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Full Length Research Paper

Alleviation of UV-B stress in *Arabidopsis* using tea catechins

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Ultraviolet-B (UV-B) 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. Effect of different levels of tea catechins on morphological damage and expression of chalcone synthase gene in *Arabidopsis thaliana* plants exposed to UV-B were investigated. Tea catechins at levels 250 - 1000 mgL^{-1} screened off UV-B resulting in less expression of chalcone synthase gene and lighter stress of the UV-B exposed plant. The application of tea catechins was propitious to the recovery of the UV-B damaged plants, especially at concentration 250 mg L^{-1}. The protective mechanism of tea catechins against UV-B stress is considered to be their sunscreen property instead of their free radical scavenging and antioxidant properties.

Key words: Tea catechins, *Arabidopsis*, UV-B, chalcone synthase, sunscreen, gene expression.

INTRODUCTION

Sunlight plays an important role in the development of higher plants (Batschauer et al., 1996). However, it comprises high energetic photons in the short wavelength UV range which causes damage to plants (Stapleton, 1992). Ultraviolet radiation is a part of the non ionizing region of the electromagnetic spectrum of the solar radiation. UV-C region of the spectrum comprises light of wavelength below 280 nm which is highly absorbed by the ozone in the stratosphere and rarely reaches the earth's surface. UV-B, a light spectrum with 280 - 320 nm, is attenuated by the ozone layer and its reach to the earth's surface is increasing because of depletion of the stratospheric ozone layer (Frederick et al., 1989; Stolarski et al., 1992; Caldwell et al., 1989). UV-C radiation with wavelength 320 - 390 nm is not attenuated by the stratosphere and hence it is unaffected by the changes in the ozone layer (Stapleton, 1992).

UV-B has greater damaging effects on living organisms because the cell macromolecules such as DNA and protein have strong absorption at 280 - 320 nm, compared to UV-A. Over exposure to UV-B radiation can cause skin damages from sunburn and premature wrinkling to carcinogenesis (Choquenet et al, 2008). UV-B can potentially interfere with growth, development, photosynthesis, flowering, pollination and transpiration of plant (Jansen et al., 2001; Surplus et al., 1998; Kliebenstein et al., 2002). Plants have developed mechanisms which protect themselves against UV-B since they are directly exposed to light on which they are dependent for photosynthesis. Plants are more resistant to UV-B because in part, they produce a variety of secondary metabolites that effectively absorb UV-B to prevent it from penetrating into the leaf mesophyll cells causing damage to macromolecules and cell organelles. Many of the UV-B absorptive secondary metabolites were biosynthesized from the phenyl-propanoid pathways (Stapleton and Walbot, 1994). Chalcone synthase (CHS) gene is a key gene encoding enzyme for the flavonoids biosynthesis pathway (Stapleton, 1992) and it is positively regulated by UV-B (Kliebenstein et al., 2002). The elevated accumulation of phenolic sunscreens may at least partly be caused by a constitutively elevated CHS transcript level (Bieza and Lois, 2001).

Tea catechins or tea polyphenols are products of the phenyl-propananoid and flavonoids pathways. Studies

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Table 1. Composition of tea catechins used (mg g^{-1})[a].

GC	EGC	C	EC	EGCG	GCG	ECG	CG	Total	Caffeine
172.22	192.56	55.66	109.98	70.12	28.00	39.15	9.17	676.85	0.74

[a]GC: (-)-Gallocatechin; EGC: (-)-epigallocatechin; C: (+)-catechin; EC: (-)-epicatechin; EGCG: (-)-epigallocatechin gallate; GCG: (+)-gallocatechin gallate; ECG: (-)-epicatechin gallate; CG: (+)-catechin gallate.

Table 2. Primers designed by the primer select program of the DNA star software.

