

Effects of supplementary UV-B radiation on growth and protein biosyntheses in rice (*Oryza sativa* L.)

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We examined the effects of supplementary ultraviolet-B (UV-B) radiation on the changes in synthesis and degradation of ribulose-1, 5-biphosphate carboxylase /oxygenase (Rubisco) and light-harvesting chlorophyll a/b binding protein of PSII (LHCII), as well as mRNA levels for small and large subunits of Rubisco (*rbcS* and *rbcL*, respectively) and LHCII (*cab*) with leaf age in UV-sensitive rice (Norin 1) and UV-resistant rice (Sasanishiki). Both Rubisco and LHCII were actively synthesized until the leaf had fully expanded, and then decreased with leaf age. Synthesis of Rubisco, but not LHCII, was significantly suppressed by UV-B in Norin 1. The degradation of Rubisco was enhanced by UV-B around the time of the leaf maturation in the two cultivars. The levels of *rbcS* and *rbcL* were reduced by UV-B at the early leaf stages after emergence in both cultivars. The level of *cab* was first present at the highest level in the two cultivars, but drastically decreased due to UV-B treatment immediately after leaf emergence in Norin 1. It was proved that synthesis and degradation of Rubisco and LHCII greatly changed with leaf age: Rubisco synthesis was significantly suppressed by supplementary UV-B radiation at the transcription step during the early leaf stages. It was also suggested that the difference between the two rice cultivars in sensitivity to UV-B in the synthesis of Rubisco might be due to the specific suppression not only after transcription but also at transcription.

Key words: leaf age, light-harvesting chlorophyll a/b binding protein of PSII, *Oryza sativa* L., ribulose-1, 5-biphosphate carboxylase/oxygenase, ultraviolet-B radiation

INTRODUCTION

Ultraviolet-B (UV-B) radiation can damage plants, decreasing biomass and productivity. We found previously that rice cultivars vary widely in the sensitivity to supplementary UV-B radiation in 198 rice cultivars belonging to five Asian rice ecotypes from the Bengal region, Indonesia and Japanese lowlands and uplands [3].

Among Japanese lowland rice, the 'Sasanishiki' cultivar (one of the leading cultivars in the northern part of Japan) was more resistant to the inhibitory effects of supplementary UV-B radiation on growth than the Norin 1 cultivar, although these cultivars are closely related ([1], [2]). We also found that supplementary UV-B radiation resulted in the reduction in the amounts of total leaf nitrogen, chlorophyll, soluble protein and ribulose-1, 5-biphosphate-carboxylase/ oxygenase (Rubisco) in fully expanded leaves in rice [1]. It was especially noticed that the amount of Rubisco was remarkably decreased by supplementary UV-B radiation in UV-sensitive Norin 1. This was not the case in UV-resistant Sasanishiki. It was

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also shown that the amount of light-harvesting chlorophyll a/b binding protein of PSII (LHCII) was not lowered as much as the Rubisco content in the two cultivars. In this experiment, we investigated quantitatively the effects of supplementary UV-B radiation on the synthesis and degradation of Rubisco and LHCII as a function of leaf age using ^{15}N tracer and the changes in the mRNA levels for the small and large subunits of Rubisco (*rbcS* and *rbcL*, respectively) and LHCII (*cab*). Furthermore, differences in UV-B sensitivity between the Sasanishiki and Norin 1 rice cultivars on the biosynthesis of the same two proteins were addressed.

MATERIALS & METHODS

Plant Materials and Growth Conditions ---- Sasanishiki and Norin 1 rice cultivars were used as experimental plants. Plants were grown hydroponically under visible radiation in growth chambers [1]. After emergence of the 8th leaves, the plants were grown with or without supplementary UV-B radiation (1.12W) filtered through UV-29 glass filter (Toshiba Glass, Tokyo, Japan). UV-B radiation was provided by UV-emitting fluorescent tubes (FL20SE, Toshiba Electric, Tokyo, Japan).

^{15}N -labeling of the plants ---- Plants, which had just emerged the 8th leaf in non-labeled nutrient solution, were used for ^{15}N -labelling experiments. ^{15}N -labeling was performed in the following two ways with labeled nutrient solution containing 2 mM $(^{15}\text{NH}_4)_2\text{SO}_4$ (30.3 atom % excess) instead of 1 mM NH_4NO_3 in non-labeled nutrient solution as described by Takeuchi et al. [4]. The 8th leaf on the main stem was collected from the plants on the different 3 days following the ^{15}N -treatment, and was provided for analyses.

