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RESEARCH ARTICLE

Strong Solar Irradiance Reduces Growth and Alters Catechins Concentration in Tea Plants over Winter

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Abstract

An experiment was conducted to investigate the effect of sunlight and chilling on growth, photosynthesis and chemical composition of tea plants growing in South Korea. The plants were grown in full sun (mid-day photosynthetic photon flux density, PPFD of 563 μmol^{-2}s^{-1}), slight shade (PPFD of 99 μmol^{-2}s^{-1}) or heavy shade (HS, PPFD of 5 μmol^{-2}s^{-1}) in a field at the foot of pine forest from October to March, 2012. Mean and minimum daily temperature fell below 0 °C in December and January. Shading had a small or no effect on shoot and leaf growth. Shading increased the concentration of chlorophyll compared with plants in full sun, with relatively stable values within a treatment over time. Shading increased chlorophyll fluorescence, with lower values in all treatments in late January. The concentration of total catechins was lower under shading, and lower in early January. Low temperatures appear to decrease chlorophyll fluorescence independently of light levels. Growth under heavy shade was dependently of stored reserves and would be unsustainable over the long term. Overall quality as reflected by the concentration of catechins was best under full sun conditions.

Key words : *Camellia sinensis*, antioxidant, low temperature, light intensity, shading

Introduction

Tea is an evergreen woody plant belonging to the genus *Camellia,* family Theaceae, produced mainly in sub-tropical to temperate regions of the world (Xue et al. 2010). Due to its many beneficial effects and good taste, green tea is one of the most popular beverages in East Asia, including South Korea, China, and Japan. This traditional beverage has become popular in western countries because several components such as polyphenols have beneficial effects on human health (Cho et al. 1993; Fukai et al. 1991; Khokhar and Magnusdottir 2002; Senanayake 2013; Yang and Wang 1993). Because tea plants have poor tolerance of low temperature, production can be a problem in temperate regions that have cold winters. Although light is essential for photosynthesis in plants, excess light can lead to photoinhibition (Barber and Andersson 1992). In addition, photoinhibition under low temperatures can affect tea quality by altering leaf chlorophyll and carotenoids (Shen et al. 2015). In South Korea, the annual mean temperature for production must be higher than 13°C (Lee et al. 2005). Therefore, the effects of low temperature and high light in winter should be investigated for producing tea in this environment.

Polyphenols including catechins are major components of tea leaves (Goto et al. 1996). Catechins are the most abundant polyphenols, may account for up to 30% of the dry weight of the leaves (Graham 1992; Senanayake 2013), and influence the taste of the end product (Hunter et al. 1992). There are several catechins in green tea, including (−)-epicatechin (EC), (−)-epicatechin-3-gallate (ECG), (−)-epigallocatechin (EGC), (−)-epigallocatechin-3-gallate (EGCG), (+)-catechin,

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and (+)-gallocatechin (GC) (Dalluge et al. 1998). EGCG is the most abundant catechin accounting for 65% of the total catechin content (Bazinet et al. 2007; Eisei and Yuko 2004; Khokhar and Magnusdottir 2002; Wei et al. 2011). Our study was conducted to document the variation in the catechin content of tea leaves grown under different light conditions over several months in South Korea. We also collected information on chlorophyll content, chlorophyll fluorescence, and growth over winter.

Materials and Methods

Plant materials and growth analysis

Tea seeds collected in Gyeongnam province, South Korea, were planted and raised in sandy loam soil at 10 × 20 cm spacing with no fertilizer and no pesticide for 3 years. The seedlings were transplanted and grown at the experimental site for 3 years in full sun (FS, unshaded), slight shade (SS), and heavy shade (HS) with pine tree canopy. Mean values of photosynthetic photon flux density at day time (from 10:00 to 14:00) were measured perpendicularly at the soil surface by a quantum sensor (LI-250, LI-COR) from Dec. 2014 to Feb. 2015 in the FS, SS, and HS locations. Leaf chlorophyll content was measured using a chlorophyll meter (SPAD 502, Minolta, Japan), and chlorophyll fluorescence (Fv/Fm) was measured for the second to the uppermost leaf using a fluorescence meter (OS-30P, Opti-science, USA). Soil moisture content was determined using a TDR moisture meter (TDR-100, Spectrum technology, USA). Leaf color was determined based on the Hunter values (Hunter 1975). Plant height, stem, and leaf size were measured from 10 randomly selected plants on Dec. 20. 2014

