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Influence of Changes Weather Conditions on Physiological and Biochemical Characteristics of Darjeeling Tea (*Camellia sinensis* L.)

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Abstract
Photosynthesis is one of the most important processes on earth. Life as we know it would not have existed without the process, the sun is the ultimate source of energy on earth and green plants through the process of photosynthesis are able to convert solar energy in to photochemical energy which is stored in the form of carbohydrate, providing food for all other heterotrophic organisms including human. The experiment was carried out at Sungma Tea Estate, Darjeeling during 2013-14 to study the effect of climatic conditions on physiological and biochemical parameters of Darjeeling tea cultivation. We had determined net photosynthesis (Pn), stomatal conductance (g_s), transpiration (*E*), water use efficiency (WUE) and leaf water potential (ψ_L) in tea during the sunny, cloudy weather, under shade tree, rainy and foggy weather. The data correlated with environmental parameters such as air temperature, rainfall, Relative humidity, photosynthetically active radiation (PAR) and vapour pressure deficit. The maximum photosynthesis (12.54 µ mol $m^{-2} s^{-1}$) was recorded in cloudy when humidity was high, air temperature; soil moisture and photosynthetically active radiation (PAR) were moderate. In rainy day, higher temperature prevailed but Pn was minimum (3.60 µ mol $m^{-2}s^{-1}$). Foggy weather leaves received much less PAR than the requirement, hence recorded the lowest rates of Pn (6.65 µ mol $m^{-2} s^{-1}$). Water use efficiency (0.59 µ mol/m mol^{-1}) was lowest in rainy weather, which increased with shade, showing flexibility in adaptation to different light environment. Higher values of g_s (0.61 mol $m^{-2}s^{-1}$) were recorded during rainy weather and lowest value observed in sunny (0.15 mol $m^{-2}s^{-1}$) weather. Higher value of *E* was recorded during rainy and lower in foggy weather. Maximum leaf water potential was recorded in rainy and foggy weather as compared to sunny. The chlorophyll content (Chl) was found highest in rainy followed by shade and foggy weather while it was lowest in sunny days. Tea shows considerable flexibility in its adaptation to different light environments as shown with physiological and biochemical changes.

***Key words**: Chlorophyll, Environmental conditions, photosynthetic activity, tea and water use efficiency.*

1. Introduction
A popular beverage made from the leaves of evergreen shrub or tree *Camellia sinensis*, family *Theaceae*. Under natural conditions, a tea plant grows to a small tree but it is configured into a bush by sequential pruning and other silvicultural practices, viz. tipping, plucking and by harvesting the optimum vegetative produce. Cultivated tea plants are usually pruned to a height of around 1- 1.5 m to make crop maintenance and harvesting (Plucking) easier and to increase yields. However, in their natural state, tea plants will grow to small trees. The Assam variety can grow into a loosely branched tree about 15 m tall. It is a lowland plant, and requires high rainfall and good drainage. The Chinese variety grows to a much smaller size, reaching a height of 3–5 m. Tea is a plantation crop having a wide distribution extending from low elevations to levels exceeding 2000 m (Wijeratne et al., 2007). Darjeeling tea plants experiences various types of climatic conditions in Darjeeling hills such as low temperature, low soil moisture in winter foggy climate, high humidity and low levels of solar radiation. All physiological processes including photosynthesis need suitable temperature. Under non-limiting conditions of light and carbon dioxide, photosynthesis increases with the rise of temperature till it reaches maximum and thereafter declines. The effect of temperature on photosynthesis for different areas can not be generalized as the optimum range would possibly vary depending on the overall environmental conditions of the area. Along with optimum soil conditions, well distributed rainfall and RH, a mean ambient temperature of 20 – 23 ^{0}C has been identified as conducive for growth of Darjeeling tea. Tea is a rain fed plantation crop and tea yield is greatly influenced by weather. Tea is a shade loving plant which had originated in the forest under story. Tea shows comparatively lower photosynthetic rates than many other crops (De Costa et al., 2007). However, many recent reports on beneficial effects on the physiological, Biochemical aspects and yield suggest the requirement of shade in tea plantations. Barua and Gogoi (1979) found that the removal of shade caused a 50 % drop of tea yield when compared to that of shaded tea plants in North East India. Barua (1969) reported that the yield of tea shoots under 35% light intensity was higher than that in plants grown with full sun. Mohoti and Lawlor (2002) and Karunaratne et al. (2003) reported that the net photosynthetic rate was significantly lower ($p<0.05$) in unshaded tea compared to that of shaded tea in higher elevations of Sri Lanka. The present investigation on how different conditions of climate influence on physiological and biochemical changes of Darjeeling tea cultivation.

