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Relationship between Ultraviolet-B Sensitivity and Cyclobutane Pyrimidine Dimer Photorepair in Rice

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Among *Indica* rice cultivars (*Oryza sativa* L. cvs.) that belong to the *aus* ecotype from the tropical Bengal region, where the amount of ultraviolet-B (UV-B) radiation in the solar radiation is relatively great, Marich-bati cultivar has exhibited resistance to UV-B radiation, while Surjamkhi cultivar appeared to be less resistant. We have examined the susceptibility to cyclobutane pyrimidine dimer (CPD) induction by UV-B radiation and the ability to photorepair CPDs using these two cultivars. UV-B radiation produced similar dimer levels in the leaves of the two cultivars. In contrast, the ability to photorepair CPDs in the UV-sensitive Surjamkhi cultivar was lower than that in the UV-resistant Marich-bati cultivar. These results were similar to our previous data, namely, that a UV-sensitive Japanese rice cultivar (*Oryza sativa* L. cv. Norin 1) cultivated in the moderate climate of Japan is deficient in its ability to photorepair CPDs. Thus, these results suggest that a strong correlation exists between the sensitivity to UV-B and the photorepair deficiency, and that a low ability in CPD photorepair may be a principal factor in determining the UV-B sensitivity in rice plants.

INTRODUCTION

The depletion of stratospheric ozone caused by the emission of chlorofluorocarbons and other trace gases has resulted in increases in the amount of UV-B radiation reaching the Earth's surface[1)]. Measurements of global ozone between 1979 and 1993 at high and mid-latitudes in both hemispheres[2)] indicate significant increases in UV-B radiation. UV-B radiation can damage plants, resulting in decreased growth and productivity of higher plants that are commercially valuable[3,4)]. Over a five-year period, we investigated the effects of supplemental UV-B radiation on the growth and yield of Japanese rice cultivars in the field in a cool rice-

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growing region of Japan. The findings of that study indicated that supplemental UV-B radiation has inhibitory effects on the growth and grain development of rice[5].

UV-B radiation is capable of directly altering the structure of DNA. Cyclobutane pyrimidine dimers (CPDs) that are formed between adjacent pyrimidines on the same DNA strand are major UV-induced DNA lesions in most organisms, including higher plants[6–8]. Such damage can be lethal or mutagenic to organisms, and can also impede replication and transcription. In rice plants grown under solar radiation, the CPD levels remain between 3 and 6 CPD Mb^{-1} ($1.3 \times 10^3 - 2.6 \times 10^3$ CPD per rice genome) throughout the day, and are elevated with UV-B supplementation (1 W m^{-2})[9]. Plants possess mechanisms to cope with such UV-induced DNA damage. Photorepair is the major pathway in plants for repairing CPDs (reviewed by Britt[8]). In photorepair, a single enzyme, photolyase is activated by light ranging from 300 nm to 600 nm to monomerize dimers.

Previous investigation of the sensitivity to UV-B radiation of 198 rice cultivars of five Asian rice ecotypes from the Bengal region, Indonesia, and Japanese lowland and upland yielded the following results: (1) the cultivars grown in Indonesia or on higher mountains in the Bengal region, where the amount of UV-B radiation in solar radiation is greater, were not always more resistant to UV-B, and there were differences in the UV-B sensitivity among cultivars that belonged to the same ecotype[10]. This result indicates that the differences in the sensitivity to UV-B radiation were not related to the geographical locations at which the cultivars are grown; (2) among Japanese rice cultivars, Sasanishiki is more resistant to UV-B radiation, while Norin 1 is less resistant, although these two cultivars are closely related[11,12]; (3) the photorepair capacity in UV-sensitive Norin 1 was lower than that in UV-resistant Sasanishiki[13–15]. However, the molecular origin of the difference in UV-B sensitivity among rice cultivars is unclear, as is whether other UV-sensitive rice cultivars also show reduced CPD photorepair.

We conducted a preliminary experiment on the sensitivity to UV-B radiation of *Indica* rice cultivars that belong to the *aus* ecotype from the Bengal region. It was found that Marichbati *Indica* cultivar was resistant to UV-B radiation and that the Surjamkhi *Indica* cultivar was more sensitive to UV-B radiation. In this work, we analyzed the susceptibility to CPD induction by UV-B radiation and the ability to photorepair CPDs, and investigated the relationship between UV-B sensitivity and photorepair ability using these two *Indica* rice cultivars.

