MATERIALS AND METHODS

Site of study : The field experiment was carried out in Springside area of Kurseong town in the tea plantation. Laboratory analysis works were done in Depts. of Botany and Tea Management, University of North Bengal and Dept. of Ecology, Kalyani University.

Geographical description : The field is located at 26°55'N and 88°12'E and at an altitude of around 1240 m above median sea level. The topography is of rolling and folded mountains.

Edaphic character : The soil is umbric Dystrochrept, has moderate permeability and is moderately well drained. Infiltration rate is 6-10 mm hr^{-1} and run off is high. Soil texture is coarse and loamy. The top soil is 45 cm thick and subsoil is stony and bouldery. Physico-chemical properties of soil of the experimental area are presented in Tables 1 and 2.

The Crop : Study was carried out on tea plant (Camellia sinensis (L) O. Kuntze), Family Ternstroemiaceae under Order Guttiferales. There are altogether 30 clones of tea cultivated in Darjeeling area. Of these, three major clones are mostly grown in the gardens. These clones are selected for present investigation. A brief description of these clones are given as follows :

A. Bannockburn 157 (A clone developed in Bannockburn Tea Estate)

A medium sized, dark green glossy leafed China hybrid clone having similarity to domesticated Camellia. It has medium sized frame " shaving brush" type with dense plucking points and many trailing lower branches. This clone is rated to be strongly resistant against drought. A very early flusher and keeps flushing till late December. With adequate irrigation in dry weather, this clone starts flushing in mid-January.

A

B

C

Fig. 2 : Young (4-year old) plants of Bannockburn-157 (A), Phoobshering-312 (B) and Tukdah-78 (C) clone.

B. Phoobshering 312 (A clone developed in Phoobshering Tea Estate)

A China hybrid clone with leaf of medium size, semi-erect, dark green having pronounced serrations and matty foliage and wavy margin. Widespread and compact frame. It thrives well on all aspects but distinctly prefers the northern slopes and high altitudes.

C. Tukdah 78 (A clone developed in Tukdah Tea Estate)

This is very vigorous China hybrid cultivar with erect leaf of dark green colour, margin flat. This clone has good resistance to drought. A fairly good spreader with lax frame.

An overall characteristics of these three clones are given in Table 3 and Fig. 2.

The experimental designs and details in respect of mature and young plants and experiment on nutrients spray are presented in Tables 4 & 5.

Table 1 : Physical properties of soil of the experimental site

Parameters	Name of horizon & depth (cm)				
	A_P 0-13	A_C 13-31	B_1 31-97	B_2 97-114	C 114-130
1. Bulk density (g cm^{-3})	1.39	1.36	1.36	1.33	1.37
2. Water holding capacity (%)	39.23	37.56	38.21	36.32	35.01
3. Porosity (%)	40.02	38.26	39.59	37.29	36.31
4. Water retention characteristic					
i) 0.1 bar	32.71	29.74	29.96	31.24	28.22
ii) 0.3 bar	25.62	21.32	20.01	22.23	20.62
iii) 3.0 bar	15.21	14.22	12.16	14.23	13.19
iv) 15.0 bar	4.65	5.01	3.21	3.11	3.04
5. Particle size distribution (%) (<2.0 mm fractions only)					
i) Coarse sand (0.25-2 mm)	52.2	50.9	48.6	50.8	59.9
ii) Fine sand (0.25-.05 mm)	21.0	22.6	22.7	23.0	18.2
iii) Coarse silt (.05-.02 mm)	8.5	8.9	7.8	7.2	8.7
iv) Fine silt (.02-.002 mm)	5.6	7.2	6.3	5.3	6.3
v) Clay (<0.002 mm)	12.7	10.4	14.6	14.7	6.9
6. Ratio of :					
i) $\frac{\text{Fine sand}}{\text{Coarse sand}}$	0.402	0.444	0.467	0.430	0.303
ii) $\frac{\text{File silt}}{\text{Coarse silt}}$	0.658	0.808	0.807	0.693	0.724
7. Textural class	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam

Source : Saha et al. (1995)

Table 2 : Physico-chemical and chemical properties of soil of the experimental site

