

# Effects of temperature on UV-B-induced DNA damage and photorepair in *Arabidopsis thaliana*

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**Abstract:** DNA damage in the form of cyclobutane pyrimidine dimers (CPDs) and (6-4) photoproducts (6-4PPs) induced by UV-B radiation in *Arabidopsis thaliana* at different temperatures was investigated using ELISA with specific monoclonal antibodies. CPDs and 6-4PPs increased during 3 h UV-B exposure, but further exposure led to decreases. Contrary to the commonly accepted view that DNA damage induced by UV-B radiation is temperature-independent because of its photochemical nature, we found UV-B-induction of CPDs and 6-4PPs in *Arabidopsis* to be slower at a low than at a high temperature. Photorepair of CPDs at 24°C was much faster than that at 0°C and 12°C, with 50% CPDs removal during 1 h exposure to white light. Photorepair of 6-4PPs at 12°C was very slow as compared with that at 24°C, and almost no removal of 6-4PPs was detected after 4 h exposure to white light at 0°C. There was evidence to suggest that temperature-dependent DNA damage and photorepair could have important ecological implications.

**Keywords:** *Arabidopsis thaliana*; cyclobutane pyrimidine dimers (CPDs); DNA repair; (6-4) photoproducts (6-4PPs); temperature; UV-B radiation

## Introduction

There is growing awareness of the potential biological effects of stratospheric ozone depletion as part of global climatic change (Leun, 1995). The most important consequence of the ozone depletion is an increase in the amount of UV-B (280—315 nm) radiation reaching the earth's surface (Björn, 1999a). Even modest increase in UV-B radiation is likely to cause significant biological damage (Björn, 1996). Investigations during the past two decades has demonstrated that UV-B radiation has many direct and indirect effects on plants, including damage to DNA. The most common DNA lesion caused by exposure to UV-B is the formation of dimers between adjacent pyrimidines in the same strand, i. e., the cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidinone adducts (6-4PPs) (Britt, 1996). Unrepaired dimers are lethal to cells because they deform the DNA helix, interfering with both replication and transcription. Both types of DNA damage can be reversed by subsequent exposure to radiation ranged from 360—420 nm (UV-A to blue light). This phenomenon is termed photoreactivation or photorepair and is due to the actions of one or more proteins termed "photolysis". These enzymes specifically recognize and bind to pyrimidine dimers (Britt, 1999).

DNA damage and repair has been investigated in several plant species (Mitchell, 1993; Pang, 1991; Takeuchi, 1996; Taylor, 1996), but information on the effects of environmental factors such as temperature is limited. Interaction of increased solar UV-B radiation with other climatic change factors such as global warming is scarce. In the present study, we examined the temperature effects on the formation and photorepair of DNA damage induced by UV-B radiation in *Arabidopsis thaliana*.

## 1 Materials and methods

### 1.1 Plant material and growth conditions

*Arabidopsis thaliana* ecotype Columbia-0 (Col-0) was used in all experiments. Seeds were surface-sterilized with 75% ethanol, rinsed with water, and incubated for 2 d at 4°C, then distributed in commercial mixture medium and covered with glass for 48 h to ensure high humidity for an even germination. After growing for 10 d, young plants were transplanted to 6 cm × 6 cm plastic pots (5 plants in each pot) and grown in a greenhouse under 800 μmol/(m<sup>2</sup> · s) photosynthetically active radiation (PAR, 400—700 nm), supplied by 400 W dysprosium lamps (Osram Powerstar, Germany). Spectrum of this type of dysprosium was shown in our former paper (Li, 2002a).

Fully expanded leaves (4th—6th leaf) were used as plant materials. Detached leaves with abaxial surface up floating on distilled water in Petri dish, were exposed to UV-B radiation at a distance of 20 cm from the lamps, and without any other illumination.

