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Research Report

Photosystem II Photochemical Efficiency and Photosynthetic Capacity in Leaves of Tea Plant (*Camellia sinensis* L.) under Winter Stress in the Field

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Abstract. The effects of winter stress on photosynthesis of the tea plant (*Camellia sinensis* L.) were determined over the course of a year. Changes in gas exchange and chlorophyll fluorescence parameters largely tracked seasonal changes of temperature, precipitation, and atmospheric vapor pressure deficit. Intrinsic photosystem II (PSII) efficiency (estimated from the ratio of variable over maximal chlorophyll fluorescence, F_v/F_m) was at maximal levels (0.80-0.83) during the summer, then decreased in the fall and remained below 0.6 from January to March. The low levels of F_v/F_m in the winter were accompanied by the strongest quenching of maximal (F_m) and initial (F_o) fluorescence, presumably reflecting engagement of photoprotective thermal energy dissipation. Net photosynthetic rate, transpiration rate, and stomatal conductance were highest in the summer and lowest from late fall to early spring. These data suggested that PSII photochemical efficiencies and photosynthetic capacity of tea plant were limited under low temperature in the winter. On the other hand, a greater water use efficiency, lower light compensation point, and lower light intensity at which photosynthesis became saturated might be advantageous for tea plants acclimated to the lower precipitation levels and light intensities of winter. Analysis of the relationships between temperature or humidity and photosynthetic variables suggested that tea plants might benefit from irrigation in winter.

***Additional key words*:** intrinsic PSII efficiency (F_v/F_m), net photosynthetic rate, water use efficiency, winter stress

Introduction

The tea plant (*Camellia sinensis* L.) is an important commercial evergreen shrub crop, with tea being one of the most popular and inexpensive beverages produced from its young leaves (Barua, 1989). Its cultivation has spread to more than 30 countries (Barua, 1989; Graham, 1992) including China, Japan and Korea, and is increasing because of its positive effects on human health (Juneja et al., 1999). Tea is drunk for its desirable sensory properties, its probable health benefits, as well as for cultural reasons (Sánchez et al., 2013). Teas are popular around the world with purported benefits to slimming, beauty, mental relaxation and relief from stress (Weisburger, 1997). Health concerns in various countries have led to increased consumption of green tea to address respiratory health, elevated cholesterol, and elevated blood pressure (Sánchez et al., 2013; Weisburger, 1997). Tea contains various biochemical constituents such as polysaccharides, vitamins B, C, and E, and polyphenols, among which catechins, caffeine and tannin are present in large amounts (Sánchez et al., 2013). Catechins in particular have attracted much attention due to their therapeutic value as anti-cancerous, anti-oxidative, anti-allergenic, and anti-bacterial compounds (Fujiki et al., 1998; Ikigai et al., 1993; Lotito and Fraga, 1998). In general, the function of constituents and/or their effects on human health are different depending on many factors such as tea cultivar, leaf age, collecting season, cultivation conditions (soil, water, and minerals), climate, and geographical location.

During growth of the tea plant, many environmental factors may cause stress and potentially affect plant growth. Temperature stress is considered as one of most important environmental factors affecting plant growth and productivity (Wijeratne et al., 2007). Several studies on the response of different tea cultivars to cultivation or climatic conditions, including diurnal changes of net photosynthetic rate and chlorophyll fluorescence, have been conducted (Barman and Saikia, 2005; Mohotti and Lawlor, 2002; Oh et al., 2012, 2013a). In Korea, cultivation of the tea plant occurs primarily

in the southern part of the Korean peninsula and on Jeju Island, corresponding to the northern limit of distribution of many tropical and/or subtropical crop species. Tropical and sub-tropical plants (such as the tea plant) exhibit a marked physiological dysfunction when exposed to low or nonfreezing temperature below about 10 to 12°C (Lyons, 1973). Low temperature in winter, late fall and/or early spring therefore has the potential to negatively impact the growth, development, and productivity of tea plants. Low temperature (between 0 and 10°C) is a dominant environmental factor for the survival, and thus distribution, of woody plants (Grace, 1987). In general, reproductive structures, roots, and young leaves are particularly sensitive to cold. Low temperature can cause many physiological and biochemical dysfunctions that disrupt all major components of photosynthesis, including thylakoid electron transport, carbon reduction cycle, and stomatal conductance for CO_2 (Allen and Ort, 2001). In this study, a seasonal investigation of chlorophyll a fluorescence and photosynthetic capacity in tea leaves was undertaken under field conditions to examine the impact of winter conditions on this evergreen shrub.