Primer	Base sequence	Product (bp)
CHS forward primer	AGA GAC AGG CTC AGA GA	350
CHS reverse primer	CAG AGA AGC CAT GTA AG	
Actin forward primer	GTT GGG ATG ACC CAG AAG GA	500
Actin reverse primer	CTT ACA ATT TCC CGC TCT GC	

using animals and human have shown that both black and green tea polyphenols have protective effect against chemical and ultraviolet light-induced skin cancer (McKay and Blumberg, 2002). Tea catechins have been shown to have protective effects against UV-B when applied on animal skins (Pickard, 1996), however, their function in plants is unknown (Katiyar et al., 2001). In this investigation, we evaluated the effectiveness of different levels of tea catechins in the protection of *Arabidopsis* against UV-B damage. The alleviating effect of tea catechins on the UV-B stress was evaluated by assessing the morphological response patterns of the plants and the expression profiles of the inducible CHS gene.

MATERIALS AND METHODS

Materials

The plant used was *Arabidopsis thaliana* ecotype *Columbia.* The seed was aseptically inoculated on petri-dishes on ½ MS salts germination medium for 4 days at 4ºC under dark and then under a 12 h 3500 lux white light/dark regime at 20 ± 1ºC. The white light was supplied by cool-white fluorescent lamps filtered through a thick glass to remove light of wavelengths less than 320 nm. Eight day old rosettes were transplanted onto vermiculite medium containing 20% of compost and grown in a growth incubator (Model ZRX-1000DC, Qianjiang Instruments and Equipment Co. Ltd., Hangzhou, China) at 20 ± 1ºC under 12 h white light/dark regime and 75% relative humidity. Rosettes of 21 day old were used to do the tests.

The powder of commercial tea catechins was obtained from Hangzhou Sinotea Company Ltd. (Hangzhou, China). The chemical composition of the powder was analyzed by HPLC (Liang et al., 2007) and listed in Table 1. The catechins powder was dissolved in distilled water at concentrations of 250, 500 and 1000 mg L^{-1} before use.

UV-B treatment

The plants in the pots (3 pots each treatment) were sprayed with the above tea catechins solutions until the leaf surfaces were completely wet and distilled water was used as control. The plants were then left to stands under white light for up to 2 h before exposed to UV-B at 20 ± 1ºC. UV-B light was supplied from UV-B fluorescent lamps (BLE-IT158 Spectronics Corporation, Westbury, New York, USA) and its fluency was 1.2 KJ m^2 h^{-1}. Rosesette sampling was carried out just prior to initiation of UV treatment and thereafter at various intervals of time for a period of 24 h. The samples (100 mg each and 3 samples every treatment) were immediately frozen in liquid nitrogen and stored at -70ºC until used for RNA extraction. Following 6 h of UV-B treatment, the plants treated with different levels of tea catechins were left to recover under the previous growth conditions. The plants were visually scored for leaf damage and vigor after one week.

Recovery assessment

The plants exposed to UV-B for various time intervals were transferred back to the initial 12 h white light/dark regime at 20 ± 1ºC for seven days to recover growth. The recovery rate was calculated according to the percentage of the growth recovered plants to total plants exposed to UV-B.

Test of CHS gene expression

Total RNA was extracted from the rosesette samples using TRIzol Reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) following the manufacturers instructions. The total RNA was treated with DNaseI (Worthington Biochemical Corporation, Lakewood, NJ, USA) to remove DNA contamination and re-extracted with phenol/chloroform/iso-amyl alcohol (25/24/1) and precipitated using ethanol. Equal loading of the total RNA were checked using a spectrophotometer and by electrophoresis on 1.2 % agarose gels (Borthakur et al., 2008).

The cDNA was synthesized using First Strand cDNA Synthesis Kit (Biobasic Inc., Canada) following the manufacturers instructions. Approximately 3 µg total RNA and 0.5 µg oligo-pd$(T)_{18}$ were used in a 20 µl reaction volume. The reverse transcription was carried out with 20 units of Maloney Murine Leukemia Virus (MML-V) reverse transcriptase for 1 h and the reaction was stopped by incubating the cDNA mixture at 70ºC for 10 min.

Chalcone synthase (CHS) and actin genes primer sequences (Table 2) were designed from *Arabidopsis* cDNA in the GenBank. The primers were designed to amplify 350 and 500 bp cDNA fragments of CHS and Actin cDNA respectively. The reaction composition for PCR is presented in Table 3. The number of PCR cycles

Table 3. PCR composition.

