Temperature-dependent formation and photorepair of DNA damage induced by UV-B radiation in suspension-cultured tobacco cells

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Introduction

Damage induced by UV-B radiation can be classified into two categories: damage to DNA (which can cause heritable mutations) and damage to physiological processes (Stapleton 1992). The two most frequent types of DNA lesions induced by UV-B radiation are the cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidone adducts, commonly referred to as 6-4 photoproducts (6-4PPs). Repair of DNA damage to prevent the biological effects caused by UV-B radiation is essential for plants to survive. Both two types of lesions can be repaired by light-dependent enzymes called photolyases (a process called photorepair or photoreactivation). Although the existence of photorepair has been reported in many plants (Britt 1996) and the properties of plant photolyase were intensively investigated recently (Hada *et al*. 1999), information on the effects of environmental factors such as temperature on photo-reactivation is limited. At high latitudes and high elevations, temperature appears to be a major factor determining survival, growth, reproduction and thus geographic distribution of plants. Effects of temperature on DNA damage and repair may be critical for UV-B effects on plants in cold climates. In the present study, to estimate effects of temperature on DNA damage and repair, cell suspension cultures have been employed to avoid interference by other environmental and developmental factors.

Materials and Methods

Suspension culture of tobacco cells

Suspension cultures of *Nicotiana tabacum* cells (BY-2) were used for all experiments. The cells were cultured on a 8-day subculture interval in modified Murashige and Skoog (MS) medium. Suspension-cultured cells were grown in darkness at 24°C.

Light sources and irradiation

UV-B radiation, 1.74 W m-2 in the range 280-315 nm (also containing UV-A, 315-400 nm, 2.28 W m-2, but only 16 µW m-2 below 280 nm) was obtained from a 40 W Q-Panel UV313 lamp (Largo, Göteborg, Sweden) and filtered through 0.13 mm cellulose diacetate film. The radiation corresponds to 0.418 W m-2 at 300 nm as weighted by a DNA action spectrum. Fig. 1 shows the spectral irradiance of UV-B lamp with and without cellulose diacetate filtering. White light, 150 W m-2 in the interval 400 to 700 nm, used for photorepair experiment was supplied by a 400 W lamp (Osram Powerstar, HQI-T/Germany) and filtered through a 10-cm depth of water in a transparent polystyrene container to remove excess infrared radiation from the lamp. Fig. 2 shows the spectral irradiance of this white light. UV-C radiation was obtained from a low pressure mercury lamp with almost all emission, 4.39 W m-2, concentrated to 254 nm. This corresponds to about 175 W m-2 at 300 nm as weighted by a DNA action spectrum. Suspension cultured tobacco cells were irradiated in Petri dishes either with UV-B or UV-C at a distance of 20 cm from the lamps, and without any other illumination.

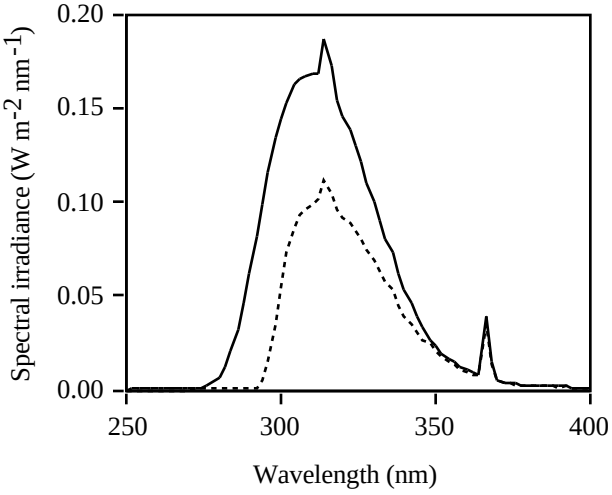

Fig. 1. Spectral irradiance of UV-B lamp with cellulose diacetate filter (broken line) and without any film (full line).