Materials and Methods

Study Site

This study was conducted on a commercial tea field (Jangwon Co., Ltd.) in Hannam-ri (33°18'641"N, 126°41'228"E, 166 m elevation) on Jeju Island, located off of the southern tip of the Korean peninsula. The site is characterized by a subtropical climate with an average annual temperature of 16.2°C and annual total precipitation of 1,851 mm.

Plant Material

Tea plants (*Camellia sinensis* L. 'Ryofu') were grown for 6 years under natural light and temperature after propagation from cuttings. Organic fertilizers were applied 6-7 times between February and September. Although tea is often irrigated between March and April and from September to October under regular cultivation in this area, no irrigation was employed in this study site even when there were fewer precipitation events. The leaves for characterization were selected between 10:00 and 12:00 h local time on clear days from the south-facing 0.5 m height branches of five shrubs that received direct solar light during most of the day. Leaves that developed in spring 2011 were selected from August through April, and leaves that developed in spring 2012 were selected from May through July. After assessment of chlorophyll a fluorescence (see below) and chlorophyll content (using a SPAD-502 chlorophyll meter; Minolta, Osaka, Japan), leaves were collected for leaf area, dry weight, and specific leaf area determinations.

Weather Data

Daily air temperature and precipitation levels were collected from August 2011 until July 2012 by a Davis Vantage Pro2 Weather Station (Davis Instruments, Hayward, CA, USA) that was installed in the tea field near the study site. When chlorophyll a fluorescence was measured in the field, the ambient air temperature and humidity were also recorded above the sampled shrubs using a TR-72 Thermo Recorder (T&D Co., Matsumoto, Japan).

Chlorophyll a Fluorescence Measurements

Chlorophyll fluorescence was measured in situ from intact, attached leaves using a Plant Efficiency Analyzer (PEA; Hansatech, King's Lynn, Norfolk, England). After 20 minutes of dark adaptation, leaves were exposed to a saturating light pulse of 1s actinic light (1,500 $\mu mol \cdot m^{-2} \cdot s^{-1}$) to obtain the chlorophyll a fluorescence transients that were subsequently analyzed using the JIP-test (Kruger et al., 1997; Strasser and Strasser, 1995). Chlorophyll fluorescence parameters (F_o, F_m, and F_v/F_m) and JIP-test parameters (RC/CS_0, ABS/CS_0, TR_0/CS_0, ET_0/CS_0, and DI_0/CS_0) were processed by the software Biolyzer 3.0 (R. M. Rodriguez, Bioenergetics Lab., Geneva, Switzerland).

Gas Exchange and Steady-State Light Response Curves

The light-saturated rate of net photosynthetic CO_2 uptake (A_{max}), transpirational rate of water loss (E), and stomatal conductance (gs) were determined under a photosynthetic photon flux density (PPFD) of 1,200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and ambient CO_2 level with a portable gas exchange system (LCpro$^+$, ADC BioScientific Ltd., Hoddesdon, UK) equipped with an LED light source (Oh et al., 2012). In each trial, the fully expanded leaves were initially equilibrated in the leaf chamber for 5 min to acclimate to a PPFD of 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in order to activate ribulose-1,5-biphosphate carboxylase/oxygenase and open the stomata (Pearcy, 1990; Valladares et al., 1997). The vapor pressure deficit (VPD) and water vapor pressure (WVP) were also recorded together with photosynthetic measurements. Instantaneous water use efficiency (WUE) was calculated by dividing A by E.

To obtain photosynthetic light response characteristics, photosynthetic rates were measured at 10 PPFD levels between 1,200 and 0 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (achieved by wrapping the leaf chamber with black cloth). The leaves were allowed to equilibrate for 5 min to each light level before gas exchange measurements, and five measurements were taken with 1 min intervals at each light level. The relationship between photosynthesis and irradiance for leaves was described by a non-rectangular hyperbola equation as seen below (Marshall and Biscoe, 1980).

$$A = [\alpha I + A_{max} - \{(\alpha I + A_{max})^2 - 4\alpha\theta I A_{max}\}^{0.5}]/2\theta - R_d$$