Component	Final concentration
PCR buffer [50 mM KCl, 10 mM Tris-HCl (pH = 8.3)]	1X
$MgCl_2$	2 mM
dNTPs	200µM
Forward primer	0.5 µM
Reverse primer	0.5 µM
Taq polymerase	1 unit/25 µl reaction vol.
cDNA	1 ul/50 µl reaction vol.
PCR quality water	To make the reaction vol.

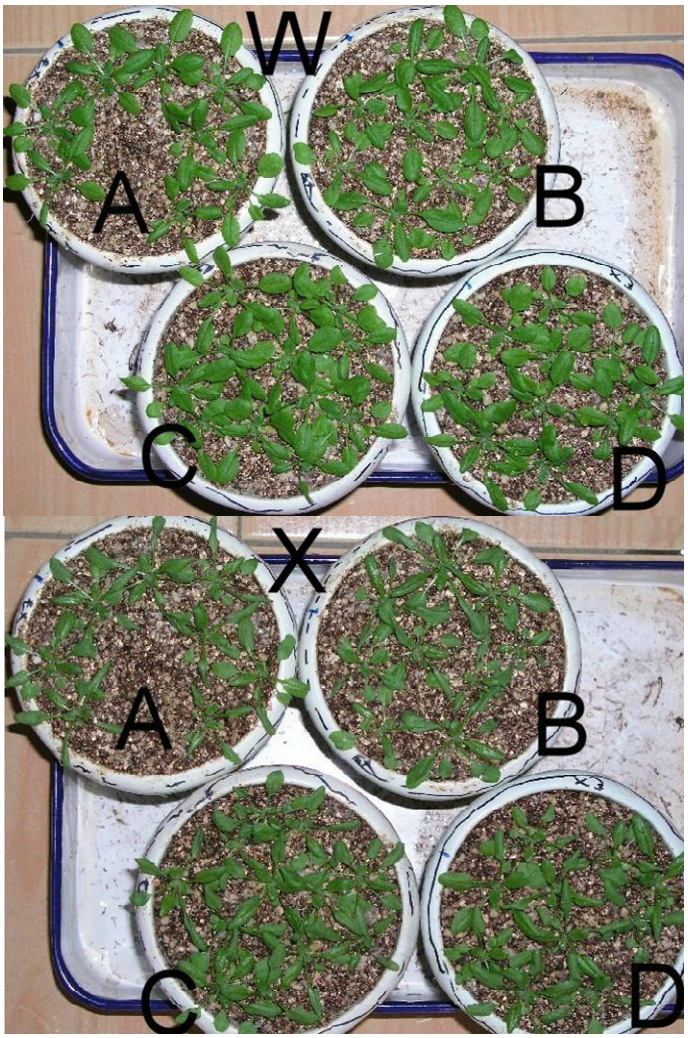

Figure 1. Variation in stress response of *Arabidopsis* plants exposed to UV-B. The levels of tea catechins: A = 0 mg L^{-1}; B = 250 mg L^{-1}; C = 500 mg L^{-1}; D = 1000 mg L^{-1}. UV-B exposure time: W = 6 h; X = 24 h.

was 30. The PCR was carried out in duplicates. Electrophoresis was carried out on 1.5% agarose gel using equal quantities (15 µl)of the PCR product. The representative and repeatable results were presented.

RESULTS AND DISCUSSION

Alleviation of UV-B stress by tea catechins in *Arabidopsis*

The degree of leaf damage increased with time of exposure to the UV-B. Effects of UV-B were initially visible on the plant leaves after 6 hours of UV treatment. Plants treated with catechins showed less stress than the control. However, the difference between various levels of tea catechins was not significant to be distinguished (Figure 1W). Clear variation in effects of different levels of catechins was realized after 24 h of exposure to UV-B. Leaves of plants treated with higher levels of catechins were less curled and more light dehydrated than the control (Figure 1X).

When the plants exposed to UV-B for various time intervals were transferred back to 12 h white light/dark regime at 20ºC to recover over a period of 7 days, most of the plants exposed to UV-B more than 12 h were highly scorched and dehydrated and died. The plants exposed to UV-B for 6 h or less partially recovered and those treated with catechins had greater recovery rate compared to the control plants (Figure 2, Table 4). The treatment of 250 mgL^{-1} catechins had the highest recovery rate and the control the lowest whereas those treated with 500 and 1000 mgL^{-1} catechins were in between (Table 4). These suggest that catechins had protective effects against UV-B damage, but high concentration of catechins was not favourable for the recovery growth of UV-B damaged plants.

