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Differences in the Amino Acid Sequences of CPD Photolyases of UV-sensitive and UV-resistant Rice Cultivars

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There is a difference in the inhibitory effects to supplemental UVB (wavelengths 280 to 320 nm) among Japanese rice (*Oryza sativa* L.), the cultivar Norin 1 is less resistant while the cultivar Sasanishiki is resistant. UVB induces photodamage in DNA. Cyclobutane pyrimidine dimer (CPD) is a major UV-induced DNA lesion. Photorepair, which is mediated by photolyase, is the major pathway in plants for repairing CPD. We have analyzed CPD induction and repair in Sasanishiki and its close relative Norin 1 using alkaline agarose gel electrophoresis. Norin 1 is deficient in CPD photoreactivation and excision, thus UV sensitivity correlates with deficient dimer repair [1]. The photorepair deficiency in Norin 1 results from a functionally altered photolyase with a photoflash analysis [2]. In this paper, we examined the UVB-sensitivity of several other UV-sensitive and -resistant cultivars and found that the CPD photolyase activity was deficient in UV-sensitive ones. It was also evident that there was a variation in the deduced amino acid sequences of CPD photolyases of the UV-sensitive and -resistant cultivars, whereas each deduced amino acid sequence of the UV-sensitive cultivars and of the UV-resistant ones was the same. These results suggest that the difference in the CPD photolyases of UV-sensitive and -resistant rice might be due to the structural alteration of CPD photolyase.

Key words: cyclobutane pyrimidine dimer, *Oryza sativa* L., photolyase, photorepair, UVB

INTRODUCTION

The depletion of stratospheric ozone because of the emission of chlorofluorocarbons and other trace gases was resulted in increases in solar UVB at the earth's surface. Studies in greenhouses and growth chambers have shown that enhanced UVB has deleterious effects on the growth and development of higher plants, as well as on photosynthesis. In our field experiments over five-year period, we investigated the effects of supplemental UVB on the growth and grain development

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of Japanese lowland rice cultivars in the field in a cool rice-growing region of Japan. Supplemental UVB had a positive effect, which was be enhanced by unusual climatic conditions such as lower temperature and less sunshine [3]. We also found that there was a difference in the sensitivity to supplemental UVB among Japanese rice cultivars. The cultivar Sasanishiki is more resistant to supplemental UVB, while Norin 1 is less resistant, although these two cultivars are closely related [4].

UVB damage induces photodamage in DNA, including cyclobutane pyrimidine dimer (CPD) and the (6-4)-pyrimidine-pyrimidone photodimer. Such damage can be lethal or mutagenic to organisms; it can also impede replication and transcription, a possible mechanism for the adverse effects observed in higher plant. Photoreactivation is the major pathway, in plant, for repairing UVB-induced DNA damage [5]. This one-enzyme repair path is mediated by an enzyme photolyase, which bind to a dimer to form a complex that is stable in the absence of light. Previous data showed that the ability to photorepair CPDs was greater in Sasanishiki than in Norin 1, despite there was no difference in the susceptibilities to CPD induction by UVB between these two cultivars [6]. We recently found that the deficiency in photorepair in Norin 1 resulted from a structure/function alteration of photolyase rather than of nonspecific repair, photolytic, or regulatory elements with photoflash analysis *in vivo* and *in vitro* and the thermostability of photolyase-CPD complex [2].

In this paper, we report the difference in the deduced amino acid sequences of CPD photolyases of UV-sensitive and -resistant rice including Sasanishiki and Norin 1.

MATERIALS AND METHODS

Plants were grown under visible light (350 μ mol PAR $m^{-2}s^{-1}$) with or without supplemental UVB in a phytotron. UVB was provided by UV-emitting fluorescent tubes (FL20SE; Toshiba, Tokyo, Japan) filtered through a UV29 cutoff glass filter (Toshiba Glass, Tokyo, Japan). The UVB intensity at the level of the plant was 1.12 W m^{-2} as measured by a spectroradiometer (SS25, 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.

The CPD frequencies were determined as previously described [2]. In brief, to assay photolyase activity *in vitro*, cell extract was mixed with UV-irradiated λ DNA, incubated for 15 min at 28℃ to form photolyase-CPD complexes, and then exposed to blue radiation which was adjusted to about 60 μ mol PAR $m^{-2}s^{-1}$. The levels of pyrimidine dimers were determined by using *Micrococcus luteus* UV endonuclease to cleave the dimer at CPD sites. DNA were then denatured by adding alkaline mixture and subjected to static-field electrophoresis. After gels were stained with ethidium bromide, an image of the fluorescence of ethidium bromide bound to DNA was recorded by using an improved version of an electronic imaging system. CPD repair was measured as a function of time [7].

RESULTS AND DISCUSSION

We first examined the effects of supplemental UVB on growth of Japanese lowland rice cultivars,

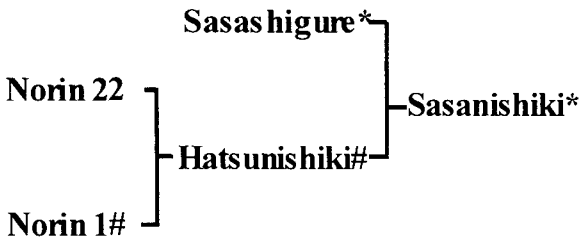

Figure 1. A part of phylogenic tree of Japanese rice cultivars. UV-sensitive (#) and UV-resistant (*) cultivars are closely related.

Sasashigure and Hatsunishiki, which were closely related to Sasanishiki and Norin (Figure 1), and found that Sasashigure was resistant, while Hatsunishiki was less resistant.

We next examined the photorepair activities in the fourth leaves and in those crude enzyme solutions of Sasashigure and Hatsunishiki. Both *in vivo* and *in vitro*, CPD photorepair activities were higher in Sasashigure than in Hatsunishiki. These CPD photorepair activities of UV-resistant cultivars Sasanishiki and Sasashigure were higher than those of UV-sensitive cultivars Norin 1 and Hatsunishiki.

We further examined the sequence of CPD photolyase gene of Sasanishiki [T. Hirouchi *et al*, to be submitted], which was also compared with that of Norin 1. Consequently we found a difference in amino acid sequences of CPD photolyases of these two cultivars. Moreover, it was evident that the deduced amino acid sequences of the UV-resistant cultivars (Sasanishiki and Sasashigure) and the UV-sensitive ones (Norin 1 and Hatsunishiki) were the same, respectively.

It was thus suggested that the structural alteration of CPD photolyase due to change in its amino acid sequence must lead to the deficiency in CPD photolyase activity in UV-sensitive rice.

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