Parameters	Name of horizon & depth (cm)				
	A_P 0-13	A_C 13-31	B_1 31-97	B_2 97-114	C 114-130
1. pH (1:2.5)					
i) With distilled water	4.91	5.01	5.23	5.20	5.12
ii) With 1 N KCl	4.52	4.61	4.66	4.72	4.65
2. Organic carbon (%)	1.21	1.13	1.03	1.01	0.91
3. Organic matter (%)	2.09	1.96	1.78	1.75	1.58
4. Total nitrogen (%)	0.110	0.092	0.091	0.091	0.082
5. Available phosphate (%)	0.021	0.024	0.027	0.023	0.020
6. C : N	11.00	12.28	11.32	11.09	11.09
7. Cation Exchange Capacity (CEC) (Cmol kg^{-1})	13.62	13.15	10.01	9.23	8.32
8. Exchangeable cations (mol kg^{-1})					
Ca^{++}	1.65	1.16	1.05	1.09	1.32
Mg^{++}	0.30	0.41	0.48	0.21	0.11
K^{++}	0.156	0.184	0.181	0.207	0.204
9. Free Iron Oxide (%)	7.26	7.64	7.38	7.92	6.01
10. Mobile Iron (%)	0.72	0.75	0.75	0.73	0.76
11. Mobile Aluminium (%)	0.60	0.58	0.68	0.37	0.22

Table 3 : Characteristic of Tea clones and for experimental varieties of Tea crops

Characteristic	B157	P312	T78
1. Mean area of an individual leaf (cm^2)	22.5	20.0	18.9
2. Length of the Lamina (cm)	7.2	7.7	7.2
3. Breadth of the Lamina (cm)	3.1	2.6	2.5
4. Length/Breadth Ratio	2.3	3.0	2.9
5. Mean internode length below plucking surface (cm)	2.2	2.8	2.0
6. Mean leaf angle from vertical (degrees)	28.9	40.0	35.3
7. Fresh weight of individual leaf (g)	0.73	0.65	0.57
8. Oven dry weight of individual leaf (g)	0.28	0.25	0.23

Table 4 : Experimental designs and details of mature and young plants (Randomised Block Design)

	Mature Plants	Young Plants
Year of planting	1985	1990
Spacing	90 cm x 60 cm x 60 cm	Same as mature plants
Planting pattern	Regular double hedge	-do-
Replication	5 (five) - Each plant one replication	-do-
Number of plants	Fifteen - 5 each of each variety (B157, P312, T78)	-do-
PARAMETERS STUDIED :		
Physiological parameters	Net photosynthesis, Stomatal conductance, Stomatal resistance, Transpiration, Leaf water potential, Leaf temperature, Growth rate analysis	-do-
Biochemical parameters	Total free amino acid, Free proline, Ascorbic acid, Total chlorophyll and Epicuticular wax	-do-
Meteorological parameters	Relative humidity Air temperature, Photosynthetic photon flux density, maximum/ minimum & Soil temperature, wind velocity, sunshine hour and rainfall.	-do-

Table 5 : Design and details of the nutritional experiment (Randomised Block Design)

Year of planting	:	1992
Age of the plants at the time of planting	:	1½ years
Replication	:	3 (three)
Treatment	:	Spray of molybdenum, potassium, phosphorus, sulphur and zinc spray @ 2% in April/May and September/October at fortnightly interval (total 4 sprays) with 1 litre capacity ASPEE hand sprayer.
Varieties	:	Bannockburn 157, Phoobshering 312 and Tukdah 78
No. of pots	:	Total 54 pots
Size of pot	:	Circumference of upper rim - 2 m Diameter (inner) upper open face- 32 cm Height from ground level - 30 cm Weight of empty pot - 3 Kg Weight of soil per pot - 10 Kg
Parameters studied	:	Net photosynthesis, Stomatal conductance, transpiration, girth & height.
Period of recording	:	Pre-monsoon, monsoon, Post-monsoon and winter. Girth and height once a year.

Fig. 3 : A portable photosynthesis system (LI6200, LICOR Inc., USA) used for recording net photosynthesis, stomatal conductance and resistance, transpiration, leaf temperature, etc.

Measurement of Physiological Parameters

A portable photosynthesis system (LI 6200 - LICOR Inc., U.S.A.) was used to measure the rate of net photosynthesis, stomatal conductance, stomatal resistance and leaf temperature (Fig. 3).

When a plant photosynthesizes, it takes up CO_2. As it respires, it gives off CO_2. The net exchange of CO_2 between a leaf and the atmosphere is measured with the LI-6200 by enclosing a leaf in a closed chamber and monitoring the rate at which the CO_2 concentration in the air changes over a fairly short time interval (typically 10-20 seconds). The net photosynthesis is then calculated using this rate of change the amount of leaves area enclosed, the volume of the enclosure, temperature and pressure. When a leaf is enclosed in the chamber, the humidity within the chamber from the change in humidity with time and rate of flow of dry air. Transpiration rate is then used with the leaf and air temperatures to calculate stomatal conductance. Photosynthetic photon flux density leaf temperature, air temperature, relative humidity are measured with the help of quantum sensor, leaf temperature thermocouple, air temperature sensor and humidity sensor.