### 1.2 UV-B and white light irradiation

UV-B radiation (also containing UV-A) was obtained from 6 UVB-313 lamps (Q-PANEL, USA) and filtered through 0.13 mm cellulose diacetate. All radiation below 280 nm was filtered out. Measurement of spectral irradiance was same as our previous report (Li, 2002b). Irradiance of the UV-B region (280—315 nm) was 2.95 W/m<sup>2</sup>. White light, 150 W/m<sup>2</sup> in the interval 400 to 700 nm, used for photorepair experiments, was supplied by a 400 W lamp (Osram Powerstar, Germany) and filtered through a 10 cm depth of water in a transparent polystyrene container to remove excess infrared radiation. Radiation measurements were carried out with a model 754—6S spectroradiometer (Optronic Laboratories, USA). Spectral irradiances of UV-B and white light for photorepair experiments were shown in our previous report (Li, 2002a; 2002b).

### 1.3 Temperature control

The effect of temperature on the induction and photorepair of DNA damage was tested at 0, 12, and 24°C. During exposure to UV-B radiation, the Petri dish was thermostated by recycling water bath. Immediately after irradiation, samples were harvested and frozen with liquid nitrogen. Samples were taken in three duplicates, and each temperature was tested in three independent experiments.

### 1.4 DNA extraction

DNA was extracted from frozen leaves as described in our previous report(Li, 2000). DNeasy Plant Mini Kit(Qiagen GmbH, Germany) was used to extract total DNA. Extraction steps were according to the protocol provided by Qiagen. The DNA extracted by the kit had A260/A280 ratios of 1.7–1.9 and the absorbance scan showed a symmetric peak at 260 nm, confirming high purity. The DNA concentration was determined by the absorbance at 260 nm. Samples were either processed immediately or stored at -20°C. No special precautions were needed to prevent photorepair of samples once they were frozen, as frozen ground samples were dropped directly into lysis buffer and photolyase is not active in lysis buffer.

### 1.5 DNA damage assay

CPDs and 6-4PPs were quantified by enzyme-linked immunosorbent assay(ELISA) according to Mori *et al.* (Mori, 1991) with monoclonal

antibodies from clones of KTM53 and KTM50, respectively. Both antibodies were supplied commercially by Kamiya Biomedical Company, USA. The monoclonal antibody from clone KTM 53 specifically recognizes thymine dimers in double- or single- strand DNA produced by UV-radiation, but does not react with 6-4PPs. The monoclonal antibody from clone KTM50 reacts specifically with 6-4PPs produced by UV-radiation, and does not react with thymine dimers. The detailed procedure was same as in our previous report(Li, 2000). Absorbance of the reaction mixture at 492 nm was measured with a microplate reader (Labsystems, Finland).

## 2 Results

### 2.1 UV-B induced DNA damage in *Arabidopsis thaliana*

*A. thaliana* plants grown in greenhouse were exposed to UV-B radiation in dark room at 24°C. Both types of dimeric pyrimidine photoproducts were induced in plant leaves. The CPDs content of leaves increased during 3 h UV-B exposure, and a smaller increase of 6-4PPs was observed(Fig.1). Further exposure of UV-B radiation led to decrease of both types of DNA damage. The decrease is probably due to photorepair activity driven by the UV-A radiation supplied together with the UV-B. It was deduced from our result that *A. thaliana*, as quantified by dimer formation in DNA, was very sensitive to UV-B radiation.

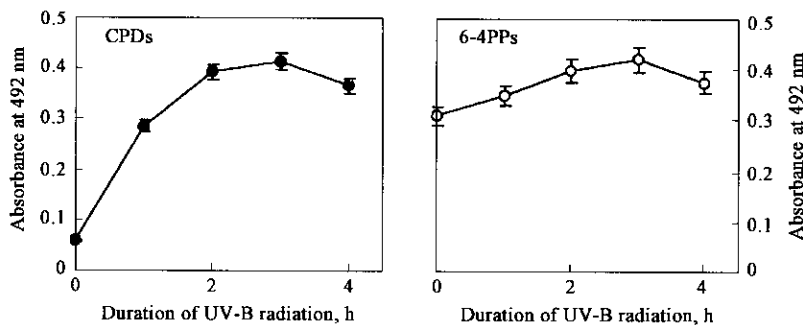


Fig.1 UV-B induced DNA damage in leaves of *Arabidopsis thaliana*. Plants were irradiated with UV-B for indicated time without any other illumination at room temperature(24°C). Fully expanded leaves(the 4th–6th leaves) were harvested and immediately frozen in liquid nitrogen for DNA extraction. To each microplate well 50 ng DNA were applied for ELISA assay of CPDs, and 100 ng DNA for 6-4PPs detection. Absorbance at 492 nm was used as a measure of antibody binding. Samples were taken in three duplicates, and each experiment was repeated for three times. Average values were given with standard errors