2. Materials & Methods
Experimental site and plant material
The study was carried out in section No. 18 at Sungma Tea Estate, Darjeeling (Lat 26^{0}52'94 SN, Long 88^{0}15'19 SE, altitude 1510 a.m.s.l) during the year 2013-14. The last five year mean annual rainfall, maximum and minimum

temperatures in the region are 2450 mm, 22.2 ^{0}C and 13.5 ^{0}C respectively. The topsoil is about 45 cm in depth and the sub soil is stony. The soil is an Umbric Dystrochrept, moderately permeable and moderately well drained. The soil texture is sandy loam.

The quality tea clone Banackborn-157 (B -157) representing 'china' type planted in 1999 was selected for the experiment. These were planted in double hedge planting method with a spacing of 90 X 60 X 60 cm on moderate slopes in the trial. After planting, recommended cultural operations, including organic fertilizers application were carried out from time to time to bring the young tea to bearing. The experimental plot composed of 150 plants each treatment and replicated four times. The plants were not irrigated as this is the general practice in this region.

Measurement techniques

During 2013, Net photosynthesis (Pn) Stomatal conductance (g_s), Transpiration rate (*E*) water use efficiency (WUE), and Vapour pressure deficit (VPD) were monitored three times in a month (beginning, middle and end) using a portable photosynthetic system (Li 6200, Li -cor, Nebraska, USA) with a well mixed 390 cm^3 chamber as described (Li-Cor Inc., 1987). This portable instrument has internal programmes to calculate physiological quantities from measurements of air and leaf temperatures, humidity and CO_2 concentrations. Assimilation rates are computed in this instrument by assuming linear rates of change in water vapour and CO_2 concentrations within the leaf chamber. All measurements were made between 10 00 and 12 00 hours when the maximum values of P_N and other physiological parameters were recorded in the diurnal study (Ghosh Hajra and Kumar, 2002). In order to generate data on climatic weather influence on gas exchange property, Pn was monitored during January (Foggy weather), April (Sunny weather and under shade tree), July (Rainy weather) and October (Cloudy weather). The intercellular CO_2 concentration (Ci) was computed in the Li-6200 using initial values of Pn, *E*, ambient CO_2 concentration, and leaf resistance. The water use efficiency (WUE) was calculated as the ratio of CO_2 assimilated to water transpired. Dark-green healthy looking mature leaves at the surface of the canopy and fully exposed to incident sunlight were used for the observations.

Four plants randomly selected from each replicated plot were assessed on every recording. Photosynthetically active radiation (PAR) and VPD were measured concurrently using the photosynthesis system. Leaves were not brought into horizontal position during the measurement to avoid sudden change in incident quantum flux.

Leaf water potential (ψ_L) was measured simultaneously with P_N using a dew point hygrometer (model C-52 sample chamber connected to an HR 33T microvoltmeter, Wescor Inc., Logan, USA) as described by Wescor Inc. (1988). Small circular leaf discs from the leaves on the opposite branches to those for P_N measurement were used and ψ_L values were expressed as megapascals (-Mpa).

Chlorophyll (Chl) content of freshly harvested maintenance foliage leaves collected from the opposite branches to those for P_N measurement was estimated according to the method described by Arnon (1949), after extraction with 80% acetone in the dark and using the Hitachi (U 2000) double beam spectrophotometer. Leaf samples were collected during different climatic conditions to understand the influence of environmental conditions on pigment. Mean measurement values with standard error are presented for precision.