Where A is the net CO_2 assimilation rate, α is the quantum yield (initial slope of the light-response curve), I is the PPFD, A_{max} is the light-saturated photosynthetic capacity, θ is a curvature factor, and R_d is the dark respiration rate. Instantaneous light compensation points (ICP) were calculated by setting $A = 0$ and solving for I in the equation (Marshall and Biscoe, 1980). The photon flux density at one-half A_{max} (k), which reflects the affinity of the enzyme complexes of photosynthesis for the substrate light, was calculated using the Michaelis-Menten function modified for photosynthetic light response (Givnish et al., 2004). The R_d/A_{max} ratio was calculated in order to assess the carbon balance at the leaf level.

Statistical Analysis

Statistical analysis was performed using the SPSS 18.0 statistical software (SPSS lnc., Chicago, IL, USA). Analysis of variance (one-way ANOVA) and subsequent Duncan's multiple range test were performed to test variations in growth and photosynthetic response to seasonal changes. Pearson's correlation analyses were applied to examine associations between temperature or humidity and selected photosynthetic and environmental variables A_{max}, E, gs, VPD, WVP, and WUE.

Results

To assess the impact of environmental conditions on leaf gas exchange and chlorophyll fluorescence, detailed information is required on the seasonality of key environmental factors. Therefore, daily precipitation and maximum and minimum air temperature were collected from August 2011 through July 2012, as shown in Fig. 1. The annual mean air temperature was 14.9°C during the study period, with monthly mean temperatures highest in August (24.9°C) and lowest in February (4.0°C). The maximum and minimum of daily air temperature were 32.8°C in September, 2011 and -4.7°C in February, 2012, respectively. From early December 2011 until late February 2012, maximum daily air temperature remained below 10°C and then rose above 10°C from the middle of March 2012 and thereafter. Total annual precipitation during this period was 1,645 mm, with most of the precipitation occurring from spring through fall (corresponding to the wet season) and little winter rainfall from December until February (dry season).

Leaf morphological variables of total area, dry weight, and specific leaf area were measured from the leaves collected for the study (Table 1). The leaf area remained statistically

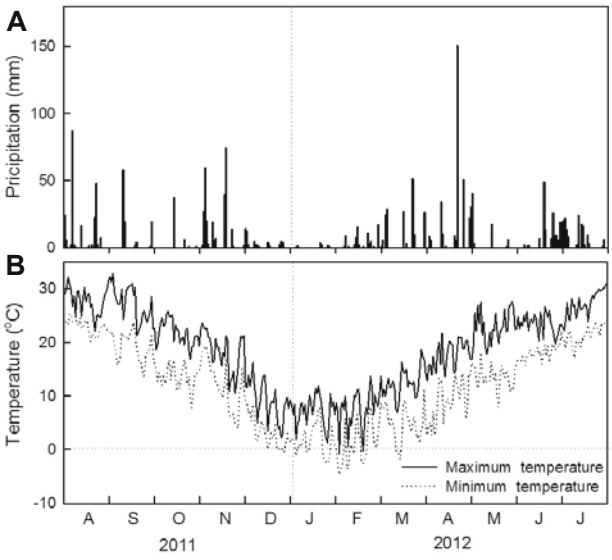

Fig. 1. Daily precipitation (A) and maximum and minimum air temperature (B) determined from August 2011 to July 2012 on the tea (*Camellia sinensis* L.) field at Hannam-ri on Jeju Island.

constant throughout the study period. In contrast, leaf dry weight remained constant from August 2011 through April 2012 but was lower in May and June 2012. Consequently, the specific leaf area was greater in May and June 2012 compared to the other characterized dates. The estimated leaf chlorophyll levels mirrored those of the specific leaf area, with significantly lower levels in May and June of 2012.

For one year intrinsic (dark-adapted) PSII efficiency (F_v/F_m) and absolute chlorophyll fluorescence yields, F_m and F_o, were measured on three independent days with weather conditions typical for each month (Fig. 2). As shown in Fig. 2A, the ambient temperature recorded at the time when chlorophyll a fluorescence was measured in the field showed roughly same trends to those in Fig. 1, with atmospheric humidity levels largely paralleling the trends in temperature. From spring through summer to early autumn, F_v/F_m was at maximal levels, then decreased during the late fall and remained below 0.6 from January through March with the lowest value in February (Fig. 2A). However, it rose rapidly with the onset of spring and reached a high level in April. The seasonal changes of F_m (Fig. 2B) and F_o (Fig. 2C) largely paralleled those of F_v/F_m. In May 2012, the F_v/F_m value of young leaves newly developed in spring was a little low probably due to a highly elevated level of F_o. Afterwards F_v/F_m rose to optimal levels in the early summer (June and July) with a decrease of F_o as leaves matured.