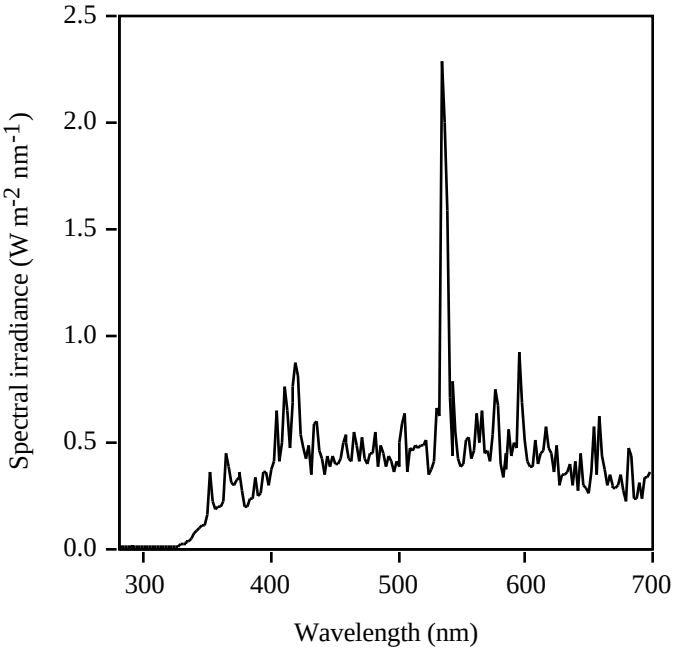

Fig. 2. Spectral irradiance of the white light.

Temperature control

Suspension-cultured tobacco cells were put in a 6-cm petri dish without lid for exposure to different light irradiation under darkness. Petri dishes were thermostated and gently agitated during irradiation exposure. The effect of temperature on the induction and photorepair of DNA damage was tested at 0, 12, 24°C. Tobacco cells were brought from culture room (24°C) to the experimental temperature in dark room with 5-min acclimation, then began light treatment.

Extraction of DNA

DNeasy Plant Mini Kit (Qiagen GmbH, Germany) was used to extract total DNA from tobacco cells.

Assay of DNA damage

The CPDs and 6-4PPs were quantified by enzyme-linked immunosorbent assay (ELISA) according to Mori *et al.* (1991) with monoclonal antibodies from clones of KTM53 and KTM50, respectively. Absorbance of reaction mixture at 492 nm was used as relative value of DNA damage.