MATERIALS AND METHODS

Plant materials and growth conditions

For an assessment of UV-B sensitivity, plants of two rice cultivars (*Oryza sativa* L. cv. Marich-bati and cv. Surjamkhi) were grown for 25 d in pots in vermiculite: fertilized soil (2:1, v/v) in a phytotron (Tabai Expec Ltd., Osaka, Japan) (12-h photoperiod, day/night temperatures 27/17°C). Plants were grown under metal halide lamps (400 W, MT400DL/BUD, Iwasaki Electric, Kyoto, Japan) providing 350 μmol PAR m^{-2} s^{-1}. Plants were grown under visible light with or without supplemental UV-B radiation from UV-emitting fluorescent tubes

(FL20SE; Toshiba, Tokyo, Japan) filtered through a UV29 cutoff glass filter (Toshiba Glass, Tokyo, Japan), above the plants. This filter reduced 290 nm radiation by 50%[16]. The UV-B intensity at the level of the plants was 1.12 W m^{-2}, as measured by a spectroradiometer (SS-25, Japan Spectroscopic, Tokyo, Japan).

A growth analysis included a determination of the number of tillers, and fresh and dry weights of the aerial parts of the plants. Experimental materials were dried at 80°C for 3 days, and then the dry weights were measured.

Experiments of CPD induction and photorepair of CPD were carried out using the fully expanded third leaves of these two cultivars. The plants were grown in pots of fertilized soil under visible light without any supplemental UV-B radiation in a phytotron, as described above.

Quantification of UV-absorbing compounds

The fully expanded third leaf was homogenized in 50 mM sodium phosphate buffer (pH 7.2) that contained glycerol (10%) and 2-mercaptoethanol (1%) at a ratio of leaf to buffer of 1:10 (g:ml) with a mortar and pestle. The UV-absorbing compounds were quantified using a method of Li et al[17]. An aliquot of the homogenate was extracted with 1% HCl in 70% methanol (final concentration) at 4°C for 24 h. The mixture was centrifuged at 1,800 *g* for 15 min, and the amount of UV-absorbing compounds in the supernatant (defined as the absorbance at 330 nm per unit of leaf fresh weight [g]) was determined spectrophotometrically (V-550 UV-visible spectrophotometer; JASCO Ltd. Co., Tokyo, Japan).

UV irradiation and photorepair

To induce CPD, the detached third fully expanded leaves were placed on wet filter paper and irradiated with unfiltered UV-B radiation emitted from a UV-fluorescent tube (FL20SE; Toshiba, Tokyo, Japan) at a rate of 0 to 13.4 W m^{-2} for 15 min. For repair experiments, the detached leaf was exposed to 9 kJ m^{-2} (10.0 W m^{-2} for 15 min) of UV-B radiation to induce approximately 30 CPD Mb^{-1}. The leaves were exposed to photoreactivating radiation from blue fluorescent tubes (20B-F, Toshiba, Tokyo, Japan) or kept in a light-tight box immediately after the UV-B exposure. The fluence rate of the blue radiation was adjusted to about 60 μmol PAR m^{-2} s^{-1}. After exposure of UV-B or blue radiation, the leaves were harvested immediately and stored in liquid nitrogen until being analyzed. All subsequent manipulations were carried out in red light to minimize uncontrolled photoreactivation. For an analysis of UV-induced CPD formation or photorepair, at least three independent experiments were carried out. The data taken from these experiments were averaged.

DNA extraction, preparation of agarose plugs, and treatment of rice DNA with UV endonuclease

Rice DNA extraction, the preparation of agarose plugs, and the treatment of DNA with UV endonuclease have been described in detail elsewhere[15]. In brief, the leaf was ground in a mortar and pestle in liquid nitrogen, and then mixed with 120 μl of lysis buffer (10 mM Tris-HCl, pH 7.5, 0.7 M EDTA, 2% sarcosyl, 13% mannitol, 1 mg ml^{-1} proteinase K [Boehringer