The JIP-analyses were performed on the chlorophyll a fluorescence transients obtained from leaves sampled in August, November, February and April, cardinal months on which F_v/F_m exhibited significant differences over the course

Table 1. Morphological variables and relative chlorophyll content of leaves of tea plant (*Camellia sinensis* L.). Data are means ± standard errors from 3-4 leaves on each study date.

Month	Leaf area (cm^2/leaf)	Dry weight (g/leaf)	Specific leaf area ($cm^2 \cdot g^{-1}$)	Chlorophyll content (SPAD value)
August	22.4 ± 2.6 a[z]	0.26 ± 0.01 a	85.2 ± 4.6 b	76.3 ± 7.1 a
September	21.8 ± 1.9 a	0.25 ± 0.02 a	86.9 ± 2.1 b	77.3 ± 1.2 a
October	21.9 ± 3.4 a	0.25 ± 0.05 a	87.5 ± 2.7 b	74.6 ± 6.5 a
November	22.0 ± 4.9 a	0.25 ± 0.08 a	88.1 ± 4.1 b	79.7 ± 4.8 a
December	23.3 ± 1.9 a	0.32 ± 0.04 a	73.9 ± 6.1 b	81.8 ± 2.6 a
January	19.7 ± 2.4 a	0.29 ± 0.06 a	71.8 ± 13.1 b	80.4 ± 5.3 a
February	21.1 ± 2.0 a	0.29 ± 0.03 a	74.2 ± 8.4 b	77.5 ± 3.6 a
March	21.5 ± 3.7 a	0.29 ± 0.04 a	75.6 ± 9.0 b	78.8 ± 3.8 a
April	18.9 ± 1.7 a	0.29 ± 0.02 a	65.0 ± 5.0 b	79.8 ± 1.3 a
May	21.0 ± 2.4 a	0.15 ± 0.01 b	137.5 ± 14.0 a	45.6 ± 5.8 c
June	21.4 ± 1.3 a	0.23 ± 0.02 ab	94.0 ± 4.3 b	60.8 ± 5.2 b
July	23.0 ± 3.9 a	0.27 ± 0.03 a	84.4 ± 8.6 b	74.6 ± 5.1 a

[z]Different letters within each column indicate significant differences between dates ($p < 0.01$).