Results

CPDs and 6-4PPs induced by UV-B in cultured tobacco cells

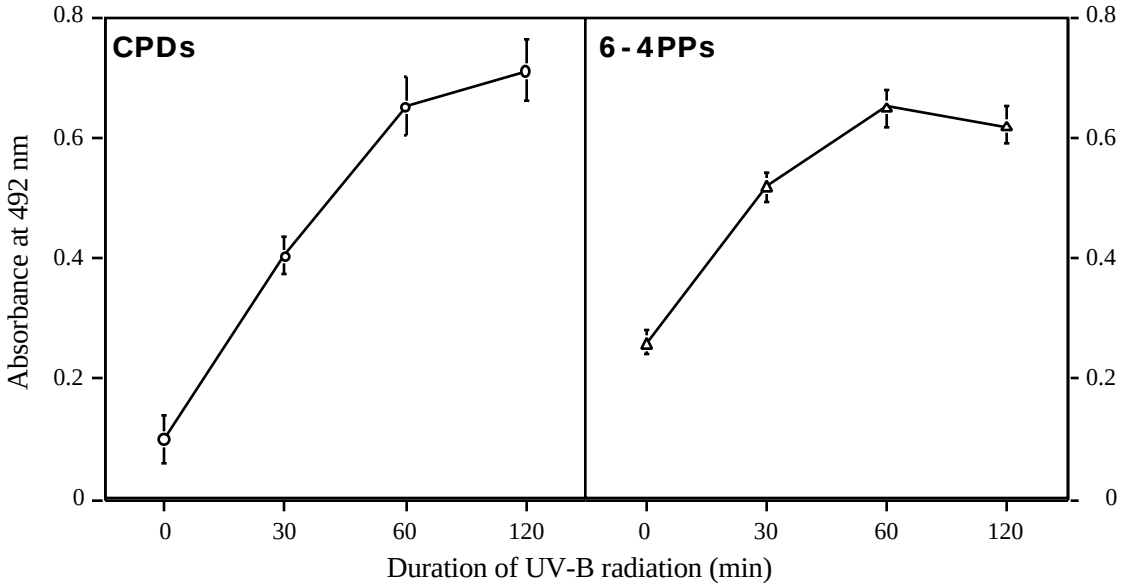

Fig. 3. UV-B induced formation of CPDs and 6-4PPs in tobacco cells. Cultured tobacco cells were irradiated with UV-B for indicated time at room temperature (24°C). To each microplate well 50 ng DNA were applied for ELISA assay of CPDs, and 100 ng DNA for 6-4PPs detection. Absorbance at 492 nm was used as a measure of antibody binding. Average values are given with standard deviations.

Effect of temperature on the formation of CPDs and 6-4PPs

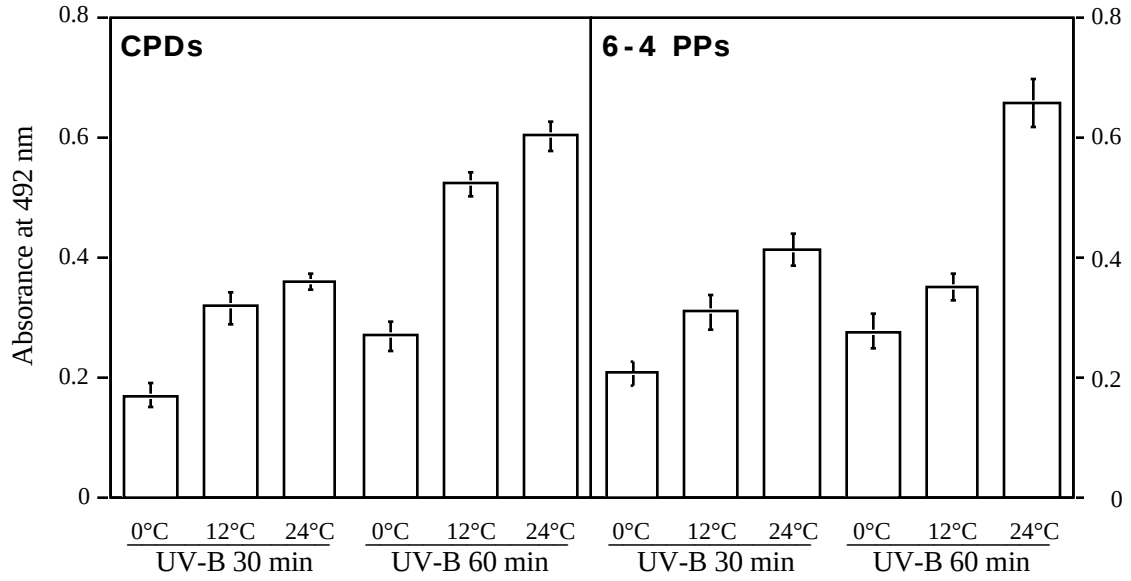

Fig. 4. Effect of temperature on UV-B-induced formation of CPDs and 6-4PPs in tobacco cells. Tobacco cells were exposed to UV-B radiation for 30 min or 60 min in darkness. Otherwise as for Fig. 3.