Effect of tea catechins on expression of CHS gene

Reverse transcription PCR was used to determine the genetic response of the *Arabidopsis* plants to UV-B. Actin transcript was used for comparison of equal loading as well normalization between treatments for comparison of the expression profiles of chalcone synthase (CHS) gene. Equal loadings of RNA were obtained except for 24 h UV-B treatments according to the expression strength of Actin (Figure 3). Over exposure to UV-B might cause serious damage of the plant, resulting in the complete

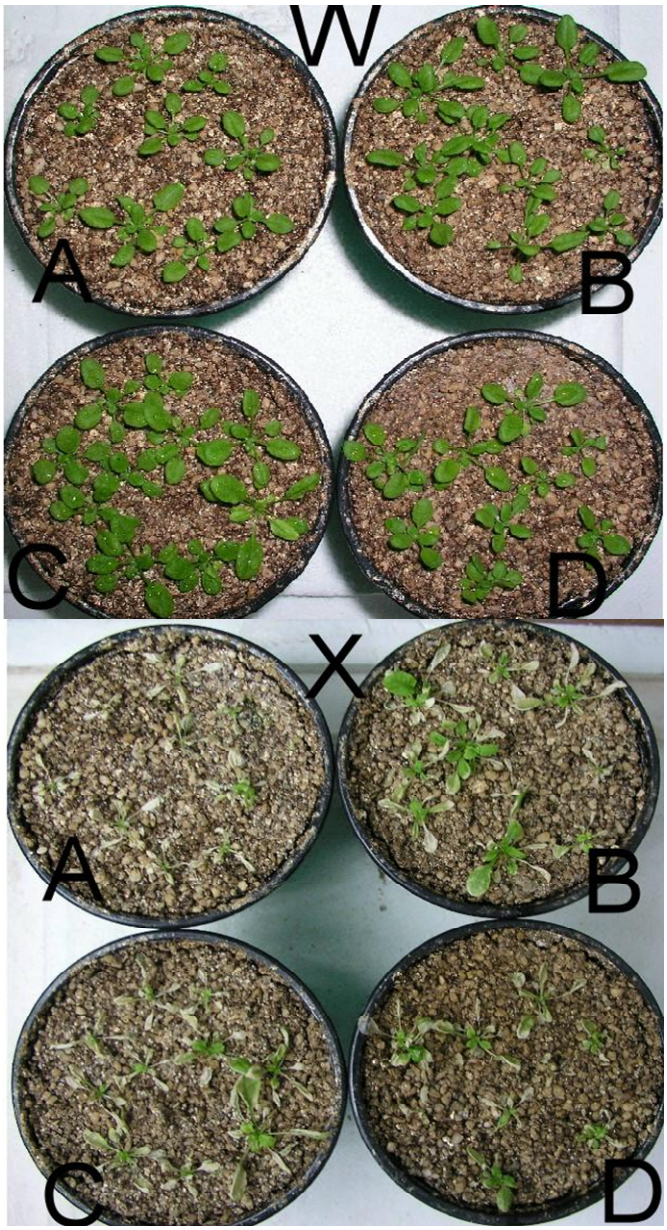

Figure 2. Variation in recovery of *Arabidopsis* plants after exposure to UV-B. The levels of tea catechins: A = 0 mg L^{-1}; B = 250 mg L^{-1}; C = 500 mg L^{-1}; D = 1000 mg L^{-1}. W: Before UV-B irradiation; X: The plants were exposed to UV-B for 6 h and then recovered under 12 h white light/dark regime at 20 ± ºC for 7 days.

suppression of RNA biosynthesis in the plants exposed to UV-B for 24 h.