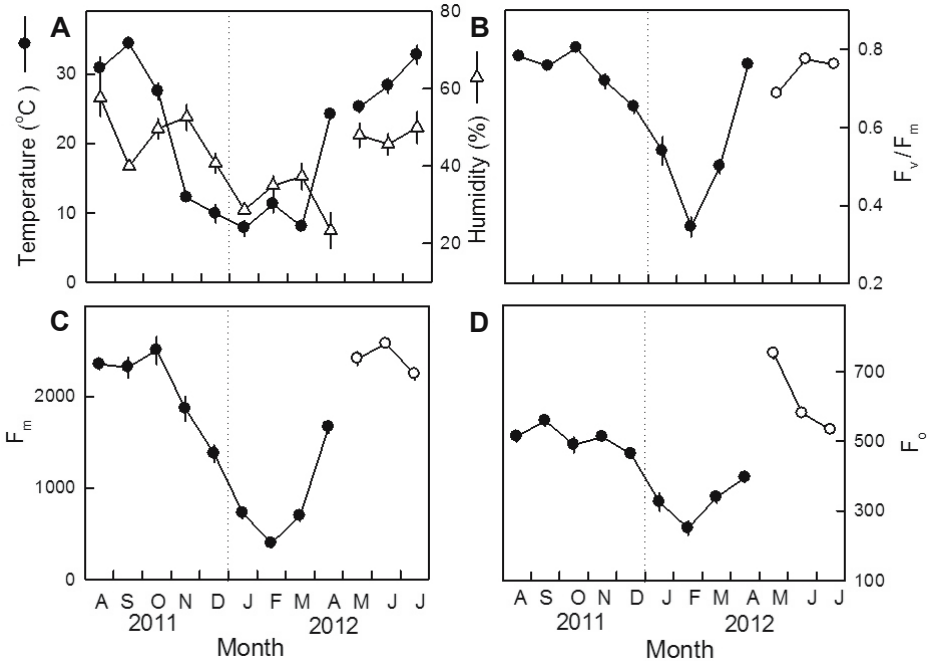

Fig. 2. Seasonal changes in ambient air temperature and humidity (A), photochemical efficiency of photosystem II (F_v/F_m) (B), maximal fluorescence (F_m, arbitrary units) (C), and initial fluorescence (F_o, arbitrary units) (D) in leaves of tea plant (*Camellia sinensis* L.). Filled circles represent leaves that developed in 2011 and open circles represent leaves that developed in 2012. The values of F_m, F_o, and F_v/F_m were determined from dark-adapted (20 minutes) chlorophyll a fluorescence transients O-J-I-P; means ± standard errors of 6–10 leaves shown.

of the year (Fig. 2A). Table 2 depicts the seasonal differences in parameters obtained from the JIP-test. The reaction centers per cross section (RC/CS), the absorption flux of photons per cross section (ABS/RC), the flux of excitation energy trapped per cross section (TR_0/CS), and the electron transport per cross section (ET_0/CS) were all high in August and/or November but were significantly lower in February. On the other hand, the dissipation energy per cross section (DI_0/CS) was elevated in November and February and was lower in August and April.

Leaf gas exchange measurements were also performed during the cardinal months of April, November, February and April. Light-saturated net photosynthetic rate (A_{max}), transpiration rate (E), and stomatal conductance (gs) were high

Table 2. Seasonal differences in JIP-analyses parameters from the leaves of tea plant (*Camellia sinensis* L.). Data are means ± standard errors of 6-10 leaves on each study date.

Month	RC/CS[z]	ABS/CS	TRo/CS	ETo/CS	DIo/CS
August, 2011	425.1 ± 19.1 ab[y]	526.8 ± 15.2 a	408.5 ± 10.3 a	308.1 ± 6.8 a	118.3 ± 5.5 bc
November, 2011	487.6 ± 28.5 a	522.2 ± 13.3 a	372.6 ± 13.8 a	251.3 ± 11.5 b	149.5 ± 10.9 ab
February, 2012	75.1 ± 16.4 c	248.3 ± 21.4 c	89.6 ± 14.0 c	67.1 ± 11.0 c	158.7 ± 9.7 a
April, 2012	370.0 ± 11.2 b	403.7 ± 13.0 b	305.4 ± 10.4 b	225.6 ± 4.7 b	98.3 ± 3.6 c

[z]RC/CS, reaction centers per cross section; ABS/CS, absorption flux of photons per cross section; TRo/CS, flux of excitation energy trapped per cross section; ETo/CS, electron transport behind Q_A^- per cross section; and DIo/CS, dissipation of energy per cross section.
[y]Different letters within each column indicate significant differences between dates ($p < 0.01$).