3. Result and Discussion

In 2013-14, the highest values of photosynthetic rate were recorded in cloudy, under shade tree, sunny, foggy and rainy weather respectively (Table 2). The maximum photosynthesis (12.54 µ mol m^{-2} s^{-1}) was recorded in cloudy when humidity was high, air temperature; soil moisture and photosynthetically active radiation (PAR) were moderate. In rainy day, higher temperature prevailed but Pn was minimum (3.60 µ mol m^{-2}s^{-1}). During sunny, Pn recorded at lowest rate than cloudy and under shade tree. When PAR in sunny weather increasing from lower intensities to saturation level (1450 µ mol m^{-2}s^{-1}) than the lower rate of Pn recorded in sunny when atmosphere was dry and plants were suffering from moisture stress. There is abundant evidence in the literature that Pn is inhibited by water stress (Balasimha et al., 1991; Sobrado, 1996). Vandana and Bhatt (1996) also reported that the sharp reduction in Pn in the sunny months in Sesbania was caused by high temperature and low R.H. In rainy, humidity (90%) and soil moisture (32.06 and 30.42 % in top and subsoil respectively) were highest (figure 2) and atmospheric temperature 24 – 25 ^{0}C) was high but Pn was at its lowest among the weather conditions. The limiting factor for Pn was probably the low sunshine hours (0.5 - 1.5 h day^{-1}) in this reason. PAR recording rainy condition was less than 100 µ mol m^{-2}s^{-1} which in turn also affected the Pn. Similarly during foggy months, particularly in forenoon hours, low temperature coupled with low PAR limited the tea leaf Pn while increase in air temperature and medium irradiance enhanced the Pn. Foggy weather leaves received much less PAR than the requirement, hence recorded the lowest rates of Pn (6.65 µ mol m^{-2} s^{-1}) (Table 2). Lower value of gs (0.15 mol m^{-2}s^{-1}) recorded in sunny weather (0.15 mol m^{-2}s^{-1}) and under shade tree (0.21 mol m^{-2}s^{-1}) (Table 2). The highest value of gs (0.61 mol m^{-2}s^{-1}) was recorded in rainy weather. The high light intensity received by leaves in sunny weather condition increase its leaf temperature significantly, which may ultimately reduce the stomatal conductance through stomatal closure. The rate of photosynthesis of tea leaves is known to decline at leaf temperatures exceeding 30 ^{0}C (Hadfield), 1975). The gs at different weather conditions more or less followed the patterns of changes in transpiration rate. Transpiration rate (E) in general increased with increase in leaf temperature and PAR in sunny (April summer month) and cloudy weather (October autumn month) as it was observed in Sesbania (Vandana and Bhatt, 1996). The decrease in gs and E was more pronounced in sunny month when both atmospheric and soil moisture were low (18 and 22% in top and subsoil respectively) and demand for water was high. In rainy weather, higher value of E (6.05 µ mol m^{-2}s^{-1}) was recorded through PAR reached minimum but the soil moisture (figure 2), air temperature and relative humidity were reasonably high (90- 92%). VPD was highest in sunny and Cloudy weather conditions (Table 2). Water use efficiency (0.59 µ mol/m mol^{-1}) was lowest in rainy weather, which increased with shade, showing flexibility in adaptation to different light environment (figure 3). WUE decreased during rainy than other weather conditions. It was highest in under shade tree due to a decrease in gs. The highest value of leaf water potential (ψ_L) (-1.12 MPa) was recorded in rainy and the lowest value (-3.05 MPa) in sunny weather (Table 2). Tea has highly sensitive stomata, which show partial closure during midday even when the plants were growing on a wet soil (Williams, 1971; Carr, 1977). Stomatal closure was slightly preceded by reduced shoot xylem leaf water potential (ψ_L), indicating that stomatal closure occurred as a response to an internal water deficit in the shoot. This indicates that the rate of root water absorption and its subsequent transfer through the xylem is not very efficient in tea even under conditions of moderate atmospheric demand (i.e. > 5mm d^{-1}). This could be due to specific characteristics in the absorbing region of the root system and or the xylem vessels. Stomatal movement is one of several important physiological processes, which respond to VPD. In fact, Carr (1977) showed that both gs and ψ_L were negatively correlated with VPD, ambient temperature (Ta) and incident PAR. Interestingly, it was found that ψ_L of tea was more sensitive to Ta and VPD when the soil was wet than when it was dry. The observation that ψ_L and gs of tea is less sensitive to Ta and VPD in a dry soil provides indirect evidence that stomatal opening of tea may be controlled by hormonal signals originating from roots (Davies and Zhang, 1991). In general, a linear relationship between ψ_L and VPD of the atmosphere was observed. Pn, in general, decreased with lower ψ_L. VPD was higher in dry month (February to April) than in wet months (June to August). The maximum value of Pn was recorded up to a VPD of 1.70 kPa; thereafter Pn declined slowly.