At the time of recording the instrument was calibrated and programmed. In situ reading of the physiological parameters were taken in the field without destruction of leaves. First, second and third leaves (recently matured leaves) were taken for observation. Recording were done during morning hours of every alternate month. Photosynthetically active period was ascertained by taking repeated readings. The leaf chamber was connected to the console. A leaf still connected to the plant, inserted in the leaf chamber and closed the chamber. The relative humidity was made constant adjusting the flow meter. The leaf was taken out and again inserted after allowing enough air to enter the leaf chamber. The log button was then pressed to record the data. The data from the measurement were automatically logged into the scratch pad in the LI-6200's memory. After the observation was complete, the instrument computed mean and range for each data. The data

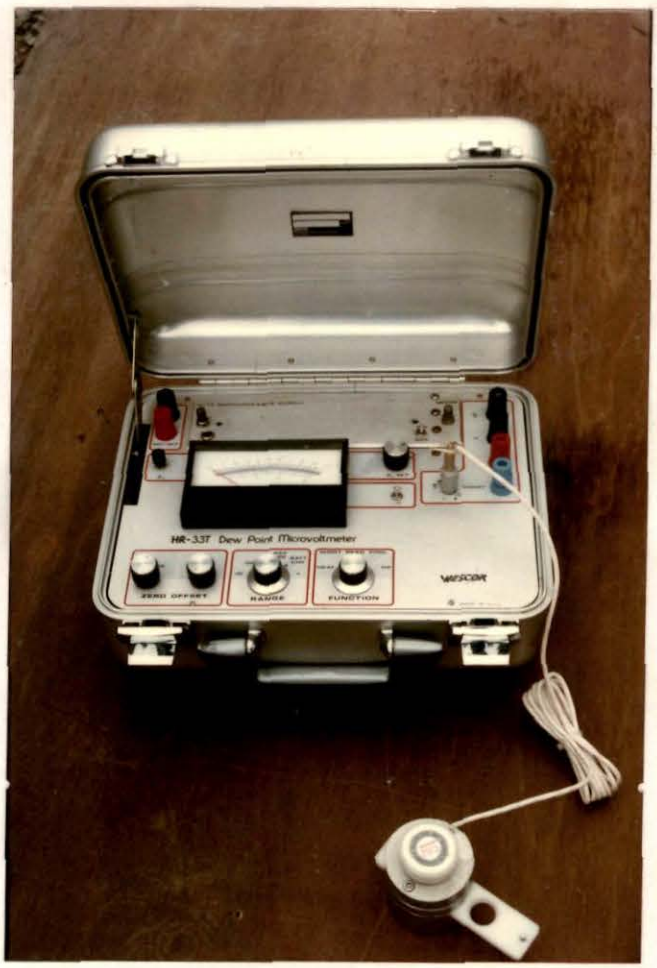

Fig. 4 : A dew point microvoltmeter (HR 33T, WESCOR Inc., USA) used for measurement of leaf water potential.

were stored and printed in the Laboratory using a printer (L800 EPSON, JAPAN). the first, second and third leaves from each plant were used separately for physiological study.

Measurement of Leaf Water Potential

A dew point microvolt meter (HR33T, WESCOR Inc.) (Fig. 4) was used for the measurement of leaf water potential. Water moves from regions of high water potential to regions of low water potential. The greater the differences in the potential of two, the greater will be the energy exchange in the transfer of water. Thermocouple hygrometer provide a means of measurement of the total water potential. The measurement is based on the energy and is an actual measurement of the water potential. The technique of measuring water potential by determining the dew point depression temperature. A thermocouple is cooled below the dew point by means of the Petlier effect (Spanner, 1951), thereby collecting microdroplets of condensed water upon the junction surface. The thermocouple temperatures converges to the dew point, where it remains with a static amount of water. The e.m.f. produced by the temperature difference between the junction at the dew point temperature and the ambient temperature is a linear function of the water potential. The proportionately constant is approximately -0.75 Volts bar^{-1}.