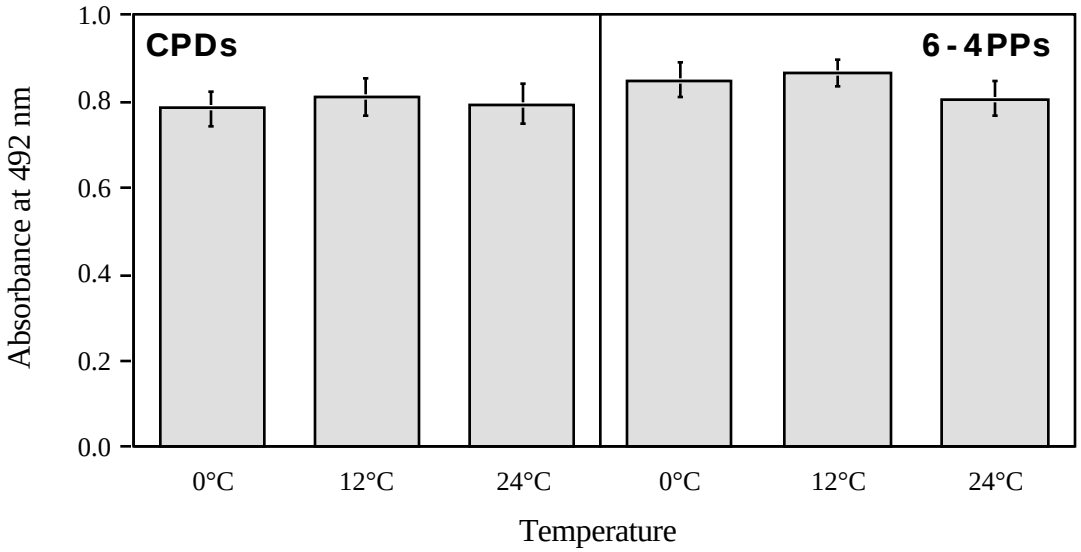

Fig. 5. DNA damage induced by UV-C radiation in cultured tobacco cells. Tobacco cells were exposed to UV-C radiation for 15 min at indicated temperatures in darkness. Immediatelyafterirradiation,tobacco cells were harvested for DNA extraction and photodamage assay. Otherwise as for Fig. 3.

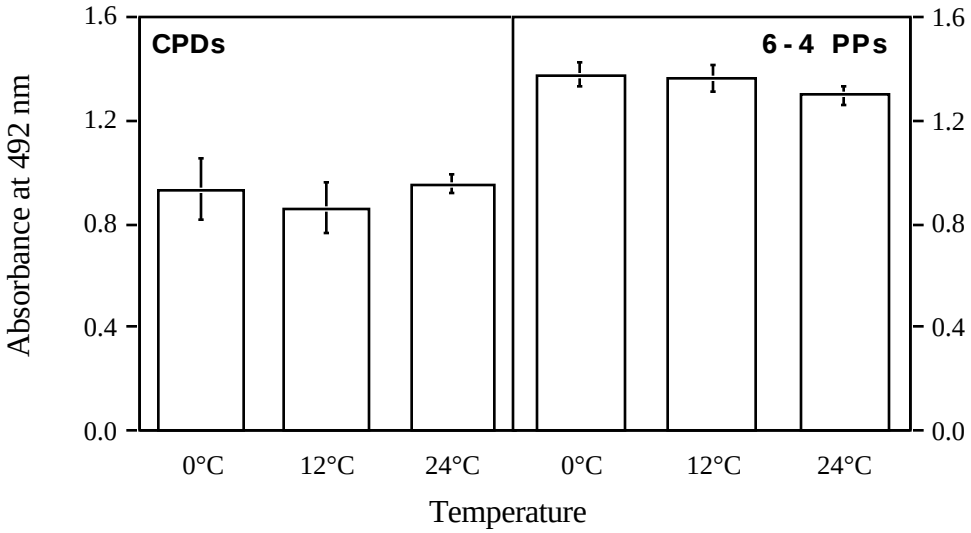

Fig. 6. Effect of temperature on the formation of photoproducts induced by UV-B radiation in DNA solution. Purified DNA in 10 mM phosphate buffered saline (PBS, pH 7.4) (30 µl, 50 ng/µl) from tobacco cells grown in darkness were exposed to UV-B radiation for 30 min at indicated temperatures under darkness. Otherwise as for Fig. 3.