Catechin analysis

Leaves used for measuring the chlorophyll content and chlorophyll fluorescence were harvested for carechin analysis. Detached leaves were stored at -70°C until analysis and freeze-dried before analysis. Extracts from ground samples were made using a mixture of methanol and water (70:30 v/v, 10 mL) for 30 min at room temperature. The extracts were filtered through a 20 μm syringe filter prior to HPLC analysis. Catechins were analyzed using a gradient HPLC system comprising two LC-10 AD VP pumps and an SPD-10Avp UV detector (Shimadzu, Japan). The column used was a NOVA-park C18 (5.0 μm, 3.9 × 150 mm) fitted with a C18 guard column. The column was eluted with a gradient of 100% solution A (9% acetonitrile, 2% acetic acid) for 10 min, to 68% of solution A and 32% of solution B (80% acetonitrile, 2% acetic acid) for 10 min, at a flow rate of 1.0 ml/min at 35°C. The signals from the eluent were monitored at 280 nm.

Statistical analysis

The growth data were statistically analysed using the Statistical Analysis System (SAS ver. 9.1) program. The one-way ANOVA and the Duncan's multiple range test were used to determine the significant differences among the treatments test at $P < 0.05$. The results were expressed as mean value of three replications.

Results and Discussion

Environmental conditions and plant growth

Light levels differed greatly among the shading treatments (Fig. 1). Mean values of PPFD over winter at FS, SS, and HS sites were 563, 99, 5 μmol $m^{-2} s^{-1}$, respectively. Both average and minimum temperature were below 0°C from mid-Dec.

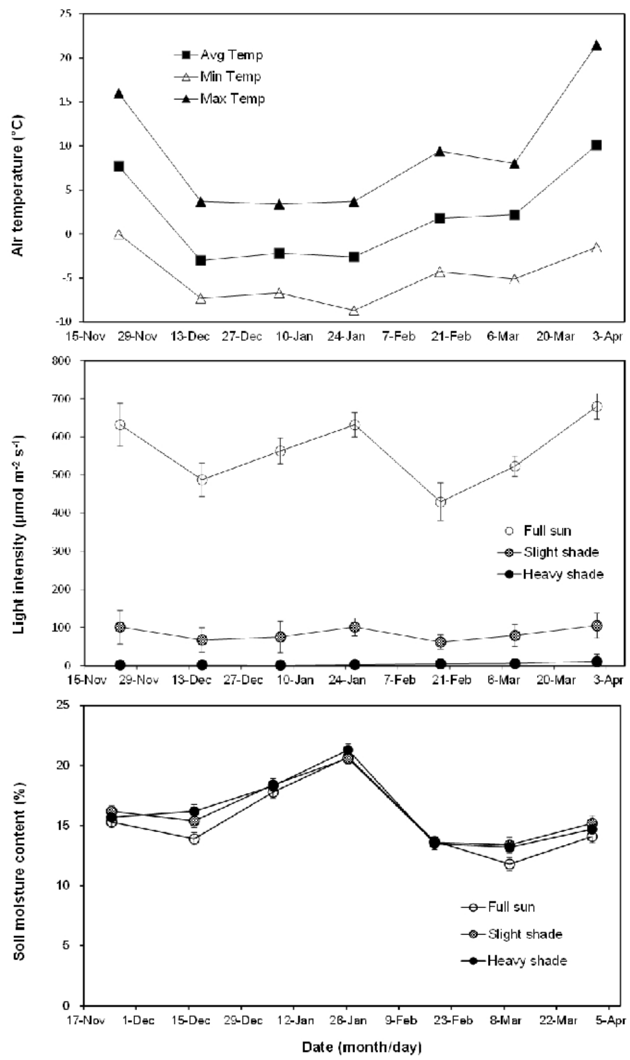

Fig. 1. Air temperature, light intensity and soil moisture content during the experimental period. Light intensity was measure measured as PPFD at the canopy level and soil moisture content was measured with the soil at 5 cm deep. Values for light intensity and soil moisture content were the mean of three replications. Values are given as mean standard error for each condition bars mean standard error.