Quantitation of Total Leaf N, soluble protein N, Rubisco and LHCII ---- There were determined as described elsewhere by Hidema et al. [1]. The Rubisco and LHCII contents were determined from SDS-soluble fraction of the

homogenate by SDS-PAGE.

Isolation of Rubisco and LHCII for ^{15}N analysis ---- Rubisco for ^{15}N analysis was isolated by an anion-exchange column chromatography packed with Bioscale Q (Bio-Rad) and LHCII for ^{15}N analysis was isolated by SDS-PAGE using continuous-elution electrophoresis system (Bio-Rad). The ^{15}N in Rubisco and LHCII were determined by emission spectrography [5] with a JASCO ^{15}N -analyzer (NIA-1, JASCO, Tokyo, Japan), and the amounts of Rubisco and LHCII synthesized or degraded based on ^{15}N -incorporation was estimated as described [4].

*Determination of transcript levels for *rbcS*, *rbcL* and *cab** ---- Transcript levels for these genes were determined by RT-PCR, using each gene-specific primer pair. The 18S rRNA was used as a positive control for RT-PCR.

RESULTS AND DISCUSSION

The 8th leaves were fully expanded around 9 days after leaf emergence in the two rice cultivars grown with or without supplementary UV-B radiation. The amount of any type of nitrogen was lowered by supplementary UV-B radiation at any different leaf stages after the emergence in UV-resistant Sasanishiki and UV-sensitive Norin 1. The amounts of total leaf N and Rubisco N was especially lowered in Norin 1 than in Sasanishiki though LHCII content was not decrease so much as the Rubisco content in two cultivars. The ratio of Rubisco to total leaf N was dramatically decreased during the early stages of leaf development in Norin 1 by supplementary UV-B radiation. It was noticed that only the Rubisco content was significantly reduced by supplementary UV-B radiation in both cultivars but not LHCII content. Therefore we examined the effects of UV-B radiation on the synthesis and degradation of two proteins using ^{15}N tracer.

It was found that the amounts of ^{15}N incorporated

Rubisco were greater at the early stages of leaf development in the two cultivars grown without supplementary UV-B radiation, meaning that the Rubisco was actively synthesized when the leaf was vigorously expanding. However, when Norin 1 was grown with supplementary UV-B radiation, the level of ¹⁵N incorporated into Rubisco was significantly dropped at the periods of 3-9 days after leaf emergence. This was not the case in Sasanishiki. As for the amount of ¹⁵N-LHCII, we could not observe the great difference between cultivars and the strong effect of supplementary UV-B radiation as compared with that of Rubisco. On the other hand, the degradation of Rubisco and LHCII was transiently enhanced by supplementary UV-B radiation at the period of 9-12 days after leaf emergence in both cultivars, but there appeared little difference between the two cultivars. In this experiment, it was proved that the synthesis of Rubisco, but not LHCII, was remarkably suppressed by supplementary UV-B radiation during early stages of leaf development especially in Norin 1, and that the degradation of Rubisco and LHCII were higher just after the leaf fully expanded in both two cultivars.

We next examined the changes in the mRNA levels for *rbcS*, *rbcL* and *cab* with leaf age after emergence. The levels of *rbcS* and *rbcL* increased in 3 days after leaf emergence, and then gradually decreased with age. The supplementary UV-B radiation suppressed transcription of Rubisco through leaf life in the two rice cultivars. In this case, we could not detect such a great reduction in the amounts of those transcripts as seen in the amount of Rubisco in this period in Norin 1 grown with supplementary UV-B radiation. It was thus suggested a possibility that the supplementary UV-B radiation might specifically suppress the biosynthesis of Rubisco at the biosynthetic step after transcription in UV-sensitive cultivar but not in UV-resistant one, which may be one of factors determining the sensitivity to supplementary UV-B radiation in rice. The level of *cab* drastically

reduced in 3 days after leaf emergence in Norin 1 grown with supplementary UV-B while there were not so greater changes in the levels of ¹⁵N-LHCII in this period. Therefore, it is speculated that the turnover rate of *cab* transcript might be long and that higher amount of *cab* transcript is needed to have been accumulated before leaf emergence. Once the LHCII had been comprised in the thylakoid membrane of chloroplast, LHCII will be kept for a longer time as a stable state.

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