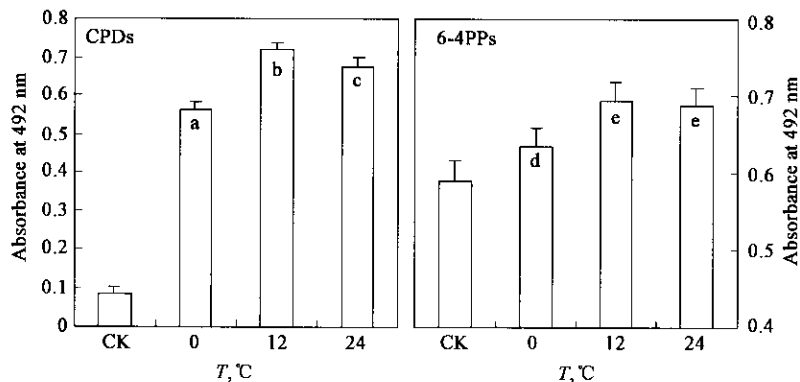


Fig.2 Effect of temperature on UV-B-induced DNA damage in *A. thaliana* leaves. Detached leaves were exposed to UV-B radiation for 2 h in darkness at different temperatures(0, 12 or 24°C). CK, as control, was kept at 24°C in dark room without UV-B radiation. Different letters under bars indicated significant difference at the  $P < 0.01$  level in the  $t$ -test; otherwise as for Fig. 1

## 2.2 Effect of temperature on DNA damage

As shown in Fig. 2, UV-B-induced DNA damage in *A. thaliana* depends on temperature. When detached leaves were exposed to UV-B radiation for 2 h at 12°C and 24°C, more CPDs and 6-4PPs accumulated than at 0°C ( $t$ -test:  $P < 0.01$ ), but the difference of 6-4PPs formation between at 12°C and 24°C was not significant. Both CPDs and 6-4PPs were induced by UV-B radiation even at 0°C.

## 2.3 Effect of temperature on the photorepair of DNA damage

Removal of CPDs and 6-4PPs in detached *A. thaliana* leaves at 24°C was more efficient than at 12 and 0°C. At the same temperature, removal of 6-4PPs is less efficient than that of CPDs (Fig. 3). During 1 h at 24°C about 50% of the CPDs, but only 13.3% of the 6-4PPs were photorepaired. Lowering of the temperature decreased the photorepair of both types of DNA damage. At 0°C 32% of the CPDs but almost no 6-4PPs were removed during 4 h white light exposure. Photorepair of the DNA damage in *A. thaliana* was strongly temperature-dependent, as expected for enzymatic processes.