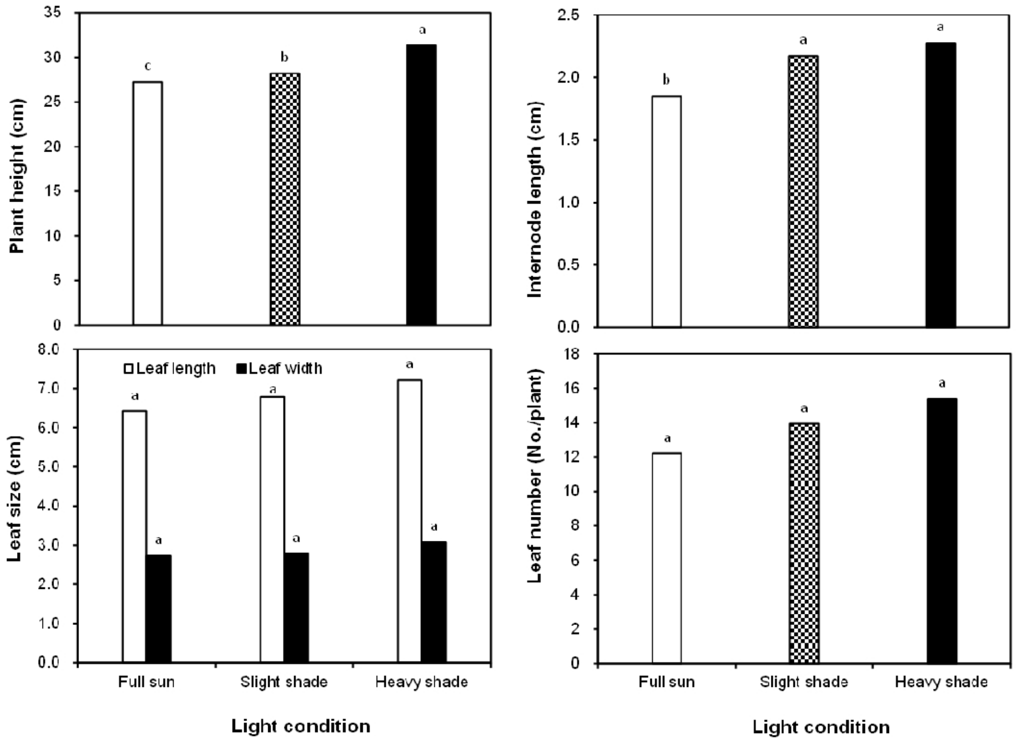

Fig. 2. Influence of light condition on tea plant height, internode length, leaf size, and leaf number of tea plants grown under different shading conditions. Within each graph, bars labeled with the same letters 514 are not significantly different at $P \leq 0.05$.

2011 to late-Jan 2012. During this freezing season, soil moisture content was higher during the period of low evaporation from the soil surface until February. Tea plants grow well in shade, which reduces photoinhibition (Lee et al. 2003; Mohotti and Lawlor 2002). Although there was insufficient light in the heavy shade for photosynthesis, shading had a small or nonsignificant ($P > 0.05$) effect on plant growth, including shoot or internode elongation, leaf production, and leaf area extension (Fig. 2) because the growth of tea plants nearly ceases due to the cold weather in winter. Soil moisture content was generally similar in the different growing conditions (Fig. 1). Tea plants should be grown in moderately wet soil (Jeyaramraja et al. 2005; Kathiravetpillai et al. 2008). Because our experimental field showed moderately wet soil conditions, the growth of tea was not limited by soil moisture.

Chlorophyll content and fluorescence, and leaf color

In general, tea and other plants that are shade tolerant have lower light saturation points than plants that prefer full sun (Janendra et al. 2007). The concentration of chlorophyll in tea plants and the efficiency of photosynthesis can be affected by environmental conditions (Lin et al. 2014) (Fig. 3). In our experiment, chlorophyll fluorescence fluctuated more strongly than chlorophyll content over the growing season. Values of quantum yield (Fv/Fm) in all plants were extremely low (less than 0.4) at the end of January, indicating that photosynthesis was inhibited. Overall values were higher under HS, intermediate under SS and lower under full sun. The inhibited