Mannheim, Indianapolis, IN, USA]). The slurry was mixed with an equal volume of 2% low-melting-point agarose (SeaPlaque; FMC, Rockland, ME, USA) in Tris-EDTA [TE] solution, and formed into plugs. The solidified plugs were incubated in a Lysis solution (10 mM Tris-HCl, pH 7.5, 0.5 M EDTA, 1% sarcosyl, 1 mg ml^{-1} proteinase K) at 45°C for at least 72 h, with daily changes of the buffer. The plugs were then rinsed with TE, and incubated with TE containing 2.5 mM phenylmethylsulfonyl fluoride (PMSF) at 45°C for 30 min. After a treatment with PMSF, the plugs were digested with UV endonuclease[18], as follows: the plugs were incubated in UV endonuclease buffer (1 mM DTT and 0.1 mg ml^{-1} BSA) for 1 h at 4°C, and then incubated in 20 μl of UV endonuclease buffer containing sufficient UV endonuclease to yield complete cleavage at all dimer sites (activity, 7×10^{14} CPD cleaved μl^{-1}) for 15 min on ice, and then incubated for 30 min at 37°C. Additional (1 μl) UV endonuclease was added and the plug was incubated for 30 min at 37°C. Duplicate plugs were incubated in the buffer without endonuclease under identical conditions. The reactions were stopped and the DNA was denatured by the addition of an alkaline stop mixture (0.5 M NaOH, 25% glycerol, and 0.25% bromocresol green) and incubation for 30 min at 37°C.

Alkaline biased sinusoidal field gel electrophoresis

Rice DNA was dispersed according to their single-strand molecular lengths by alkaline agarose gel (0.5%) electrophoresis, using static-field electrophoresis and biased sinusoidal field gel electrophoresis (BSFGE: Genofield; ATTO Co., Osaka, Japan)[14]. Molecular length markers were DNAs from *Hansenula wingei* (Bio-Rad, Hercules, CA, USA) chromosomes (smallest 1.05 Mb), T4 (170 kb), λDNA (48.5 kb), *Bgl* I digest of T4 (49.3, 40.9, 23.1, 21.0, 17.8, 12.7, and 1.3 kb), and *Hin*d III digest of λDNA (23.1, 9.4, 6.6, 4.3, and 2.3 kb). After electrophoresis, the gel was neutralized, stained with ethidium bromide, and destained.

Cyclobutane pyrimidine dimer analysis

The CPD frequencies were determined as previously described[14]. An image of the distribution of the fluorescence of ethidium bromide to DNA in the destained gels was recorded using a DNA damage analysis system made by Tohoku Electric Co. (Miyagi, Japan). CPD frequencies were calculated using the molecular length standards curve and the quantity of DNA at each migration position from the quantitative image data, as described by Quaite et al[19,20]. The CPD frequencies are expressed in units of CPD Mb^{-1}.

RESULTS AND DISCUSSION

The effects of supplemental UV-B radiation in visible light on the growth of the *Indica* rice cultivars Marich-bati and Surjamkhi were determined. Plants were grown for 25 days under visible light with or without supplemental UV-B radiation in a phytotron. Figure 1 shows the effects of supplemental UV-B radiation on the tiller number, fresh weight, and dry weight of the aerial parts of the two rice cultivars. Here, each relative value (%) is the ratio of the value for the indicated parameter for plants grown with supplemental UV-B to the value

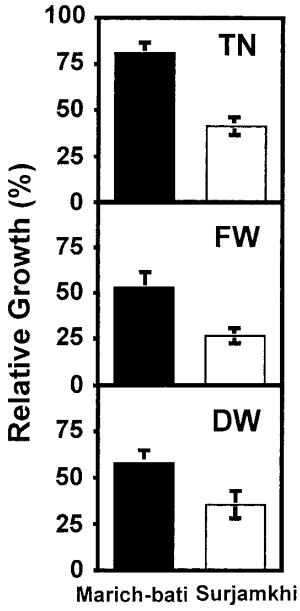

Fig. 1. Effects of supplemental UV-B radiation on the tiller number (TN), fresh weight (FW) and dry weight (DW) of the aerial parts for Marich-bati (closed columns) and Surjamkhi (open columns). Each relative value (%) is the ratio of the value for the indicated parameter for plants grown with supplemental UV-B to the value for plants of the same cultivar grown without supplemental UV-B radiation. Experimental plants were grown for 25 d in a phytotron under visible light alone or with supplemental UV-B radiation filtered through a UV29 cutoff filter.

for plants grown without supplemental UV-B radiation. A remarkable reduction in all of the growth parameters was observed in both cultivars grown under supplemental UV-B radiation. The degree of reduction in the growth parameters in Surjamkhi was significantly greater than that in Marich-bati. Therefore, it was confirmed that Surjamkhi exhibited higher sensitivity to UV-B radiation as compared with Marich-bati.

