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BRIEF COMMUNICATION

UV-B induced stress responses in three rice cultivars

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Abstract

UV-B responses of three rice (*Oryza sativa* L.) cultivars (Sasanishiki, Norin 1 and Surjamkhi) with different photolyase activity were investigated. Carbon dioxide assimilation data support that Sasanishiki was less sensitive to UV-B than Norin 1 and Surjamkhi. UV-B radiation sharply decreased the content of Rubisco protein in Surjamkhi and has no effect in Sasanishiki. The photochemical activities of photosystem (PS) 1 and PS 2 was slightly affected by UV-B treatment. The content of H_2O_2 and the activities of antioxidant enzymes, catalase (CAT), peroxides (POX) and superoxide dismutase (SOD) were enhanced after UV-B treatment. The activities of CAT and POX isoenzymes in Sasanishiki were more enhanced by UV-B radiation than those in Norin 1 and Surjamkhi.

Additional key words: catalase, $^{14}CO_2$ fixation, hydrogen peroxide, peroxidase, Rubisco, superoxide dismutase.

UV-B sensitivity of plants is determined by the balance of damage incurred and by the efficiency of repair processes that can restore the impaired functions. This balance is influenced by several factors, including the genetic background of the species, growth conditions of the studied plants, as well as the simultaneous occurrence of other stresses. UV-B sensitivity in rice cultivars is controlled by at least three recessive genes (Sato *et al*. 2003). DNA is one of the targets of UV-B radiation, which induces photodamage in DNA resulting in the production of cyclobutane pyrimidine dimers (CPDs) and pyrimidine-pyrimidone photoproducts (Britt 1996). Photoreactivation involving enzyme photolyase, is the main pathway in plants for repairing UV-B induced DNA damage. Examinations of 17 rice cultivars (Teranishi *et al*. 2004) and several wild rice species (Iwamatsu *et al*. 2008) show that the UV-B resistant cultivars had higher photolyase activities in comparison to less resistant ones. Hidema *et al*. (2005) demonstrated that CPD photorepair ability is one of the crucial factors determining UV-B sensitivity in rice. Furthermore, transgenic rice plants in which the CPD photolyase was overexpressed had higher CPD photolyase activity and showed significantly greater resistance to UV-B than wild plants (Hidema *et al*. 2007). Therefore, the CPD photolyase activity is an excellent indicator for UV-B sensitivity of rice plant but it is important to examine other factors that would promote UV-B tolerance in order to develop plants with greater UV-B resistance.

Under UV-B radiation plant cells produce reactive oxygen species (ROS) that induces oxidative damage to DNA, proteins and other cell components (Caldwell 1993, Foyer *et al.* 1994, Mahdavian 2008). To cope with oxidative stress, various ROS-scavenging systems in plants are involved. They include superoxide dismutase, catalase, ascorbate peroxidase, glutathione S-transferase, and low molecular mass antioxidants such as ascorbate, glutathione, and carotenoids (Asada 1999).

In this study UV-B response of three rice cultivars (*Oryza sativa* L. cvs. Norin 1, Sasanishiki and Surjamkhi), with different photolyase activity due to occurring muta-

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Abbreviations: CAT - catalase; CPDs - cyclobutane pyrimidine dimers; F_m - maximum fluorescence yield in dark-adapted state; F_m'-maximum fluorescence yield in light-adapted state; F_v - variable chlorophyll fluorescence; PAGE - polyacrylamide gel electrophoresis; POX - peroxidase; PPFD - photosynthetic photon flux density; PS 2 - photosystem 2; ROS - reactive oxygen species; LSU - rubisco large subunit; SSU - rubisco small subunit; SOD - superoxide dismutase; TCA - trichloracetic acid; UV-B - ultraviolet B; UVB_{BE} - biological effectiveness of UV-B radiation; Φ_{PS2} - quantum yield of PS 2 photochemistry in the light-adapted state.
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tions in CPD photolyase gene (Hidema *et al.* 2005), was investigated by measuring changes in photosynthetic rate, electron transport rate, activities of antioxidant enzymes and contents of UV-B screening pigments.