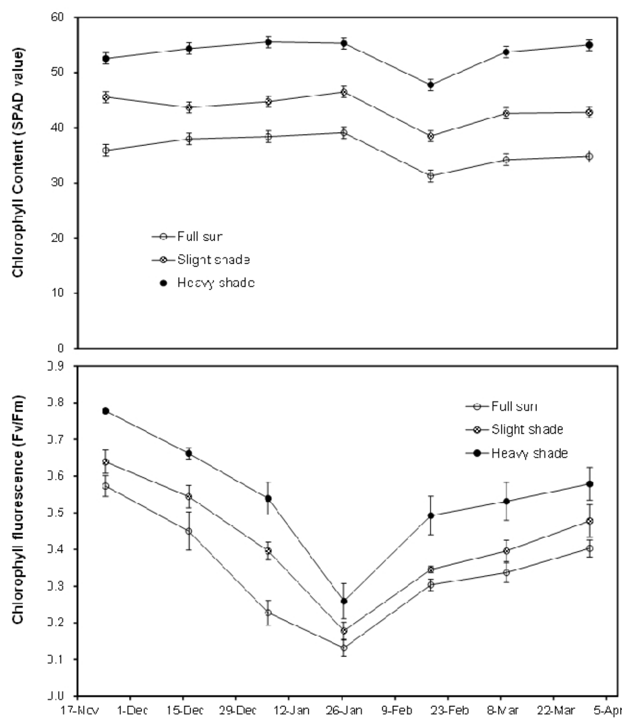

Fig. 3. Influence of light condition on chlorophyll content (SPAD value) and chlorophyll fluorescence (Fv/Fm) of tea plants. Both chlorophyll content and chlorophyll fluorescence were measured with the fully expanded uppermost leaf. Values are given as mean standard error for each condition.

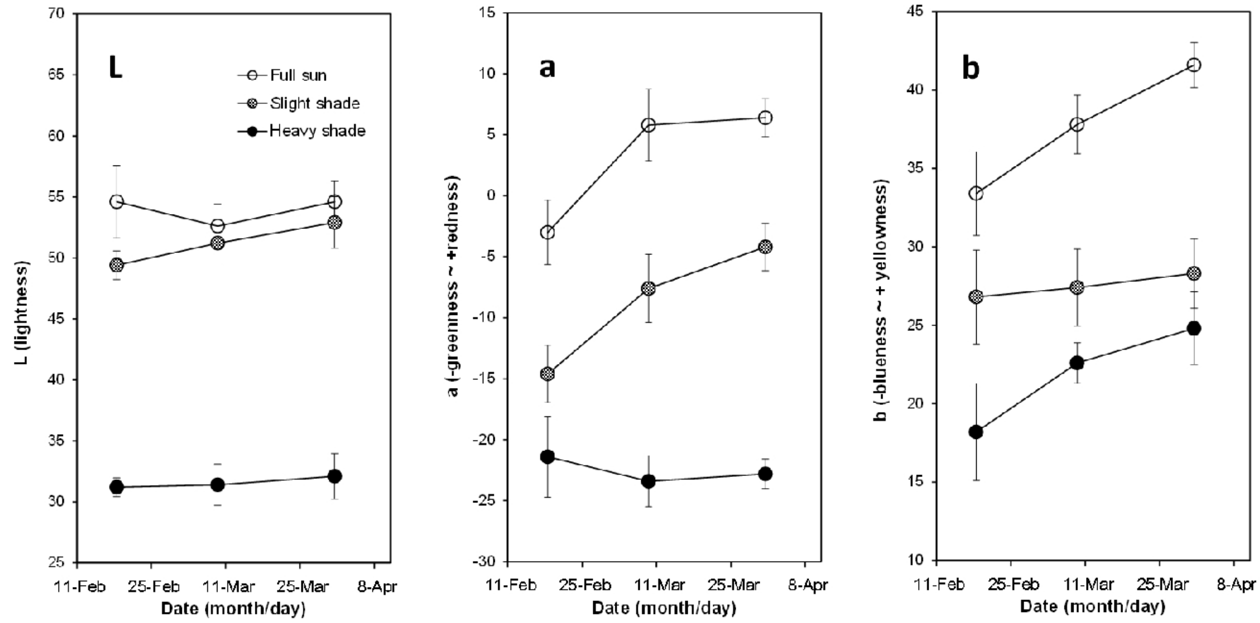

Fig. 4. Changes in tea leaf Hunter value in different light conditions during the overwintering period. A: L (lightness), B: a (+redness ~ -greenness), C: b (+yellowness ~ -blueness). Values are given as mean standard error for each condition.

Table 1. Content of catechins in tea leaves grown under different light conditions (mg/g, w/w).