We next measured the susceptibility to CPD induction and the ability to photorepair CPD in both cultivars. For the analysis, fully expanded third leaves of these two cultivars were used as experimental materials, because the second, third, and fourth leaves of rice plants are more susceptible to CPD induction, and the ability to photorepair CPD in fully expanded leaves of different stages remains approximately constant[14].

To determine the susceptibility to CPD induction, the detached leaves were exposed to 0 to 12 kJ m^{-2} of challenge UV-B radiation, and then the CPD levels in the leaves were determined. Figure 2 shows the susceptibility to CPD induction by challenge UV-B radiation in the leaves of both cultivars. UV-B radiation produced similar CPD levels in both cultivars. There was no difference in the susceptibilities to CPD induction by challenge UV-B radiation between the two cultivars.

The accumulation of UV-absorbing compounds (such as flavonoids) in the vacuoles of the epidermal and subepidermal cell layers, which are thought to act as UV-B filters[1], play a role in coping with UV-induced DNA damage[21,22]. Furthermore, flavonoid-deficient mutants of *Arabidopsis* have been found to be hypersensitive to UV-B radiation[17,23,24]. Our previous data showed that there was a significant negative correlation between the CPD levels induced by challenge UV-B exposure and the amount of UV-absorbing compounds in leaves from Sasanishiki rice cultivar grown under various light environmental conditions[16]. Table 1 gives

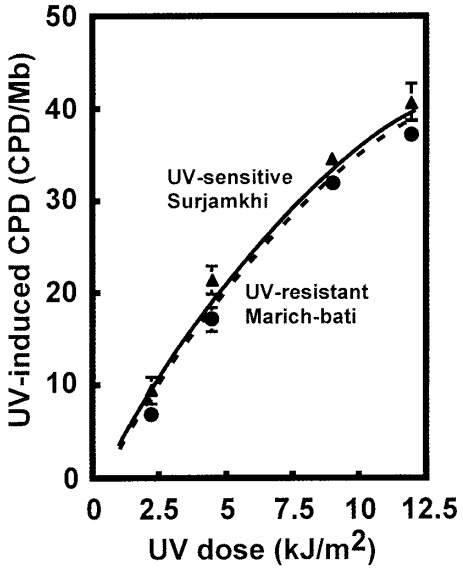

Fig. 2. Cyclobutane pyrimidine dimer (CPD) induction in third fully expanded leaves of UV-resistant Marich-bati (●) and UV-sensitive Surjamkhi (▲), as a function of exposure to UV-B radiation. When the third leaf was fully expanded, the leaf was harvested. The detached leaf was exposed immediately to 0 to 12 kJ m^{-2} of UV-B radiation. The leaf was harvested immediately after exposure to UV-B radiation, DNA was isolated, and then the CPD frequencies were determined by alkaline agarose electrophoresis. The points represent the means of at least three experiments and the vertical bars represent standard errors.

the levels of UV-absorbing compounds, assessed by measuring the absorbance at 330 nm of the acid-methanol fraction of the third fully expanded leaves between both cultivars grown without supplemental UV-B radiation. There was no significant difference in the levels of UV-absorbing compounds between the two cultivars (Table 1) and the absorption spectra between 280 and 600 nm of the acid-methanol fraction of both cultivars (data not shown). These results indicate that there were no significant differences in the dose of challenge UV-B radiation reaching to nuclear DNA between both cultivars. On the other hand, we previously found that the steady-state CPD levels in leaves of rice grown under chronic radiation in any culture were not so greatly influenced by the increased amounts of UV-absorbing compounds[16]. Thus, their importance in protection mechanisms against UV-induced biological

Table 1. The amounts of UV-absorbing compound assessed by measuring the absorbance at 330 nm of the acid-methanol fraction of third fully expanded leaves of UV-B-resistant Marich-bati and UV-B-sensitive Surjamkhi

	The amounts of UV-absorbing compounds	
Cultivar	UV-B-resistant Marich-bati	UV-B-sensitive Surjamkhi
OD330/ leaf fresh weight [g]	53.9 ± 3.4	58.1 ± 3.6

damage in rice plants has been questioned. These problems should be examined in future studies.

