

*Full Length Research Paper*

## Effect of ultraviolet B irradiation on accumulation of catechins in tea (*Camellia sinensis* (L) O. Kuntze)

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**Effect of UV-B irradiation time on accumulation of foliar catechins in two tea cultivars was investigated. Low influence rate and short term irradiation of UV-B stimulated accumulation of major tea catechins, resulting in an increase in level of total catechins. Excessive irradiation of UV-B suppressed the accumulation of tea catechins. Epigallocatechin gallate (EGCG) increased more quickly than the other catechins under appropriate UV-B irradiation. The difference in response of different tea cultivars to UV-B is discussed.**

**Key words:** *Camellia sinensis*, UV-B, catechins, tea cultivar, influence rate, irradiation time.

### INTRODUCTION

Tea catechins are flavonoid phytochemical compounds that appear predominantly in tea leaf with bitter and astringent taste as well as astringency. Due to their potent health benefits such as cardioprotective, chemoprotective, antimicrobial and preventing allergy properties (Esimone et al., 2006; Karori et al., 2007; Mbata, 2007; Mbata et al., 2008; Oyejide and Olushola, 2005; Borthakur et al., 2008), tea is increasingly being accepted by consumers. The major catechins in green tea are epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), epicatechin (EC), gallic acid (GA), gallic acid gallate (GCG), catechin gallate (CG) and catechin (C). The concentration of catechins in tea leaf depends on many factors such as tea cultivar, fertilizer application and environmental conditions.

Ultraviolet-B (UV-B, wavelengths 280-320 nm of light) has been confirmed to be harmful to living organisms and it is of concern that the amount of UV-B radiation reaching the earth's surface is increasing because of the depletion of the stratospheric ozone layer (Frederick et al., 1989). Because UV-B radiation leads to reduction in expression and synthesis of key photosynthetic proteins including the chlorophyll a/b-binding protein (Lhcb) and the D1 polypeptide of photosystem II (psbA), excessive UV-B compromises plant growth and its productivity (Stapleton,

1992; A-H-Mackerness et al., 1997). In responses to the UV-B radiation, plant increases expression of genes encoding enzymes involved in synthesis of protective phenolic sunscreens (Bieza and Lois, 2001). Tea catechins are a group of important sunscreens (Mamati et al., 2006). Understanding the effect of UV-B on the accumulation of catechins in tea plant may lead to development of techniques to increase levels of catechins in tea leaf.

The objective of this work was to investigate the effect of UV-B on the accumulation of catechins in tea leaf. The information would lead to development of techniques to regulate the biosynthesis and accumulation of catechins in tea.

### MATERIALS AND METHODS

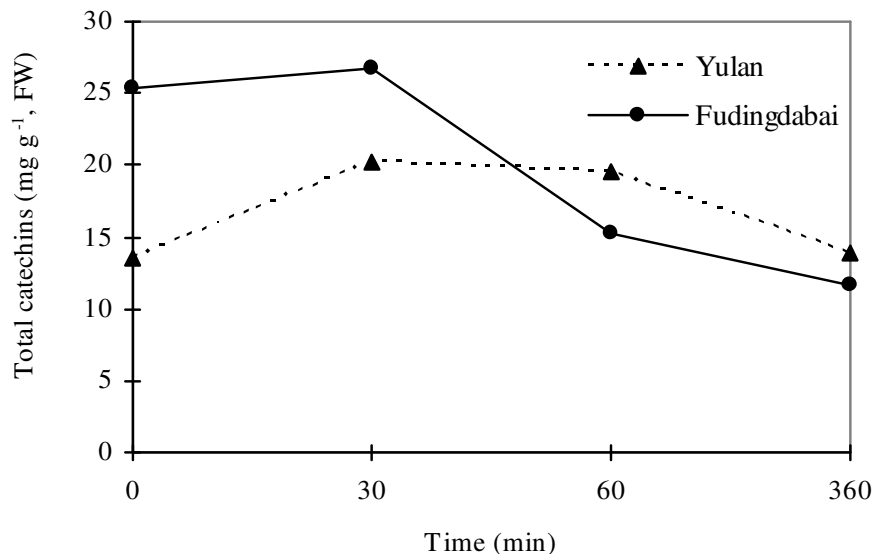
#### Plant materials

Plant materials used in the present study were one-year-old tea cuttings of *Camellia sinensis* cultivars Yulan and Fudingdabai which were planted in pots. To avoid the effect of UV-B from natural light and fluorescent lamp before UV-B treatment, the plants were placed in ZRX-300D growth incubators (Qianjiang Instrumental Corp., Hangzhou, China) under dark condition at 25°C for 3 days before UV-B irradiation treatment.