The rate of photosynthesis was not significantly between sunny weather and under shade tree treatment, but significant different ($P<0.05$) between different weather conditions (Table 2). The under shade tree leaves showed the highest rate of photosynthesis, between 600 - 800 µ mol $m^{-2}s^{-1}$ PAR. The sunny weather leaves, which received more than 1400 µ mol $m^{-2}s^{-1}$ PAR showed a photoinhibition and the rate of photosynthesis was lower. One of the benefits of under shade trees in a tea plantation was the reduction of the temperatures of the tea plant, thereby reducing excessive transpiration and moisture losses resulting in wilting. By reducing temperature and the evaporative effects of wind, shade tree benefits the plants water economy as long as the right balance is achieved between the protection given to the tea and drain that shade makes on the resources of soil moisture. Shade trees also reduce damage caused by hail and sun scorch, and reduce the soil temperature, thus helping the soil conserve both moisture and soil organic matter. The leaf fall from shade tree also provides valuable mulch to tea plantation. Darjeeling tea leaves in foggy and rainy weathers did not show saturated rates of photosynthesis. The benefits of under shade tree are also shown with the leaf temperature measurement (Table 2), which were statistically significant between the under shade condition. The sunny plants leaves had significantly higher leaf temperature, followed by under shade tree plants leaves. The stomatal conductance was significantly different between the different weather conditions. They were highest with the under shade and lowest with the sunny weather. Net photosynthetic rate mostly depended on PPFD. Pn significantly increase (<0.05) with the increase of PPFD till it reached the light saturation at 1000µ mol $m^{-2}s^{-1}$ (figure 3A). Photosynthetically active radiation (PAR) had a positive correlation (r^2=0.4156) with water use efficiency and VPD (r^2= 0.8432) (figure 3B & 3C). PAR had a positive correlation (r^2= 0.9503) with leaf temperature (figure 3D). With increase in PAR from 50 – 1500 µ mol $m^{-2}s^{-1}$, there was a linear increase of leaf temperature. At the lowest PAR, the leaf temperature was 21.07 ^{0}C against 28.91 ^{0}C in highest PAR of 1450 µ mol $m^{-2}s^{-1}$ (figure 3D). Quadratic correlation coefficient curve (figure 3E) show that the Pn values increased till the transpiration value reached 4.96 m mol $m^{-2}s^{-1}$. The curvilinear relationship between transpiration and photosynthesis was significant (r^2= 0.1819). High leaf temperature (Lt) induced higher transpiration loss in tea leaf. Curvilinear relationship between leaf temperature and Pn was also significant (r^2= 0.5053) and showed that Lt at 25.49 ^{0}C became the turning point for Pn from which it started declining (figure 3F).

Table 1. Meteorological observations recorded at Sungma Tea Estate, Darjeeling (Lat 26^{0}52'94 SN, Long 88^{0}15'19 S E, altitude 1510 a.m.s.l.) during 2013-14

Months	Air Temperature		Rainfall	Relative Humidity
	Max (^{0}C)	Min (^{0}C)	mm	%
January	16.20 (16)	4.00 (9)	7.50	82 (70)
February	21.00 (22)	6.50 (12)	39.00	86 (68)
March	23.00(20)	10.00 (15)	22.00	86 (68)
April	24.30 (21)	12.10 (16)	67.50	87 (75)
May	24.70 (20)	12.21(15)	81.50	86 (75)
June	25.00 (22)	20.81(20)	281.50	88 (86)
July	24.00 (22)	18.00 (21)	483.75	90 (88)
August	26.00 (22)	18.00 (20)	127.50	88 (86)
September	25.10 (22)	17.81(18)	399.00	88 (86)
October	22.00 (21)	14.50 (15)	17.00	88 (86)
November	21.50 (18)	8.80 (13)	0.00	84 (76)
December	15.00 (14)	8.00 (9)	0.00	86 (73)

Average value ( )

Chlorophyll a, chlorophyll b and total chlorophyll content showed a significant difference in different climatic conditions (figure 1). However, there was a tendency for pigments to be high in under shade leave than in other leaves. Chlorophyll a content was always higher in leaves of all different climatic conditions. Total Chl content was found highest in rainy followed under shade tree, foggy, cloudy and sunny weather (figure 1). Under shade leaves are known to contain more chlorophyll per unit mass than sunny leaves (Pearcy, 1998). This occurs due to increased allocation of resources to light harvesting functions as compared to those involved in electron transport and CO2 fixation capacity.