We determined the abilities to photorepair and excise CPDs at the fully expanded third leaves of these two cultivars (Fig. 3.). The detached leaves were exposed to 9 kJ m^{-2} of unfiltered UV-B radiation sufficient to induce about 30 CPD per Mb as an initial level. Immediately after exposure to UV-B radiation, each sample was irradiated with blue light for increasing lengths of time, and then the levels of CPD were determined. On the other hand, UV-irradiated leaves were kept in the dark for measurement of excision repair. The CPD levels in the leaves of Marich-bati decreased to about 50% of the initial level after 15 min of exposure to blue light. By contrast, the degree of photorepair of CPD in Surjamkhi cultivar was lower than that in Marich-bati cultivar, with only about 20% of the dimers being repaired within 15 min. This result indicates that the ability of UV-sensitive Surjamkhi to photorepair CPDs is lower than that of the UV-resistant cultivar Marich-bati. Figure 3 shows that no excision was detected in either cultivar during the time required for substantial photorepair. Many plants repair CPD by light-independent excision repair, usually more slowly than by photorepair. In Japanese rice cultivars[13], alfalfa[25], and soybean[26], excision is undetectable or

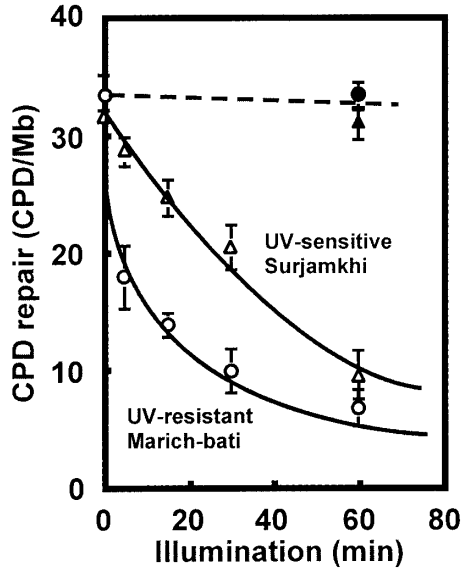

Fig. 3. Cyclobutane pyrimidine dimer (CPD) repair in the third fully expanded leaves of UV-resistant Marich-bati and UV-sensitive Surjamkhi. When the third leaf was fully expanded, the leaf was harvested. The detached leaf was exposed to 9 kJ m^{-2} of UV-B radiation to induce approximately 30 CPD Mb^{-1}. After UV-B irradiation the leaf was exposed to blue light (60 μmol m^{-2} s^{-1}) or kept in a light-tight box. Samples were harvested as a function of time; DNA was isolated, and then the CPD frequencies were determined by alkaline agarose electrophoresis. CPD levels in the leaf exposed to blue radiation: UV-resistant Marich-bati (○) and UV-sensitive Surjamkhi (△); CPD levels in the leaf kept in a light-tight box: UV-resistant Marich-bati (●) and UV-sensitive Surjamkhi (▲). The points represent the means of at least three experiments and the vertical bars represent standard errors.

low at low initial CPD levels, although the rate of excision repair can increase dramatically at high initial CPD levels.

Rice is one of the world's most important staple food grains and is widely cultivated in various regions throughout Asia; rice is grown not only in region of moderate climate at middle latitude but also tropical climates, such as Indonesia or the Bengal region where the amount of UV-B radiation in sunlight is greater. UV-B sensitivity among rice cultivars varies widely, and the difference in the sensitivity to UV-B is not related to the geographical location at which the cultivars are grown[10]. Previously, we found that the UV-B sensitivity might correlate with deficient CPD photorepair using specific Japanese rice cultivars: UV-sensitive Norin 1 and UV-resistant Sasanishiki[13,14]. In this work, we have shown that the UV-sensitive *Indica* rice Surjamkhi is also deficient in its ability to photorepair CPDs. These results thus suggest that the sensitivity to UV-B could seriously correlate with the low ability in CPD photorepair in rice plants and that the photorepair capacity is a principal factor in determining UV-B sensitivity in rice.

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