#### Test of different UV-B irradiation times

The plants were given UV-B irradiation (fluence rate 1.7  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) from four UV-B lamps (Model BLE-1T158, Spectronics corp., West-

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**Figure 1.** Effect of UV-B irradiation time on total concentration of catechins (UV-B influence rate:  $1.7 \mu\text{mol m}^{-2} \text{s}^{-1}$ ).

bury, New York, USA) for 360 min. The shoot comprising two leaves and a bud were sampled when the irradiation time was at 0, 30, 60 and 360 min. Two samples (one shoot for each sample) were taken from each treatment at the sampling time.

#### Test of different UV-B influence rates

Different influence rates were obtained by regulating the lamp number and distance between the plants and the lamps. The plants were irradiated by UV-B influence rates of 0.2, 0.4, 1.0 and  $1.7 \mu\text{mol m}^{-2} \text{s}^{-1}$  for 360 min and then sampled as above.

#### HPLC analysis of catechins and caffeine

Tea sample (0.2 g fresh weight, FW) was homogenized and extracted in a glass homogenizer with 4 mL of 4°C methanol solution (70%, v/v) for 3 min. The tissue homogenate was transferred into a 10 mL centrifuge tube and centrifuged at  $7000 \times g$  and 4°C for 15 min. The supernatant was used for HPLC analysis. The HPLC was carried out as methods described by Liang et al. (2003).

#### Data analysis

Mean values of the two samples for each treatment were presented and Tukey's test was carried out on Software of SAS for windows 8.0 (SAS Institute, Raleigh, N.C. USA).

## RESULTS AND DISCUSSION

### Effect of UV-B irradiation time on concentration of tea catechins

Figure 1 showed that under UV-B fluence rate  $1.7 \mu\text{mol m}^{-2} \text{s}^{-1}$ , total concentration of catechins (total catechins) increased during the early 30 min and then decreased during the late irradiation time. The two cultivars tested

showed a similar trend in the total level of catechins though the initial concentration of total catechins in cultivar Fudingdabai was higher than cultivar Yulan. However, the decreasing rate in total catechins of cultivar Fudingdabai was more quickly than cultivar Yulan after 30 min UV-B irradiation. Changes in composition of individual catechins revealed that epigallocatechin gallate (EGCG) in the two cultivars increased markedly during the early 30 min UV-B irradiation and the changes in other individual catechins differentiated with cultivars (Table 1). In cultivar Yulan, levels of epigallocatechin (EGC), epicatechin (EC), gallic acid (GA), gallic acid gallate (GCG), epicatechin gallate (ECG) and catechin gallate (CG) reached the highest levels at 60 min UV-B irradiation but gallic acid (GA) and catechin (C) showed a decreasing trend during the 360 min UV-B irradiation. In cultivar Fudingdabai, concentrations of gallic acid (GA) and epicatechin (EC) showed a similar tendency as EGCG, being the highest at 30 min UV-B irradiation. Catechin gallate (CG) showed an increase trend but epigallocatechin (EGC), catechin (C), gallic acid gallate (GCG) and epicatechin gallate (ECG) showed a decreasing trend during the entire 360 min UV-B irradiation in Fudingdabai (Table 1).

### Effect of UV-B influence rate on concentration of tea catechins

Total concentrations of tea catechins increased at low UV-B influence rates but decreased at high UV-B influence rates (Figure 2). However, the responses of tea cultivars to UV-B influence differed. Level of total catechins in cultivar Fudingdabai increased till UV-B influence rate was up to  $1.0 \mu\text{mol m}^{-2} \text{s}^{-1}$  while that in cultivar Yulan declined when UV-B influence rate was more than  $0.4 \mu\text{mol}$