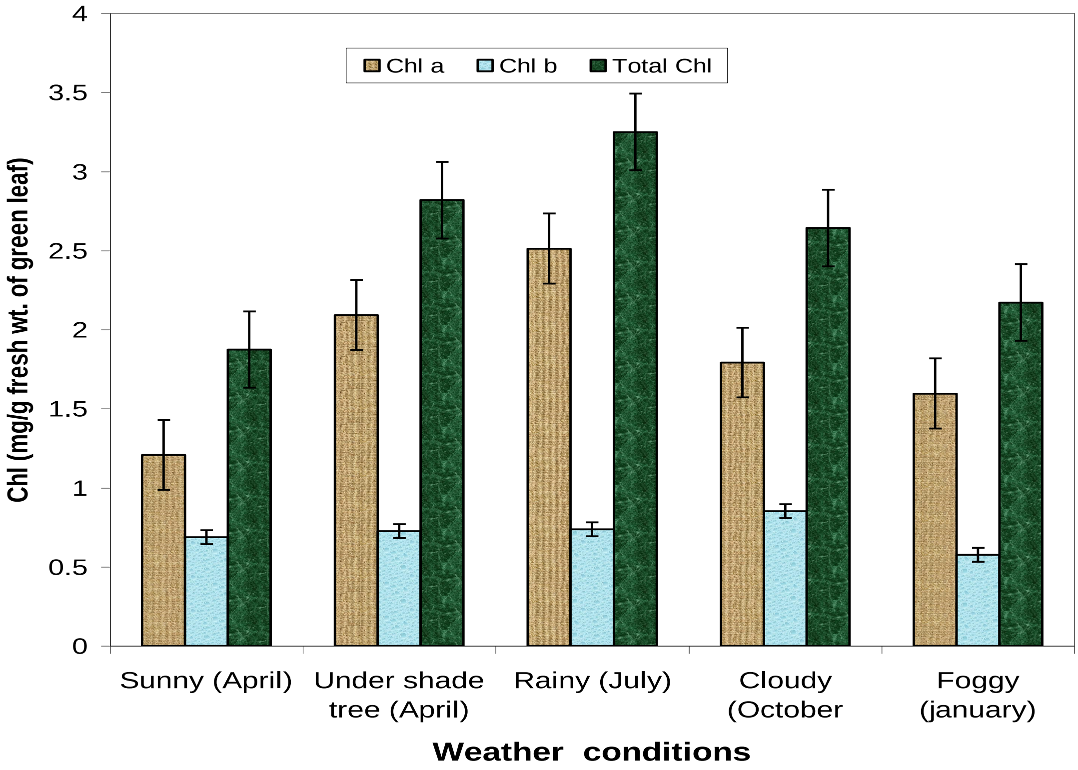

Figure 1. Changes of Chlorophyll content (Chl) in Darjeeling tea under different weather conditions. Vertical bar indicate standard error of mean

