurrence of trichomes on different parts of leaf have been noted. Thickness of cuticle also has been observed to vary in five selected clonal leaves. The cuticle of HV-39 has been recorded maximum thickness (5.41 μm), whereas lowest thickness found in T-78 (3.82 μm). Internal morphology of cuticle has been observed structural uniformity in all selected clonal leaves. Petiolar and foliar idioblast density (frequency) show differential values, maximum frequency observed in petiolar region in comparison to foliar part. Length and breadth of petiolar idioblast remain more or less constant. The same constancy has also been observed in petiolar vessels. It has also been observed that presence of idioblasts is maximum to the proximity of midrib region. Histochemical test of both the petiolar and foliar idioblasts confirm the presence of calcium oxalate crystals. It is further suggested that this anatomical evaluation of idioblasts, both found in the tea leaf petiole and lamina may be utilised in taxonomic use for identification of clones in Darjeeling hills.

The frequency and distribution of foliar stomata remain variable among the five selected clones in different parts of leaf. Highest

frequency has been found in middle fraction of the leaf and lowest frequency in the lower fraction of the leaf. Average stomatal frequency has been found highest in Thurboo-9 (112.2) and lowest has been recorded in HV-39 (86.81) per unit area. The morphological variations in petiolar sclereids are also evident in five selected clonal leaves. Considering this part of anatomical findings of foliar sclereids as well as petiolar sclereids, a tentative key to the identification of five selected clones have been proposed for the first time in Darjeeling clonal leaves of tea.

The morphological variations of petiolar sclereids have been noted to a great extent of diversification in consideration with internal morphological features. The orientation of petiolar sclereids is quite distinct in comparison to foliar sclereids. The correct and acceptable terminologies have been provided and a tentative key to the identification of five selected clonal leaves of Darjeeling hills definitely render the positive taxonomic criteria for the rapid identification of tea clones cultivated in Darjeeling hills and also other associated clones found elsewhere in the tea growing countries.

In chapter IV an attempt has been made to establish a new and rapid colorimetric method for quantitative estimation of caffeine in the tender pluckable shoot of different tea clones under consideration. It has been observed that a number of methodologies available for the purpose mentioned but most of the methodologies involve the

utilisation of costly instruments which are generally not available in all the laboratories. Moreover, all these methods involved cumbrous process without the consideration of purification of caffeine.

The proposed method is based on the principle that caffeine produces characteristic colour in presence of potassium dichromate and conc. H_2SO_4. It has also been observed that most of the authors utilise caffeine dissolved in chloroform solution. As chloroform is highly volatile it is very difficult to get accurate result. Moreover, chloroform solution is immiscible with any aqueous solution of chemical. For this reason the present method is so oriented that chloroform does not interfere at the time of its estimation. Here caffeine has been extracted with chloroform which has been concentrated and run in paper chromatography with the solvent mixture of Acetone: Petroleum ether (1:10). The region of paper containing caffeine at Rf 0.00 is generally cut into pieces treated with hot chloroform which is generally filtered and evaporated to dryness to obtain pure crystals of caffeine which again is dissolved in Conc. H_2SO_4. The reaction mixture containing acid solution caffeine and potassium dichromate develop characteristic green colour having absorption maxima (λ max) at 435 nm. Standard curve of caffeine has been prepared with the help of this method. Caffeine content as per cent of dry weight in HV-39 (3.70%), T-78 (3.90%), B-157 (4.50%) TV-19 (3.90%) and Thurboo-9 (3.70%) have been worked out. The proposed method is supposed to be a new one. It may

be considered as an easy method as only spectro-colorimeter or colorimeter is required. The O.D. value has been observed to be stable for 45 minutes.

Chapter V deals with the physiological investigation on different clones of tea for understanding their transpiration rate of leaf. This chapter also includes some observations on productivity and survival capacity of five selected clones of tea grown in the same agro-climatic condition of Clonal Proving Station (Ging) Darjeeling. Transpiration is considered as necessary evil in tea plants. Maintenance leaves in tea plants are the moderators against drought tolerance. Besides, transpiration helps in translocation of nutrients and cooling of the leaves. As a result, partition of the net dry matter into different plant organs in the tea plant is facilitated and this is very important in connection with the productivity of tea. After physiological investigation transpiration rate of different clones of tea has been worked out. Maximum of 0.201 mg/sq.cm./min. in TV-19 as compared to minimum of 0.031 mg/sq.cm./min. has been observed in HV-39. It has also been noted that leaf area does not always affect transpiration rate as it is marked in HV-39, where leaf area has been determined as highest but transpiration rate is found significantly the lowest among the five selected clonal leaves.

Quantitative estimation of dry weight and moisture content of five selected clonal leaves of different fractions have shown slight

variation. In all the cases of moisture content of different clonal leaves, it is of interest to note that maximum moisture content has been found in matured leaves, excepting B-157 where in young leaves show maximum moisture content. In older leaves, mostly moisture content has been noted lowest percentage. Whereas in young leaves, indicated an intermediated percentage of moisture content. Considering dry weight (dry matter content) of different leaf position, it has been further observed that highest dry matter production has been indicated in old leaves and lowest dry matter found in young leaves in most of the cases.