**Table 1.** Effect of UV-B irradiation time on total concentration of catechins ( $\text{mg g}^{-1}$ , FW)<sup>a</sup>.

Cultivar	Time (min)	GC	EGC	C	EGCG	EC	GCG	ECG	CG
Yulan	0	3.88±0.05	0.54±0.04	1.20±0.01	1.73±0.03	2.25±0.05	0.51±0.03	2.50±0.43	0.93±0.03
	30	2.79±0.23	1.34±0.03	1.17±0.04	8.46±0.10	3.06±0.07	0.59±0.04	2.61±0.06	0.14±0.01
	60	3.18±0.05	2.89±0.06	0.64±0.02	5.69±0.01	3.48±0.04	0.69±0.03	2.61±0.05	0.41±0.03
	360	1.67±0.08	2.00±0.05	1.16±0.02	3.05±0.02	2.63±0.03	0.59±0.17	2.57±0.11	0.26±0.11
Fudingdabai	0	2.85±0.23	1.72±0.30	1.19±0.03	3.62±0.13	4.30±0.13	0.91±0.03	10.68±0.73	0.17±0.02
	30	3.00±0.07	0.80±0.04	1.08±0.09	9.50±0.15	4.97±0.06	0.59±0.04	6.55±0.02	0.27±0.19
	60	1.64±0.04	1.01±0.11	0.98±0.04	3.07±0.03	2.75±0.06	0.71±0.01	4.68±0.05	0.42±0.01
	360	2.47±0.01	0.71±0.01	1.05±0.03	1.54±0.03	0.38±0.01	0.59±0.01	4.20±0.04	0.71±0.03

<sup>a</sup>UV-B influence rate  $1.7 \mu\text{mol m}^{-2} \text{s}^{-1}$ .

GC: galliccatechin; EGC: epigallocatechin; C: catechin; EGCG: epigallocatechin gallate; EC: epicatechin; GCG: galliccatechin gallate; ECG: epicatechin gallate; and CG: catechin gallate.

**Table 2.** Effect of UV-B influence rate on total concentration of catechins ( $\text{mg g}^{-1}$ , FW).

Cultivar	UV-B ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	GC	EGC	C	EC	EGCG	GCG	ECG	CG
Yulan	Control <sup>a</sup>	0.83±0.03	2.07±0.05	1.18±0.03	2.82±0.04	4.27±0.07	0.66±0.06	2.735±0.05	0.46±0.06
	0.2	2.48±0.03	1.68±0.04	0.72±0.02	3.61±0.06	26.42±0.72	0.29±0.04	3.67±0.06	1.18±0.04
	0.4	2.22±0.04	8.14±0.14	0.93±0.02	3.09±0.05	29.82±0.15	0.22±0.06	3.09±0.04	1.15±0.08
	1.0	0.63±0.03	4.82±0.13	0.4±0.05	2.81±0.14	27.5±0.30	0.17±0.01	3.43±0.03	1.1±0.01
	1.7	1.8±0.13	1.76±0.01	1.12±0.04	2.47±0.06	6.36±0.04	0.53±0.02	2.43±0.01	0.3±0.02
Fudingdabai	Control <sup>a</sup>	2.12±0.04	0.95±0.04	1.23±0.01	4.16±0.01	3.53±0.05	0.92±0.01	5.62±0.05	0.22±0.04
	0.2	2.08±0.03	2.99±0.07	0.68±0.01	3.66±0.01	12.93±0.10	0.67±0.01	7.76±0.02	0.06±0.01
	0.4	2.17±0.03	2.46±0.03	1.08±0.02	3.88±0.04	23.54±0.54	0.35±0.05	7.45±0.013	0.78±0.01
	1.0	2.01±0.08	4.81±0.12	0.81±0.07	0.56±0.06	29.76±0.01	0.17±0.07	5.66±0.30	1.22±0.18
	1.7	2.59±0.18	0.73±0.04	1.06±0.04	0.395±0.04	1.43±0.13	0.56±0.03	4.31±0.19	0.60±0.13

<sup>a</sup>The control plant was placed under dark conditions. The abbreviations were the same as Table 1.