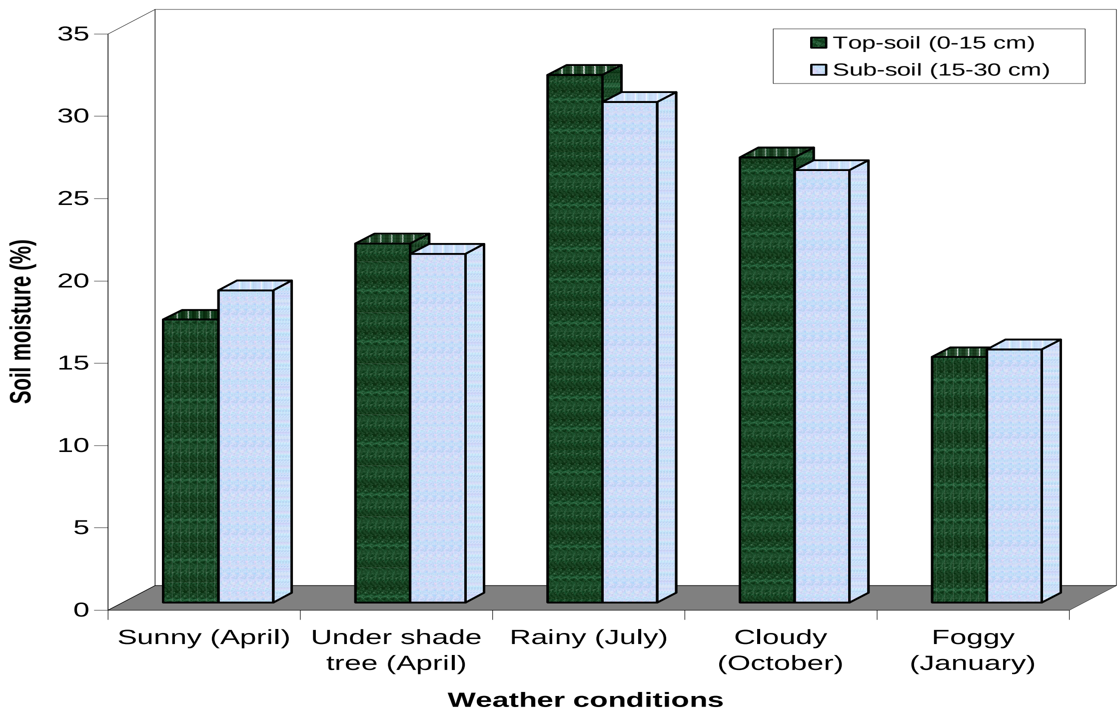

Figure 2. Volumetric water content (Sm) in plots at the time of recording of phsiological observations at Sungma Tea Estate, Darjeeling. Data in the parenthesis indicate the soil depth.

Table 2. Effect of different weather conditions on physiological parameters in Darjeeling tea.

Parameters	Weather Conditions					se	CD at 5%
	Sunny	Cloudy	Under shade tree	Foggy	Rainy		
PAR (μ mol $m^{-2}s^{-1}$)	1450.25	956.58	702.83	173.85	52.94	14.62	45.06
Pn (μ mol $m^{-2}s^{-1}$)	11.17	12.54	11.48	6.65	3.60	0.33	1.01
E (m mol $m^{-2}s^{-1}$)	4.23	4.96	3.40	3.37	6.05	0.11	0.34
gs (mol $m^{-2}s^{-1}$)	0.15	0.30	0.21	0.27	0.61	0.02	0.06
WUE (μ mol/ $mmol^{-1}$)	2.58	2.53	3.38	1.98	0.59	0.08	0.23
VPD (kPa)	2.82	1.68	1.61	1.45	0.67	0.04	0.12
Lt. (^{0}C)	28.91	25.49	23.01	21.60	21.07	0.21	0.65
ψ_L (Mpa)	-3.05	-2.54	-1.96	-1.54	-1.12	0.05	0.17

Note: Photosynthetically active radiation (PAR), Net photosynthetic rate (Pn), Transpiration rate (*E*), Stomatal conductance (gs), Water use efficiency (WUE), Vapour pressure deficit (VPD), Leaf temperature (Lt) and Leaf water potential (ψ_L).