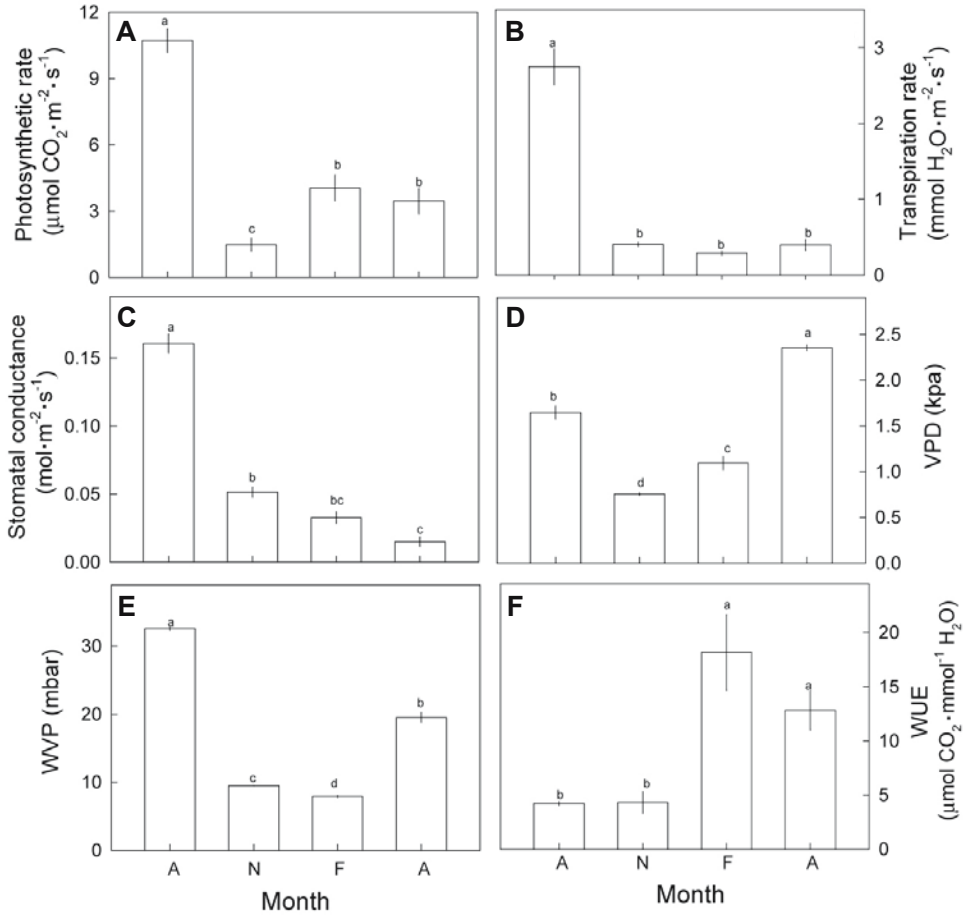

Fig. 3. Seasonal changes in light-saturated photosynthetic CO_2 uptake (A), transpirational water loss (B), stomatal conductance (C), leaf-to-air vapor pressure difference (VPD) (D), water vapor pressure of the atmosphere (WVP) (E), and water use efficiency (WUE) (F) for leaves of tea (*Camellia sinensis* L.) characterized in August, November, February, and April. Data are means ± standard errors of 4–5 leaves. Significant differences among the values are indicated by the different letters ($p < 0.01$).

in August and low during the other three months (Figs. 3A to 3C). The leaf-to-air vapor pressure difference (VPD) and water vapor pressure (WVP) were low in November and February and higher in April and August (Figs. 3D and 3E). In contrast, the water use efficiency (WUE), calculated from A_{max} divided by E, was higher in February and April (Fig. 3F).

To determine the detailed relationships between temperature or humidity and selected photosynthetic and environmental variables, Pearson's correlation analyses (Table 3) were performed with the data obtained in summer (August) or winter (February). Temperature was significantly positively correlated with A_{max}, E, gs, VPD and WVP, and negatively correlated with WUE in summer. However, in winter, temperature was significantly negatively correlated with gs, positively correlated with VPD and WVP, and showed no relationship

Table 3. Correlation analysis between temperature or humidity and photosynthetic and environmental variables for the leaves of tea plant (*Camellia sinensis* L.) grown under field conditions.

Environmental factors	Season	Photosynthetic variables					
		A_{max}[z]	*E*	*gs*	VPD	WVP	WUE
Temperature	Summer	0.706**	0.948**	0.638**	0.867**	0.505*	-0.535**
	Winter	-0.170	0.221	-0.570**	0.883**	0.651**	0.057
Humidity	Summer	-0.446*	-0.791**	-0.348	-0.975**	-0.119	0.704**
	Winter	0.521**	0.272	0.754**	-0.839**	0.229	0.115

[z]A_{max}, light-saturated photosynthetic CO_2 uptake; *E*, transpirational water loss; *gs*, stomatal conductance; VPD, vapor pressure deficit; WVP, water vapor pressure; and WUE, water use efficiency.
*,** Indicate significant correlations at $p < 0.05$ and $p < 0.01$ levels, respectively.

Table 4. Characteristics of the photosynthetic light-response curves obtained from leaves of the tea plant (*Camellia sinensis* L.) grown under field conditions. Data are means ± standard errors of 3 leaves on each study date.