At the time of recording the sample chamber is connected to the console and calibrated. Leaf disc punched from very young flush and at once inserted in the sample chamber. The leaf disc come into contact with the thermocouple. Sufficient time is allowed thereafter for thermal and vapour equilibration. The samples were kept for 20-30 minutes. After that the sample was cooled for 15-30 seconds and put to dew point. The reading was taken in microvolt unit which was converted in bar unit dividing by -0.75.

Methods of Biochemical Estimations

Each biochemical parameter was studied during pre-monsoon, monsoon and post-monsoon - three times in each season replicated five times, each genotype. Total 135 samples in a year were analysed for each biochemical parameter.

Estimation of Total Free Amino Acid (Moore and Stein, 1948)

Third leaves of the maintenance foliage were plucked and carried to the laboratory. 1g of the plant sample was weighed with the help of a top loading electronic balance and grinded in a pestle and mortar with 10 ml of 80% ethanol. The extract was filtered with the help of Whatman No. 1 filter paper. The filtrate or supernatant was saved. The extraction was repeated twice with the residue and all the supernatant were pooled. The volume was made up to 25 ml adding 80% ethanol.

1 ml of Ninhydrin solution was added to 1 ml of the extract in test tube. The mixture was boiled in a boiling waterbath for 20 minutes. 5 ml of the dilute solvent was added and the contents were mixed and kept for 15 minutes. Then the intensity of the purple colour was read against a reagent blank in spectrophotometer at 570 nm. The reagent blank was prepared by taking 1 ml of 80% ethanol.

50 mg of glycine was dissolved in 50 ml of distilled water in a volumetric flask.10 ml of this stock standard was taken and diluted to 10 ml in another volumetric flask for working standard solution. A series of volume 0.1 to 1 ml of this standard solution (concentration range 10 mg to 100 mg) was prepared and proceeded as that of the sample and spectrophotometric readings were taken.

A standard curve was drawn using absorbance versus concentration. The concentration of the total free amino acids in the sample was determined with the help of the standard curve.

Estimation of Proline (Bates et al., 1973)

Third leaves of the maintenance foliage were plucked and carried to the laboratory. 1g of the plant sample was weighed with the help of a top loading electronic balance and grinded in a pestle and mortar with 10 ml of 3% Aqueous sulphsalicyclic acid. The extract was filtered with the help of Whatman No. 2 filter paper. The filtrate or supernatant was saved. The extraction was repeatedly pooled. The volume was made up to 25 ml adding 3% sulphsalyclic acid.

2 ml of filtrate was taken in a test tube. 2 ml of glacial acetic acid and 2 ml of acid ninhydrin was added. This set was boiled in boiling waterbath for 1 hour. The reaction was terminated by placing the tube in icebath. 4 ml of toluene was added and stained well for 20-30 seconds. The toluene layer was separated with the help of Pasteur pipette and warmed to room temperature. The red colour intensity was measured at 520 nm in an U/V spectrophotometer against a reagent blank.

50 mg of proline was dissolved in 50 ml of 3% sulphosalicyclic acid in a volumetric flask. 10 ml of this stock standard was taken and diluted to 10 ml in another volumetric flask for working standard solution. A series of volume 0.1 to 1 ml of this standard solution (concentration range 10 mg to 100 mg) was prepared and proceeded at that of the sample and spectrophotometric readings were taken.

A standard curve was drawn using absorbance versus concentration. The concentration of free proline in the sample was determined with the help of the standard curve.

Estimation of Ascorbic Acid (Harris and Ray, 1935)

Third leaves of the maintenance foliage were plucked during morning hours, labelled and carried to the laboratory. 2g leaves were weighed with the help of a top loading electronic balance and crushed in a clean mortar pestle 20 ml of 5% metaphosphoric acid

solution. The extract was filtrated using Whatman Filter paper No. 1. The extract was repeated twice with the residue. The volume of the supernatant was made up to 100 ml adding 5% metaphosphoric acid solution. The ascorbic acid content was titrated using 10 ml of dye solution (dichlorophenol indophenol).

Estimation of Chlorophyll (Arnon, 1949)

Leaves of the maintenance canopy were plucked during morning hours and carried to the laboratory 0.5g leaf samples were weighed with the help of a top loading electronic balance.