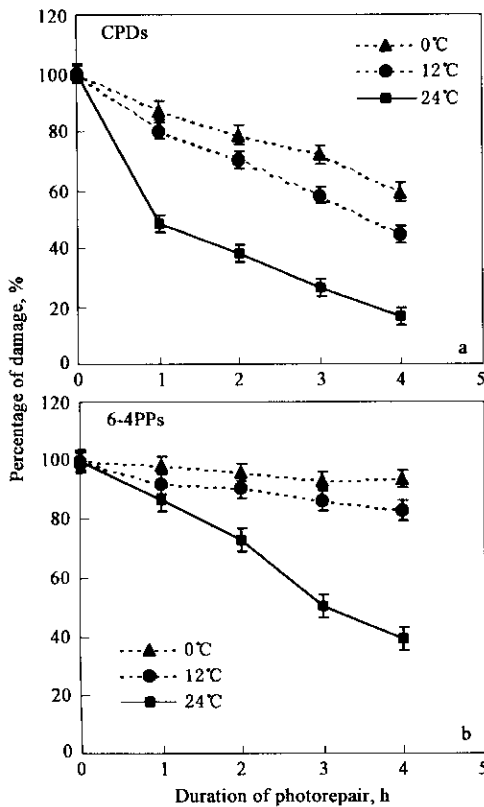


Fig. 3 Effect of temperature on the photorepair of DNA damage in *Arabidopsis thaliana* leaves. Detached leaves were exposed to UV-B for 2 h and subsequently irradiated with white light for indicated time at different temperatures, otherwise as for Fig. 1

## 3 Discussions

Temperature is one of the major environmental factors controlling survival, growth, reproduction, and thus geographic distribution of plants. The study of combined temperature and UV-B radiation could be of importance with respect to possible effects of climatic change, especially global warming and increasing levels of UV-B radiation caused by the depletion of stratospheric ozone layer. Caldwell (Caldwell, 1994) stated that UV-B radiation may render plants more resistance to heat

stress. Inhibition of chlorophyll synthesis and photosynthesis by UV-B in cucumber seedling was less pronounced at 25°C as compared to 20°C (Takeuchi, 1993). The present investigation provided molecular evidence for temperature-dependence of UV-B-induced DNA damage and photorepair.

Although much is known about UV-induced DNA damage and subsequent repair in microorganisms and in mammalian cells, we know considerably less about process in plants (Landry, 1997). UV-B induced DNA damage have been reported in a variety of plants (Pang, 1991; Quaitie, 1992; Stapleton, 1997; Taylor, 1996), but no clear pattern emerges from the literature with respect to temperature effects on the formation of DNA damage. It was recently reported no significant differences between temperatures over the range 0–25°C in the amounts of CPDs and 6-4PPs induced by UV-B in *Palmaria palmata* (a macroalgae) (Pakker, 2000). In the present study, we found that accumulation of DNA damage in detached *A. thaliana* leaves was temperature-dependent, with less damage at 0°C than 12°C or 24°C (Fig. 1). Temperature-dependent UV-B-induced DNA damage has been confirmed by our research group in tobacco leaf discs (Li, 2002b) and suspension-cultured cells (Li, 2002a). This discovery could be of important ecological implications. In natural ecosystem, low temperature often occurs at high altitude where solar UV-B radiation is higher than regular level, particularly in winter and early spring when ozone layer depletion is more serious than other seasons in a year. Even if radiation levels are low in high latitudes, radiation damage there may be important, because the low temperature there inhibits photorepair.

It was observed in our investigation that 6-4PPs is not photorepaired as rapidly as the CPDs, and that efficiency of photorepair of both type of dimers significantly decreased at lower temperature (Fig. 3). Photorepair of 6-4PPs practically came to a halt at 0°C, which indicated that this lesion can become particularly important in cold climates. Photorepair at low temperature of 0°C was apparently inhibited, damage formation of both types of dimers was decreased (Fig. 2 and Fig. 3), which could be interpreted as plant adaptation to its environment. Difference of the repair capacity could contribute to changes in species distribution in areas affected by the enhanced solar UV-B radiation.

To understand how increased solar UV-B radiation may affect ecosystem structure, a much wider survey of UV-induced DNA damage and repair in more species will be required. Research at the ecosystem-level for solar UV-B is barely beginning. In addition to temperature, numerous environmental factors may weaken or enhance the responses of plants to UV-B radiation. Therefore, experimentation on different environmental factors is essential if the ecological effects of enhanced UV-B radiation are to be fully evaluated.