Effect of temperature on the photorepair of CPDs and 6-4PPs

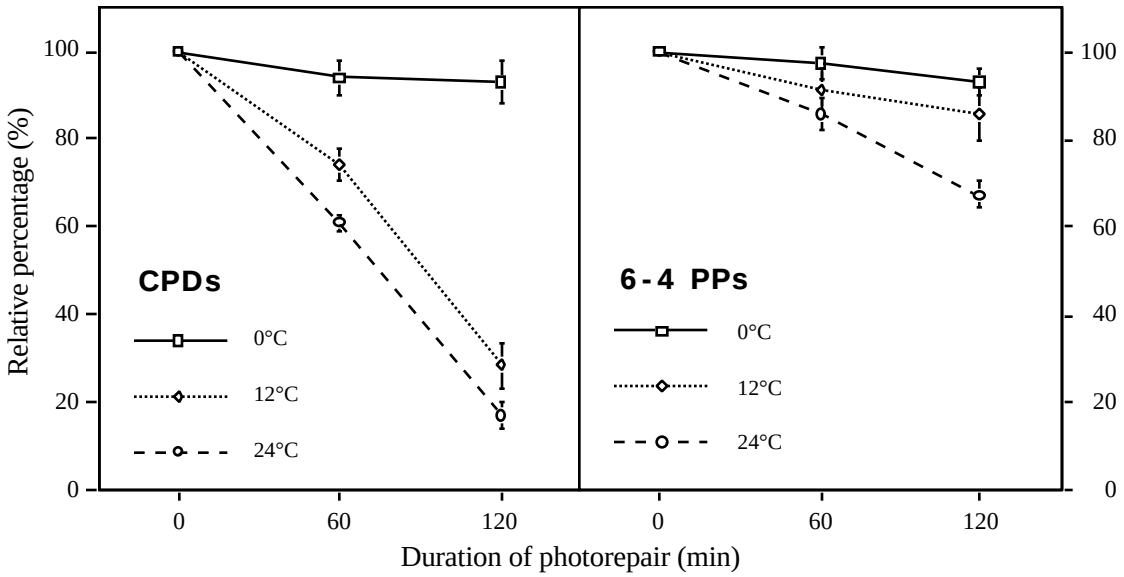

Fig. 7. Effect of temperature on the photorepair of CPDs and 6-4PPs in tobacco cells. Tobacco cells were irradiated with UV-B for 1 h and subsequently irradiated with white light for indicated time at different temperatures. Otherwise as for Fig. 3.

Conclusions

1. It was confirmed in the present investigation that CPDs and 6-4PPs were induced in tobacco cells by UV-B radiation, and the photorepair of CPDs is more efficient than that of 6-4PPs. This agrees with the situation in leaves from several plants. To our knowledge, this is the first report on DNA damage and photorepair detected in cultured plant cells.

2. UV-B radiation induces formation of CPDs and 6-4PPs in cultured tobacco cells even at 0°C, but low temperature significantly decreases the formation of DNA damage. Our results from cultured tobacco cells indicate that DNA damage induced by UV-B is temperature-dependent. One possible explanation for the temperature dependence is a more vulnerable state of DNA (such as DNA in uncoiled for gene transcription or cell division) at higher temperature.

3. Temperature has great influence on photorepair of DNA damage in tobacco cells. Low temperature significantly decreased the removal of CPDs and 6-4PPs, and there was no photorepair of CPDs and 6-4PPs detected at 0°C. Despite the low radiation levels of UV-B in subarctic and arctic regions, it is possible that any damage caused will not be repaired so effectively, because of the the inhibition of photorepair by low temperature.

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References

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