Month	A_{max}[z] (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$)	R_d (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$)	R_d/A_{max} ratio	ICP (μmol $\cdot m^{-2} \cdot s^{-1}$)	*k* (μmol $\cdot m^{-2} \cdot s^{-1}$)
August, 2011	14.5 ± 2.1 a[y]	-1.8 ± 0.6 a	0.12 ± 0.02 a	24.9 ± 3.4 a	198 ± 56 a
November, 2011	2.6 ± 0.9 b	-0.7 ± 0.4 ab	0.24 ± 0.08 a	19.3 ± 8.6 a	30 ± 21 b
February, 2012	4.7 ± 0.8 b	-0.5 ± 0.2 ab	0.13 ± 0.07 a	13.5 ± 7.9 a	145 ± 97 ab
April, 2012	3.7 ± 0.7 b	-0.2 ± 0.1 b	0.05 ± 0.02 a	6.3 ± 2.8 b	146 ± 76 ab

[z]A_{max}, light-saturated rate of net photosynthetic CO_2 uptake; R_d, rate of respiratory CO_2 evolution determined in darkness; ICP, light compensation point; and *k*, PPFD at 50% of photosynthetic light saturation.
[y]Different letters within each column indicate significant differences between dates ($p < 0.05$).

with A_{max}, *E*, and WUE. On the other hand, humidity was significantly negatively correlated with A_{max}, *E* and VPD, positively correlated with WUE, and exhibited no correlation with *gs* or WVP in summer, while in winter humidity was significantly positively correlated with A_{max} and *gs*, negatively correlated with VPD, and had no relationship with *E*, WVP or WUE.

The photosynthetic light response characteristics, including A_{max}, dark respiration rate (R_d), R_d/A_{max} ratio, instantaneous light compensation point (ICP), and the PPFD of half A_{max} (*k*), were obtained from the leaves of tea plant in August and November, 2011, and February and April, 2012 (Table 4). The values of A_{max} and R_d were highest in August and remained low from November until April. The R_d/A_{max} ratio was a little high in November, though there was no significant difference among seasons. However, the values of ICP and *k* were lowest in early spring (April) and in early winter (November), respectively.

Discussion

Optimal temperatures for photosynthesis and growth of tea plant (*Camellia sinensis* L.) were previously found to be between 25-30°C (Barman et al., 2008; Barua, 1989). Photosynthesis and growth declined markedly at temperatures below 20°C (Barman et al., 2008) or above 35°C (Hadfield, 1968). Moreover, the optimal temperature required for producing tea leaves of high quality is considered to be 18-25°C (Carr and Stephens, 1992; Watson, 1986). Likewise, in the closely-related *C. japonica*, the optimal temperature for photosynthesis was between 15-25°C, below or above which temperature photosynthesis declined significantly (Liu et al., 2003). The average temperature in the main producing districts of tea should not be below 13°C (for the coldest month) nor above 30°C (for the warmest month; Barua, 1989). Tea plants exhibited a cessation of growth in the country of Georgia when the mean air temperature fell below 13°C (Labedev, 1961), and its metabolism was severely reduced at lower temperatures. Such influence may be caused by low air and/or soil temperature, affecting both shoot hydration and photosynthetic metabolism (Allen and Ort, 2001). In the current study site, the maximum daily temperature during winter (December until February) was below 10°C, and minimum daily temperature fluctuates around 0°C, suggesting that the metabolism and/or growth of the tea plants were far from optimal during the cold season.

It is widely accepted that low temperature induces a decrease of the net photosynthetic rate associated with decreased activity of Calvin cycle enzymes (Berry and Björkman, 1980; Kingston-Smith et al., 1997). This effect can be transient and fully reversible upon return to warmer temperatures, but acclimation to lower temperature on a seasonal basis can involve either 1) upregulation of some key photosynthetic enzymes to maintain higher rates of

photosynthesis than would otherwise be possible at the lower temperature in some species (e.g., herbaceous biennials and winter annuals) or 2) downregulation of photosynthesis in sclerophytic evergreen species (Adams et al., 2001, 2002, 2004; Cohu et al., 2013, 2014). The latter can, furthermore, be accompanied by an increase in photoprotective energy dissipation that is reflected in a decreased F_v/F_m as the excess absorbed excitation energy is diverted to thermal energy harmlessly released to the environment (Adams et al., 2006, 2013). In the current study, both PSII photochemical efficiency and light-saturated photosynthesis were reduced in the winter when temperatures were lowest, water availability was presumably less (due to reduced levels of precipitation), and the vapor pressure difference between leaves and the atmosphere was greatest.