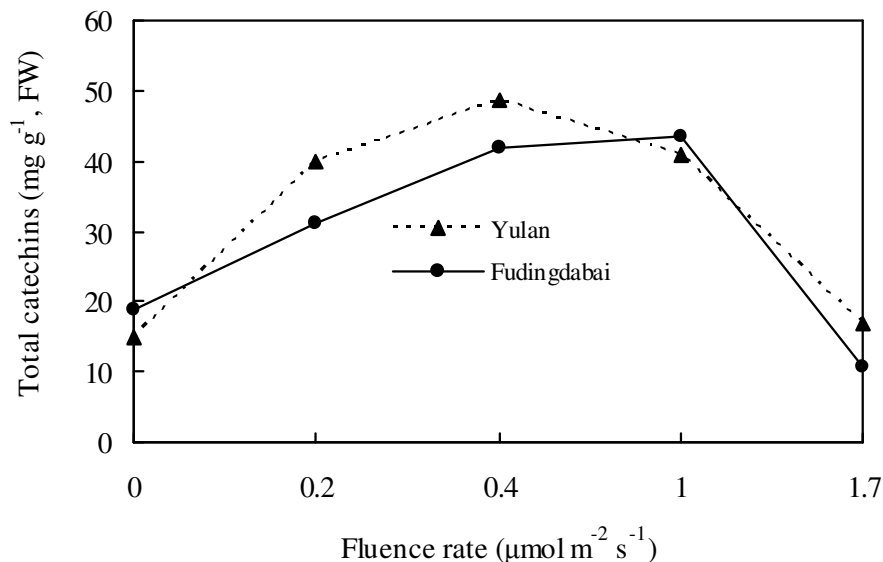
$\text{m}^{-2} \text{s}^{-1}$ . When the UV-B influence rate was higher than  $1.0 \mu\text{mol m}^{-2} \text{s}^{-1}$ , total catechins declined sharply, especially in cultivar Fudingdabai (Figure 2).

Changes in composition of individual tea catechins varied with cultivars. For cultivar Yulan, EGCG and EGC, the two major catechins, reached the highest level when UV-B influence

rate was  $0.4 \mu\text{mol m}^{-2} \text{s}^{-1}$ , and GC, EC, ECG and GC were the highest at UV-B of  $0.2 \mu\text{mol m}^{-2} \text{s}^{-1}$ , while C and GCG decreased with increase in UV-B influence rate. For cultivar Fudingdabai, EGCG was at the highest level at UV-B influence rate of  $1.0 \mu\text{mol m}^{-2} \text{s}^{-1}$ , and EGC and ECG were at the high level when UV-B influence rate was  $0.2 \mu\text{mol m}^{-2} \text{s}^{-1}$ . The other individual catechins

showed decline tendency with increase in UV-B influence (Table 2).

UV-B radiation has been confirmed to have physiological effects on plants, including suppression of photosynthesis, stomatal conductance and transpiration (Cechin et al., 2008) as well as down-regulation of photosystem II activity (Yang and Yao, 2008). These paralleled with high-



**Figure 2.** Effect of UV-B influence rates on total concentration of catechins (UV-B irradiation time: 360 min).

er levels of protective sunscreens. Tea catechins are a group of important protective sunscreen compounds (Mamati et al., 2004; Mamati, 2005). Chalcone synthase (CHS) is the first enzyme in the flavonoid-specific branch of the phenolpropanoid pathway during biosynthesis of flavonoids and catechins. It was confirmed that UV-B elevated levels of expression of CHS mRNA and accumulation of flavonoids and sinapates flavonoids (Bieza and Lois, 2001; Dixon and Paiva, 1995). The UV-B stimulated the expressions of genes related to enzymes involved in biosynthesis of catechins and resulted in elevation in accumulation of tea catechins during the early stage of UV-B irradiation. However, excessive UV-B irradiation damaged the plants and partial catechins were consumed as sunscreens, resulting in decrease in level of total catechins. The quick decline of total catechins in cultivar Fudingdabai after 30 min UV-B irradiation suggests that it was more sensitive to UV-B damage than cultivar Yulan. Examination of leaf structure found that the epidermal wax layer on leaf of cultivar Yulan was thicker than that of cultivar Fudingdabai. The difference in UV-B tolerance between the two cultivars might be related to epidermal structures of their leaves.

## Conclusion

The above study showed that low influence rate and short period UV-B irradiation stimulated accumulation of major catechins, resulting in increase in level of total catechins. However, excessive UV-B irradiation including high influence rate and long period irradiation, suppressed the accumulation of tea catechins. Tables 1 and 2 showed that EGCG increased more quickly than the other

catechins under appropriate UV-B irradiation. EGCG was one of the most important antioxidants in tea leaf. This finding will be interesting for developing techniques to elevate EGCG level in tea leaf by artificial supplement of UV-B prior to leaf harvest. The UV-B influence and treatment time should be varied with cultivar when applied in field-grown tea plants. Based on the present study, 30-min treatment using UV-B influence less than  $1.7 \mu\text{mol m}^{-2} \text{s}^{-1}$  will be a choice.

## ACKNOWLEDGMENT

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