Date	Light condition	EGC[a]	C	ECG	EC	EGCG	Total
24 Oct.	Full sun	58.8bc[c]	9.7a	16.8c	76.4c	58.6c	220.3c
	Slight shade	33.7a	18.9b	14.2b	60.7b	45.7b	173.2b
	Heavy shade	30.8a	27.8c	8.5a	16.3a	37.7a	121.1a
16 Dec.	Full sun	64.1c	5.2a	21.5c	59.8c	39.2c	189.8c
	Slight shade	28.8b	15.8b	11.4b	45.9b	27.2b	129.1b
	Heavy shade	0.4a	23.0c	ND[b]a	14.7a	16.0a	54.1a
6 Jan.	Full sun	68.0c	6.6a	22.4c	50.4c	34.2c	181.6c
	Slight shade	37.0b	14.0b	10.5b	42.4b	27.0b	130.9b
	Heavy shade	14.4a	20.1c	4.9a	18.3a	20.9a	78.6a
26 Jan.	Full sun	108.1c	8.6a	19.0c	54.6c	39.1c	229.4c
	Slight shade	44.7b	15.8b	11.5b	32.1b	19.0b	123.1b
	Heavy shade	14.5a	29.8c	5.3a	17.1a	15.4a	82.1a

[a]EGC: epigallocatechin, C: catechin ECG: epicatechin gallate, EC: epicatechin, EGCG: epigallocatechin gallate. [b]Under limit of detection. Each value is the mean of three replications. Same letters within a light condition in a column are not significant different by Duncan's multiple range test at 0.05% probability.

leaves changed their color (Fig. 4). Leaves in the shades were greener than the leaves in the full sun. Low temperatures enhance photoinhibition of PSII under strong light (Öquist et al. 1993). Our result showed that the low temperature enhanced photoinhibition in both shaded and unshaded plants, however, the effect was less in shaded leaves. Therefore, it can be assumed that the reduction in growth (Fig. 2) of plants under full sun might be due to the inhibited photosynthesis because the plants reduced chlorophyll concentration and increased other pigments to avoid photoinhibition at low temperature (Fig. 4).

The lowered chlorophyll concentration that a result for diminishing the photoinhibition affects photosynthesis even at more favorable light and temperature at full sun site. In leaf chromatic feature, low lightness values were associated with the higher concentration of chlorophyll (Figs. 3, 4). Red leaves might be due to degradation of chlorophyll and accumulation of anthocyanins and carotenoids. The higher L value indicates more degradation of chlorophyll, and higher a and b values indicate more carotenoids and anthocyanins (Fig. 4). The changed color properties indicate that leaves exposed to more direct sunlight might be more vulnerable to higher photoinhibition stress.

Catechin concentrations

Tea has functional components that provide health benefits to human. Catechins vary in concentration depending on environmental conditions and genotype (Wei et al. 2011). The concentration of total catechins was higher in unshaded than in both slight and heavyily shaded leaves (Table 1) and opposite to the trend in photosynthetic efficiency. Total catechin concentration was higher in late October and late January than in early January in both shaded and unshaded plants. The differences between FS and HS were greater in winter from December to January than in October. In early January, the concentration of catechins in unshaded leaves was nearly twice that in heavy shaded leaves. Low temperature-induced oxidative stress increases the concentrations of phenolic compounds and antioxidant activity (Blokhina et al. 2003). Our experiment suggests that tea plants exposed high irradiance in winter can have high concentrations of catechins. Among catechins, the concentrations of epigallocatechin (EGC) and epicatechin gallate (ECG) were more sensitive to the shading and low temperature than epicatechin (EC) and epigallocatechin gallate (EGCG) (Table 1). The concentration of EC and EGCG decreased from late November to early January, whereas EGC and ECG increased. Apart from their nutritional benefits to human health, catechins protect leaves from damage by UV light, and their synthesis is affected by light and temperature (Kleiner et al. 1999), and growing conditions (Wei et al. 2011). The biosynthesis of catechins changes differentially under light and dark conditions. The concentration of epicatechins (ECs) declined, while the concentration of catechins (Cs) increased in the dark (Hong et al. 2014). Catechins were the major polyphenol compounds induced by oxidative stress in photoinhibiting conditions during overwintering (Powles 1984; Suzan et al. 1989; Wei et al. 2011). The result implies the tea plants increase the production of antioxidants that protect tissues from photoinhibition by accompanying a reduced growth instead of dead.

Conclusion

The results of this study showed that shading only have a small effect on the growth of tea plants growing in Korea from October to March. In contrast, concentration of chlorophyll and chlorophyll fluorescence were higher in shaded than in non-shaded leaves. High chlorophyll level of shaded leaves were associated with less inhibition of leaves in winter. Chlorophyll fluorescence that low in all treatments in late January and higher in November and April were higher in shaded leaves than semi-shaded and unshaded leaves. The concentration of total catechins was higher in unshaded than in shaded leaves, and lower in early January than at other times. Low temperatures appear to reduce potential photosynthesis in tea independently of light levels. Growth is less sensitive to light during the cold season than the synthesis of catechins.

Acknowledgements

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