Rice seedlings were grown hydroponically (½ Hogland solution) under white fluorescent lamps (PPFD 160 μmol m^{-2} s^{-1}) with a 12-h photoperiod, day/night temperature of 25/22 °C and 60 % air humidity for 30 d. Then the seedlings were irradiated with UV-B (312 ± 25 nm; fluorescent tubes TL 20W/12 R, *Philips*, Hamburg, Germany) for 5 h (UVB_{BE} 14.4 kJ m^{-2} d^{-1}). The rate of photosynthesis was determined as $^{14}CO_2$ fixation by the method described by Moll (1986). Chlorophyll fluorescence induction of leaf disks was measured with a pulse amplitude modulation fluorometer (*PAM 101-103*, *H. Walz,* Effeltrich, Germany) as described previously (Fedina *et al.* 2007). The redox state of P_{700} was monitored as 810/860 nm absorption changes. A *Walz ED 700DW-E* emitter/detector unit was connected to a *PAM 101E* main control unit (Klughammer and Schreiber 1998). Proline content was determined by the method of Bates *et al*. (1973), hydrogen peroxide content by the method of Esterbauer and Cheeseman (1990). Native PAGE in 7.5 % gel was carried out by the method of Davis (1964). Peroxidase (EC 1.11.1.7) isoenzymes were detected according to procedure of Ornstein (1964). Superoxide dismutase (EC 1.15.1.1) isoenzymes were detected by the method of Greneche *et al*. (1991). Catalase (EC 1.11.1.6) isoenzymes were stained as described by Woodbury *et al*. (1971). SDS-PAGE was conducted on 12.5 % acrylamide gels according to the description of Laemmli (1970). UV-B absorbing compounds were determined according to the procedure of Mirecki and Teramura (1984).

The $^{14}CO_2$ fixation rate of Surjamkhi was higher in comparison to Norin 1 and Sasanishiki when the three cultivars were grown under visible radiation alone. UV-B treatment significantly reduced the photosynthetic activity of all cultivars. The $^{14}CO_2$ fixation declined by 72 % in Surjamkhi, by 63 % in Norin 1 and by 52 % in Sasanishiki (Table 1). The amounts of Rubisco large (LSU) and small (SSU) subunits were similar in the non UV-B treated rice cultivars (Fig. 1*A*). As a result of UV-B irradiation the strong reduction in Rubisco LSU and SSU content was observed in Surjamkhi and relatively low in Sasanishiki. UV-B treatment did not influence PS 1 activity in cultivar Sasanishiki, since there was no change in far-red induced P_{700} oxidation (Fig. 1*B*). Significant reduction in ΔA_{830} was established in Surjamkhi, while in Norin 1 this reduction was very slight. The photochemical activity of PS 2 in Surjamkhi decreased as a result of UV-B treatment (Table 1). As a result of UV-B irradiation Φ_{PS2} was reduced by 11 and 24 % in Norin 1 and Surjamkhi, respectively, while in Sasanishiki it was not affected. The decrease of the Φ_{PS2} was accompanied by a corresponding increase in $1 - (F_v'/F_m')$, which indicated increased thermal energy dissipation in the antennae. The fraction of absorbed light energy which is not used for photochemistry (LNU) increased twice in Surjamkhi after UV-B treatment (Table 1).