Quantitative estimation of dry-matter content of the pluckable tea shoot of five selected clones manifest some variable results. T-78 shows lowest dry-matter (16.38) and highest dry-matter content has been observed in Thurboo-9 (22.44), obviously, highest percentage of moisture content has been noted in T-78 (82.63) and lowest amount recorded in Thurboo-9 (77.81). Appearance of colour after drying of the pluckable tea shoots of five clones has also been observed. With the help of this observation, tea samples can be easily identified and sorted out by observing the residual colour of the pluckable shoots after withering process prior to rolling (which is commonly practiced by Darjeeling tea factories during manufacture of orthodox tea).

Moisture content has been found to be the marginal value in matured leaves with the young tea leaves, while the same is generally

decreased in old leaves (with the ageing of tea leaves), which appears to be a new finding in tea leaves at the clonal level in Darjeeling hill conditions. Moisture content of tea leaf may be considered as one of the 'biological marker' of ageing of tea leaves. Survival percentage of different clones of tea after five years of maintenance in the same agro-climatic condition of Clonal Proving Station (Ging) has been worked out. B-157 has been observed to show maximum survival value (99.0%) and lowest value of 74.5% has been observed in HV-39. However T-78 also show high survival percentage (94.5) in comparison to TV-19 (85.5%) and Thurboo-9 (78.5%). The average yield during three consecutive years of experimentation (1996, 1997, 1998) has been observed to be maximum of 2946 kg./ha. (T-78) as against lowest yield of 1313 kg./ha. in B-157. On the basis of overall performance of yield pattern and survival value, T-78 clone may be considered the best for cultivation in Darjeeling hills. B-157 though showing high survival percentage is considered poor yielder and overall performance of HV-39, TV-19 and Thurboo-9 may also be considered noteworthy and satisfactory in Darjeeling hills.

Chapter VI deals with the biochemical investigation on different clones of tea that has been taken into consideration for this part of work. Biochemical characteristics of tea are of importance for understanding the very basis of tea quality which is related to the interactions between biochemical components of the tea leaves during

different phases of commercial processing. It is well recognised that all the chemical constituents which are responsible for producing characteristic taste and flavour of tea are generally known as secondary metabolites and these are generally derived from the primary metabolites like sugar, aminoacids etc. These metabolites again are dependent on the photosynthetic efficiency of a particular clone of tea. Besides tea leaves contain large number of enzymes which are of importance as they influence and help in building tea quality during commercial processing.

In all the selected five clonal leaves, chlorophyll-b content has been found to be greater than chlorophyll-a. Higher amount of both the chlorophylls (a and b) has been observed in old leaves and lower chlorophyll amount in young leaves. In matured leaves, relatively intermediate chlorophyll content has been observed. Chlorophyll-a and chlorophyll-b ratio has been observed high in Thurboo-9 (old leaves), whereas lowest ratio is recorded in mature leaf of the same clone. The total chlorophyll content of tea leaf has been suggested as one of the 'biological marker' considering tea-leaf senescence. The observation indicates that total chlorophyll content is steadily increased with the age of the tea leaves in all five selected clones of Darjeeling hills.

Soluble sugar content has been found to be lowest in young leaves and maximum in matured leaves manifested in all the selected clones excepting Thurboo-9.

The gradual decrease of soluble sugar content in tea leaf may be due to high conversion of sugar to secondary metabolites. In five selected clonal leaves of Darjeeling hills, the phenol content has shown some interesting result. No significant variation has been noted among the individual leaf of the five clones. It has been further observed that the maximum phenol content is found in young leaves and the phenol content drastically decreased in mature and old leaves and which appears to be due to ageing of tea-leaf.

The variations in protein content and amino acids content at the clonal level are significant. Protein content has been found to be the highest in the mature leaf and lowest in old leaf. Young leaf have comparatively intermediate protein content. Amino acid quantity is found to be highest in young leaves and lowest in mature leaves, an intermediate amount of total amino-acid has been found in old leaves. A contrasting relationship has been obtained between the protein content and amino acid content of five selected clonal leaves of Darjeeling hills. It has been observed that in each selected clonal leaves, protein content is found to be maximum in mature leaves, while amino acid content is minimum in the same leaf. This protein content and amino acid relationship has been observed predominantly in HV-39 mature leaf.

Polyphenol oxidase activity of different leaf status (young, mature and old) of five selected clones of Darjeeling hills has been

observed a wide range of variation. It has been observed that average PPO activity shows about 1.85 and 3.5 times higher in mature and old leaves respectively in comparison to young leaves where relatively low PPO activity has been observed. Enhancement of PPO activity in the mature and old leaves in comparison to young tea leaves of five selected clones have positively been correlated with natural ageing of tea leaves and may be regarded as the best 'biological marker' of natural ageing of tea leaves at the clonal level.

Peroxidase activity has been observed to be maximum in the young leaves of five selected clones and is seen to be decreased gradually in mature and old leaves. Peroxidase has been regarded as one of the key enzyme and attributes diverse biochemical functions in plants, specially the oxidation of phenolic compounds. As the mature and old leaves of tea contain much less amount of phenolic compound as compared to that of young leaf, the peroxidase activity is also much less in mature and old leaves, may be have some reasonable correlation.

Catalase activity shows more or less marginal difference in activity in different types of leaf. Maximum catalase activity has been noticed in young leaves and in the old leaves, minimum activity has been recorded. Relatively intermediate nature of activity has been observed in mature leaves. Insignificant variation in catalase activity may be regarded as the characteristics feature of this enzyme found in the five selected clonal leaves of Darjeeling hills.