From spring to summer, F_v/F_m values were near or within the optimal range (0.80-0.83) found in unstressed leaves of higher plants utilizing the C3 pathway of photosynthesis (Björkman and Demmig, 1987). The decreased levels of PSII efficiency observed during the winter were most likely due to increased levels of thermal energy dissipation, given that these were accompanied by decreases in both F_m and F_o, as was documented in the response of several other species in response to winter stress that involved increased levels of the photoprotective carotenoids (zeaxanthin and antheraxanthin) of the xanthophyll cycle (Adams and Demmig-Adams, 1994; Oh et al., 2013b; Verhoeven et al., 1996; see Kitajima and Butler, 1975, for a detailed analysis of chlorophyll fluorescence). In addition, the decreases of RC/CS, ABS/RC, TR_0/CS and ET_0/CS, and the increase of DI_0/CS in winter (February), suggested that most PSII reaction centers were transformed to inactive forms, the reaction centers or electron transport chain could neither absorb the energy nor reduce Q_A, and that more excitation energy could be dissipated as heat (Strasser et al., 2000). These findings are also consistent with previous studies that have characterized a decrease in PSII proteins involved in electron generation and acceptance of those electrons into the photosynthetic electron transport chain (the oxygen evolving complex and the D1 protein of the PSII reaction center) in evergreen species during the winter (Adams et al., 2013; Zarter et al., 2006a, 2006b). A selective removal of such key proteins, coupled with sustained engagement of zeaxanthin and antheraxanthin in thermal energy dissipation (indicated by the quenching of the F_m and F_o levels of chlorophyll fluorescence), should provide powerful protection against the generation of the potentially damaging reactive oxygen species superoxide and singlet excited oxygen (Adams et al., 2004, 2006, 2013).

Several other studies have also reported that *A* and F_v/F_m were reduced in tea plants (Hazra and Kumar, 2002; Joshi and Palni, 1998; Joshi et al., 2000) as well as in other plants in response to low temperature or winter conditions (Adams et al., 2013). The winter decrease of *A* in the present study was accompanied by decreases of *E* and *gs*. Reductions in *A*, *E*, and *gs* have also been reported in orange plants experiencing low temperature stress (Ribeiro et al., 2009). Stomatal closure caused by low temperature could compromise gas exchange and CO_2 fixation, stimulating downregulation of Calvin cycle enzymes, and thereby contributing to a decline in photosynthetic capacity (Allen and Ort, 2001). Alternatively, the lower rates of both photosynthesis during winter could simply be due to the inhibitory effect of lower temperature on the Calvin cycle enzymes (Allen and Ort, 2001), resulting in higher levels of CO_2 within the leaf leading to increased stomatal closure. It is also, of course, possible that decreased stomatal opening during the winter months is triggered by reduced water availability and/or the increased vapor pressure deficit between the leaves and the atmosphere. Regardless of the proximal cause for decreased stomatal opening during the winter, the resulting increase in water use efficiency is likely beneficial in minimizing water loss during the driest months of the year.

The lower rates of dark respiration during winter could also be due to the impact of lower temperatures on the enzymes of glycolysis and the tricarboxylic acid cycle (Atkin and Tjoelker, 2003). Interestingly, the ratio of dark respiration to light-saturated photosynthesis was low all the year over, indicating that tea leaves might keep a positive carbon balance during the whole year (Catoni and Gratani, 2014; Gratani et al., 2008) and thereby able to assimilate carbon even in winter.

It is clear that winter weather represents stressful conditions for the tea plant. Given the results of the correlation analyses, tea plants seem to be exposed to stress caused not only by low temperatures but also water stress due to little rainfall coupled with low humidity during winter. It might, therefore, be possible to obtain higher yields of tea leaves during subsequent spring seasons through irrigation of the tea plants during the winter, a practice that is not currently undertaken on Jeju island. On the other hand, mild stress could be beneficial for improving the quality of tea leaves, since the level of potent antioxidants such as catechins and proanthocyanidins likely increase in response to stress. However, the impact of winter stress conditions on tea plants, and specifically on the young leaves developing in the subsequent spring, is not yet known. Thus, further studies on the employment of defense mechanisms by tea leaves, including antioxidant compounds related to oxidative stress in response to low temperature, water deficiency, frost, etc., should be undertaken. In particular, bud-burst and characterization of developing young leaves in spring should receive additional attention.

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