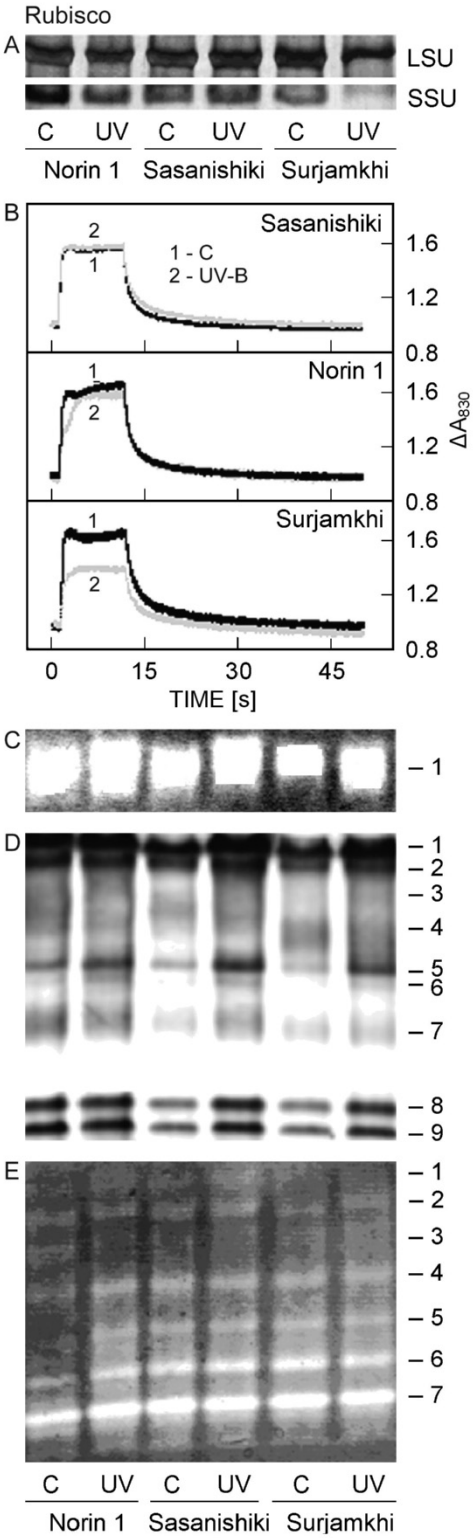

Fig. 1. Effect of UV-B on content of Rubisco large (LSU) and small (SSU) subunits (*A*), activity of photosystem 1 (*B*), and isoenzyme profiles of catalase (*C*; 50 μg protein was loaded in each lane), peroxidase (*D*; 50 μg protein was loaded in each lane) and superoxide dismutase (*E*; 25 μg protein was loaded in each lane) in cultivars Norin 1, Sasanishiki and Surjamkhi.

Table 1. The effects of UV-B irradiation on the rate of $^{14}CO_2$ fixation [mg(CO_2) g^{-1}(f.m.) h^{-1}], changes in the maximum quantum efficiency of PS 2 photochemistry (F_v/F_m), the actual efficiency of PS 2 electron transport (Φ_{PS2}), thermal energy dissipation in the antennae (1 - F_v'/F_m'), the light energy not used for photochemistry (LNU), the absorbance at 300 nm and 535 nm (characteristics of flavonoid and anthocyanin contents, respectively), contents of H_2O_2 [μmol g^{-1}(f.m.)] and proline [mg g^{-1}(f.m.)] in 3 rice cultivars. Means ± SE were calculated from 3 independent experiments. Differences singnificant at * - $P < 0.05$; ** - $P < 0.01$ and *** - $P < 0.001$.

Parameters	Norin 1 control	+UV-B	Sasanishiki control	+UV-B	Surjumkhi control	+UV-B
$^{14}CO_2$ fixation	7.800 ± 0.290	2.900 ± 0.230***	6.400 ± 0.120	3.100 ± 0.210***	11.500 ± 0.840	3.200 ± 0.210***
F_v/F_m	0.757 ± 0.015	0.684 ± 0.034	0.783 ± 0.012	0.758 ± 0.018	0.767 ± 0.018	0.704 ± 0.030
Φ_{PS2}	0.612 ± 0.018	0.544 ± 0.021*	0.660 ± 0.013	0.638 ± 0.027	0.668 ± 0.006	0.508 ± 0.041**
1 - F_v'/F_m'	0.286 ± 0.007	0.330 ± 0.013*	0.257 ± 0.009	0.289 ± 0.008*	0.297 ± 0.012	0.393 ± 0.023**
LNU	0.190 ± 0.023	0.210 ± 0.013	0.154 ± 0.025	0.160 ± 0.020	0.126 ± 0.023	0.259 ± 0.150***
A_{300}	2.260 ± 0.010	2.900 ± 0.120	1.860 ± 0.010	2.190 ± 0.010	2.310 ± 0.017	2.850 ± 0.010
A_{535}	0.025 ± 0.000	0.058 ± 0.003	0.023 ± 0.000	0.027 ± 0.000	0.036 ± 0.001	0.063 ± 0.001
H_2O_2	2.620 ± 0.060	3.060 ± 0.041**	2.040 ± 0.092	2.300 ± 0.130	2.440 ± 0.050	2.640 ± 0.060
Proline	0.066 ± 0.004	0.118 ± 0.014*	0.140 ± 0.017	0.180 ± 0.008	0.156 ± 0.015	0.241 ± 0.022*