Figure 3 showed that there was no expression of CHS gene when the plants were grown under white light condition. It confirmed that UV-B is a prerequisite for the inducible CHS expression. When the UV-B exposure was carried out for 2 h, the expressions of CHS were observed in all treatments and control, among which the control had the strongest expression. When the UV-B irradiation lasted for 6 h, the level of CHS mRNA accumulation in the control plant was still greater than the treatments of catechins. Among the catechins treated plants, the level of CHS mRNA decreased with increase in concentration of catechins (Figure 3). The CHS gene expression is induced by UV-B (Stapleton, 1992; Stapleton and Walbot, 1994; Kliebenstein et al., 2002). Less expression of CHS in plants treated with catechins indicates that the extract of tea catechins played a role as UV-B screen which absorbed UV-B and prevented it from penetrating into the leaf mesophyll cells, thus affecting the transcriptions of CHS mRNA. The decrease in level of CHS mRNA accumulation with the increase in concentration of catechins suggests that more UV-B was screened off by higher levels of catechins.

Table 4. Effect of tea catechins on recovery of UV-B stressed plants after seven days.

Tea catechins (mg L^{-1})	Recovery rate (%) [a]
0	17b
250	47a
500	39b
1000	32c

[a]The recovery rate was the percentage of the plants that recovered to total plants number exposed to UV-B. The data with different alphabetical letters were significantly different at ρ = 0.01.

However, extended period of exposure to UV-B beyond 12 h of treatment suppressed the expression of CHS gene, including those treated with catechins (Figure 3). It suggests that the self defense system of the plants such as enhancing CHS expression and biosynthesis of sunscreens were seriously destroyed by the over exposure to UV-B. This was consistent with the results of recovery growth, in which no plant was recovered from treatments beyond 12 h UV-B irradiation.

UV-B is known to induce formation of reactive oxygen species (ROS) and to influence the processes of biochemistry and physiology in plant, resulting in lower productivity of plants (Landry et al., 1995). The harmfulness of UV-B is alleviated by various plant adaptation mechanisms including the repair mechanisms following DNA damage (Schmitz-Hoerner and Weissenbock, 2003), elimination of the ROS (He and Hader, 2002) and prevention of penetration of the damaging radiation through absorption by accumulation of active compounds (Landry et al., 1995; Schmitz-Hoerner and Weissenbock, 2003). Animal test showed that green tea polyphenols scavenged ROS, inhibited cytochrome P-450 and DNA binding carcinogens (Picard, 1996). Tea extracts have been shown to have powerful antioxidant properties (Atawodi, 2005; Oyejide and Olushola, 2005; Chowdhury et al., 2006; Karori et al., 2007; Zahral et al., 2007) and to have free radical scavenging properties (Rice-Evans et al., 1997). The present study showed that tea catechins screened off UV-B, resulting in alleviation of UV-B stress and less CHS gene expression in *Arabidopsis* exposed to

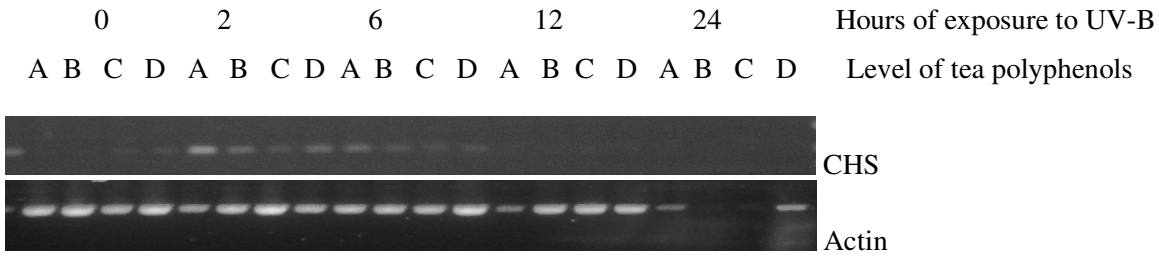

Figure 3. Expression profile of chalcone synthase gene in *Arabidopsis*. Levels of catechins: A = 0 mg L^{-1}; B = 250 mg L^{-1}; C = 500 mg L^{-1}; D = 1000 mg L^{-1}.

UV-B. However, the recovery of UV-B damaged plants was not correlated to the concentration of catechins used. The mechanism of protective effect of applied tea catechins against UV-B stress in *Arabidopsis* might be due to their UV-B screening property instead of their free radical scavenging and antioxidant properties.

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