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## References:

Björn L O, Callaghan T V, Gehrke C *et al.*, 1999a. Ozone depletion, ultraviolet

- radiation and plant life[J]. *Chemosphere: Global Change Science*, 1: 449—454.
- Björn L O, 1999b. UV-B effects: receptors and targets[M]. In: *Concepts in photosynthesis and photomorphogenesis*(Singhal G. S. *et al.* ed.). New Delhi, India; Narosa Publishing House. 821—832
- Björn L O, 1996. Effects of ozone depletion and increased UV-B on terrestrial ecosystems[J]. *Intern J Environmental Studies*, 51: 217—243.
- Britt A B, 1999. Molecular genetics of DNA repair in high plants[J]. *Trends Plant Sci*, 4: 20—25.
- Britt A B, 1996. DNA damage and repair in plants[J]. *Ann Rev Plant Physiol Plant Mol Biol*, 47: 75—100.
- Caldwell M M, 1994. Modification of the cellular heat sensitivity of cucumber by growth under supplemental ultraviolet-B radiation[J]. *Plant Physiol*, 104: 395—399.
- Jansen M, Gaba V, Greenberg B, 1998. Higher plants and UV-B radiation: balancing damage, repair and acclimation[J]. *Trends Plant Sci*, 3: 131—135.
- Landry L G, Stapleton A E, Lim J *et al.*, 1997. An *Arabidopsis* photolyase mutant is hypersensitive to ultraviolet-B radiation[J]. *Proc Natl Acad Sci USA*, 94: 328—332.
- Leun J, Tang X, Tevini M, 1995. Environmental effects of ozone depletion: 1994 assessment[J]. *AMBIO*, 24: 138—142.
- Li S, Paulsson M, Björn L O, 2002a. Temperature-dependent formation and photorepair of DNA damage induced by UV-B radiation in suspension-cultured tobacco cells[J]. *J Photochem Photobiol B: Biol*, 66: 67—72.
- Li S, Wang Y, Björn L O, 2002b. Temperature effects on the formation of DNA damage in *Nicotiana tabacum* leaf discs induced by UV-B radiation[J]. *Ecological Science*, 21: 115—117.
- Li S, Wang Y, Wang X *et al.*, 2000. Cyclobutane pyrimidine dimer accumulation in relation to UV-B sensitivity in rice cultivars[J]. *Acta Botanica Sinica*, 42: 576—581.
- Mitchell D L, Karentz D, 1993. The induction and repair of DNA photodamage in the environment[M]. In: *Environmental U. V. photobiology*(Young A. R., Björn L. O., Moan J., Nultsch W. ed.). New York; Plenum. 345—377.
- Mori T, Nakane M, Hattori T *et al.*, 1991. Simultaneous establishment of monoclonal antibodies specific for either cyclobutane pyrimidine dimer or(6 - 4) photoproduct from the same mouse immunized with ultraviolet-irradiated DNA[J]. *Photochem Photobiol*, 54: 225—232.
- Pakker H, Martins R, Boelen P *et al.*, 2000. Effects of temperature on the photoreactivation of ultraviolet-B-induced DNA damage in *Palmaria palmae* (Rhodophyta)[J]. *J Phycol*, 36: 334—341.
- Pang Q, Hays J B, 1991. UV-B-inducible and temperature-sensitive photoreactivation of cyclobutane pyrimidine dimers in *Arabidopsis thaliana*[J]. *Plant Physiol*, 95: 536—543.
- Quaite F E, Sutherland B M, Sutherland J C, 1992. Action spectrum for DNA damage in alfalfa lowers predicted impact of ozone depletion[J]. *Nature*, 358: 576—578.
- Stapleton A, Thornber C, Walbot V, 1997. UV-B component of sunlight causes measurable damage in field-grown maize (*Zea mays* L.); developmental and cellular heterogeneity of damage and repair[J]. *Plant Cell Environ*, 20: 279—290.
- Takeuchi Y, Murakami M, Nakajima N *et al.*, 1996. Induction and repair of damage to DNA in cucumber cotyledons irradiated with UV-B[J]. *Plant Cell Physiol*, 37: 181—187.
- Takeuchi Y, Ikeda S, Kasahara H, 1993. Dependence on wavelength and temperature of growth inhibition induced by UV-B irradiation[J]. *Plant Cell Physiol*, 34: 913—917.
- Taylor R M, Nikaido O, Jordan B R *et al.*, 1996. Ultraviolet-B-induced DNA lesions and their removal in wheat (*Triticum aestivum* L.) leaves[J]. *Plant Cell Environ*, 19: 171—181.

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