UV-B treatment led to considerable increase of flavonoids in Norin 1 and Surjamkhi and had slight effect in Sasanishiki (Table 1). UV-B effect on accumulation of anthocyanins was more pronounced in UV-sensitive cvs. Norin 1 and Surjamkhi than in UV-tolerant Sasanishiki. Due to UV-B, free proline content increased by 78 % in Norin 1, that had low intrinsic proline content, 54 % in Surjamkhi and 28 % in Sasanishiki. The content of H_2O_2 slightly increased in all cultivars after UV-B treatment (Table 1). MDA content did not change considerably (data not shown).

Rice leaves showed very high CAT activity (Fig. 1*C*). For all cultivars tested CAT activity increased as a result of UV-B treatment. The increase was more pronounced in Sasanishiki and Norin 1 than in Surjamkhi. Nine POX isoenzymes were detected (Fig. 1*D*). UV-B enhanced the activity of all POX isoenzymes particularly of isoenzymes No. 5, 8 and 9. The activities of these isoenzymes in Sasanishiki were enhanced by UV-B radiation more than in Norin 1 and Surjamkhi. Seven SOD isoforms were detected (Fig. 1*E*). A slight increase of SOD activity was observed for isoenzyme No. 7 in all UV-B treated cultivars.

The results presented here showed that genotypic differences in these three rice cultivars were expressed in terms of their response to UV-B radiation. These new data supported that Sasanishiki was less sensitive to UV-B radiation than Norin 1 and especially Surjamkhi, as it was described previously (Hidema and Kumagai 2006). The reduction in photosynthetic activity in the UV-B sensitive rice cultivar could be due to a decrease of Rubisco content, Rubisco activation and electron transport rate. DNA lesions, such as CPD interfere with DNA replication and transcription (Britt 1999). Thus, the differences in the susceptibility to CPD induction by UV-B radiation and/or repair abilities including CPD photolyase activity could affect the protein content. From our previous data, the relative rates of CPD photolyase activity among these three cultivars were Sasanishiki > Norin 1 > Surjamkhi, although there was no significant difference in susceptibility to CPD induction (Teranishi *et al.* 2004, Hidema *et al.* 2005a,b). It is thought that under UV-B irradiation modification of proteins occurs *via* photooxidation by ROS and free radicals produced during photosensitization (Caldwell 1993, Foyer *et al.* 1994). Especially, the generation of ROS causes direct or indirect oxidative damage to DNA, proteins, *etc*. (Takeuchi *et al*. 1995, Booij-James *et al*. 2000, Mackerness 2000). Caldwell (1993) demonstrated that ROS generated by UV-B induced photodamage to Rubisco, and Desimone *et al.* (1996, 1998) showed that ROS caused proteolytic degradation of the LSU. Furthermore, Karpinski *et al.* (1997) reported that an increased H_2O_2 content was detected simultaneously with the inhibition of photosynthesis by UV-B radiation, associated with the damage and degradation of the D1 and D2 proteins of the PS 2 reaction centre. In this study, all cultivars showed an increase of the content of H_2O_2 (Table 1) and the activities of ROS scavenging enzymes CAT, POX and SOD (Fig. 1) after UV-B treatment. The higher increases in the activities of ROS scavenging enzymes in the UV-resistant Sasanishiki are important to keep oxidative stress at a lower level than in the UV-sensitive Norin 1 and Surjamkhi.

The accumulation of UV-screening pigments such as flavonoids and anthocyanins in the vacuoles of the epidermal and subepidermal cell layers plays a role in mitigating UV-B-induced damage (Reuber *et al.* 1996, Bharti and Khurana 1997). However, our data showed that there were no significant correlation between sensitivity to UV-B and accumulation of UV-absorbing compounds in these three rice cultivars. Similar results were observed in rice by Teranishi *et al.* (2004) and in cucumber by Adamse and Britz (1996). Hada *et al.* (2003) reported that excess accumulation of anthocyanins reduced the amount of blue and UV-A radiation, which is utilized by CPD photolyase for monomerization of dimers and thus lowered CPD photorepair in purple rice leaves.

We suggest that ROS scavenging enzymes are essential for rice plants to cope with UV-B during growth.

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