The samples were ground to a fine pulp in a mortar pestle with 10 ml of 80% Acetone solution. The extracts were filtered through Whatman No. 1 filter paper. The supernatant was saved. The residue was again ground and filtered. The procedure was repeated till the residue was colourless. The volume was made up to 10 ml with 80% acetone. The absorbance of the solution was read at 645 nm and 663 nm against the solvent (80% acetone) blank.

$$\text{Mg chlorophyll/g tissue} = \frac{(20.2 \times \text{Absorbance at 645 nm} + 8.02 \times \text{Absorbance at 645 nm}) \times \text{Final volume of chlorophyll extract in 80\% acetone}}{1000 \times \text{Fresh weight of tissue extracted}}$$

Estimation of Epicuticular Wax Content (Silva Fernandes et al., 1961)

Second leaves of the maintenance canopy were plucked in the morning hours and carried to the laboratory. Ten leaf blades were immersed, one at a time, each for 15 seconds in 100 ml redistilled chloroform. The extract was filtered and evaporated. The residue was weighed. The amount of wax was calculated against leaf area and against the fresh weight.

Ten leaves of each plant were measured with the help of a

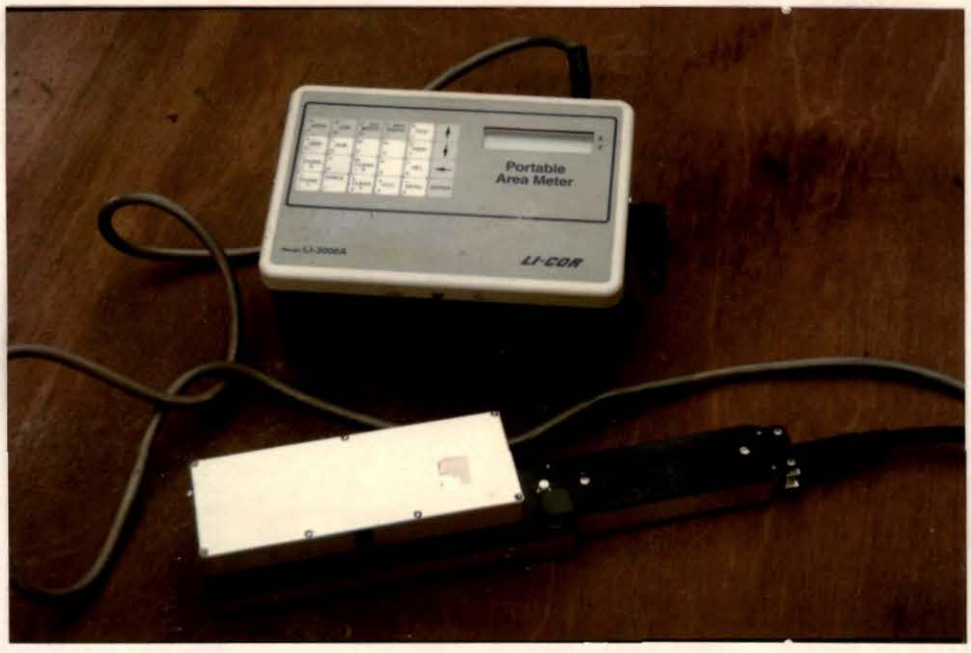

Fig. 5 : A portable area meter (LI 3000A, LICOR Inc., USA) used for measurement of leaf area, length and width.

Measurement of Environmental Parameters

Minimum, maximum, wet bulb and dry bulb thermometers (National Instruments, Calcutta) housed in a Stevenson screen, sunshine recorder (National Instruments, Calcutta), cup counter anemometer (Electromech Equipments, Pune), soil thermometer (A. Paul Instrument Co. Ltd., Haryana), and rain gauge (Ramkala, Pune) were used. All the instruments were tested and approved by Indian Meteorological Department, Pune. Following weather parameters were recorded - maximum temperature, minimum temperature, relative humidity, rainfall, sunshine, hour, soil temperature, wind velocity etc.

Soil moisture was estimated by oven dry method every alternate months. Soil was derived at a depth of 0-15 (top) and 15-30 cm (subsoil) dried in the oven at 110°C for 48 hours. Maximum, minimum, dry bulb, wet bulb and soil temperatures, wind run were recorded every day at 6.39 and 13.39 hours while rainfall was recorded at 8.30 and 17.00 hours everyday. Fresh sunshine cards inserted everyday after sunset replacing the used card. Using a portable photosynthesis system (LI 6200, inside leaf chamber of LI 6200, LICOR Inc., U.S.A.) the carbon dioxide concentration and photosynthetic photon flux density were measured when physiological readings were taken.

Statistical Computations

Critical Difference (C.D.) or Least Significant Difference (L.S.D.), Co-efficient of Variance (C.V.) and Correlation Coefficient were calculated using the method referred by Panse and Sukhatme (1989) while in case of regression, the method referred by Gupta and Kapoor (1986) was used.