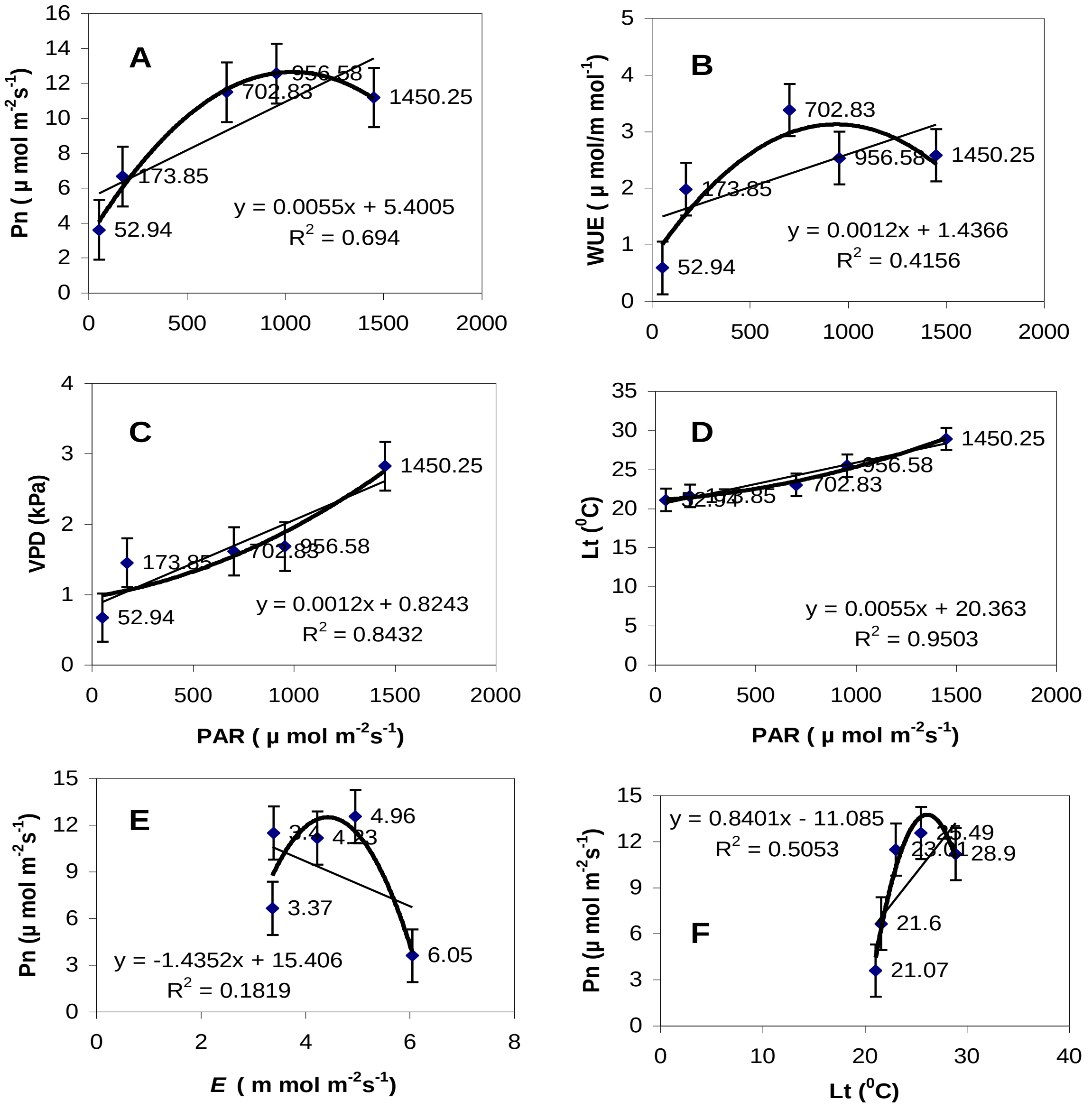

Figure 3 Relationship between (**A**) photosynthetically active radiation (PAR) and net photosynthetic rate (Pn); (**B**) water use efficiency (WUE) and PAR; (**C**) vapour pressure deficit (VPD) and PAR; (**D**) PAR and Lt; (**E**) Pn and Transpiration (*E*), (**F**) Pn and Lt under diferent weather conditions. Vertical bar indicate standard error of the mean.

4. Conclusion

The present study generated a considerable amount of important information on the effect of cloud and shade tree on the photosynthetic rates and pigment concentration in tea leaves. Overall result clearly showed the shade tree favours leaf photosynthesis rate compared to sunny weather. From the result and discussions it is clear that under field conditions, light intensity plays the vital role for leaf photosynthesis and controls the other factors for the growth of Darjeeling tea cultivation. Maintaining tea cultivation regulated under shade tree is beneficial in terms of reducing temperatures of the tea plant, reducing excessive transpiration, reducing moisture losses and minimizing the effects of photoinhibition and thereby maximizing bush health of tea plants during the long dry period in Darjeeling hill.

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