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Effects of UVB radiation on protein turnover of Rubisco and LHCII in rice

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Introduction

Ultraviolet-B (UV-B) radiation can damage plants, decreasing biomass and productivity. We previously found that rice cultivars vary widely in the sensitivity to supplemental UV-B radiation in 198 rice cultivars that belonged to five Asian rice ecotypes from the Bengal region, Indonesia and Japanese lowlands and uplands (Sato & Kumagai 1993). 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 supplemental UV-B radiation on growth than the Norin 1 cultivar, although these cultivars are closely related (Kumagai & Sato 1992; Hidema et al. 1996). It has been reported that the supplemental UV-B radiation causes reduction in the amounts of chlorophyll (Chl), and ribulose-1, 5-biphosphate carboxylase/oxygenase (Rubisco) in leaves of C3 plants (Strid et al. 1990). On the other hand, it has also been shown that the level of Rubisco in leaves changed dramatically with leaf age, and that the levels of leaf proteins in rice are balanced by synthesis and degradation throughout the leaf life (Mae et al., 1983). In this connection, we found that supplemental UV-B radiation resulted in the reduction in the amounts of total leaf nitrogen, Chl, and Rubisco in fully expanded leaf in rice (Hidema et al. 1996). It was especially noticed that the amount of Rubisco was remarkably decreased by supplemental UV-B radiation in an UV-sensitive Norin 1. This was not the case in an UV-resistant Sasanishiki. In this experiment, we investigated the effects of supplement UV-B radiation on the synthesis and degradation of Rubisco and LHCII with leaf age using ^{15}N tracer and the changes in the mRNA levels for *rbcS*, *rbcL*, and *cab*, and the cultivar difference between Sasanishiki and Norin 1 cultivars in the sensitivities to the effects of supplemental UV-B on those biosyntheses.

Materials and methods

Plant Materials and Growth Conditions ---- Sasanishiki and Norin 1 rice cultivars were used as experimental plants. Plants were grown hydroponically under visible radiation, providing 350 µmol PAR $m^{-2}s^{-1}$, without supplemental UV-B radiation in growth chambers (Hidema et al. 1996). After emergence of the 8th leaves, the plants were grown with or without supplemental UV-B radiation filtered though UV-29 glass filter (Toshiba Glass, Tokyo, Japan). UV-B radiation was provided by UV-emitting fluorescent tubes (FL20SE, Toshiba Electric, Tokyo, Japan). The UV-B intensity at the level of the plants was 1.12 W, and the biologically effective UV-B radiation was 39.5 kJ $m^{-2}d^{-1}$.

^{15}N-labeling of the plants ---- Plants, which had just emerged the 8 th leaf in non-labeled nutrient solution, were used for ^{15}N-labelling experiments. ^{15}N-labeling was performed in the following two ways. First, each plant grown in non-labeled nutrient solution for different 3

days, from 0 to 15 days after the 8th leaf emergence, was grown for 3 days in labeled nutrient solution containing 2 mM $(^{15}NH_4)_2SO_4$ (30.3 atom % excess) instead of 1 mM NH4NO3 in non-labeled nutrient solution. The 8th leaf on the main stem was collected from the plants on the different 3 days after the start of each ^{15}N labeling, and provided for analyses. In the second, plants, which were grown in non-labeled nutrient solution until the 8th leaf emergence, were grown in labeled nutrient solution containing 2 mM $(^{15}NH_4)_2SO_4$ (30.3 atom % excess) instead of 1 mM NH_4NO_3 for 3 days from 0 to 3 days after leaf emergence. Then, the plants were cultured again in non-labeled nutrient solution, and 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, Rubisco and LHCII ---- There were determined as described elsewhere (Hidema et al., 1992 and 1996). 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 in the supernatant obtained by centrifugation at 33,000 x g described above was isolated as follows. The supernatant was subjected to fractionation between 10 and 15% (w/v) solutions of polyethylene glycol-6000 in the presence of 100 mM NaCl, followed by an anion-exchange column chromatography packed with Bioscale Q (Bio-Rad). LHCII for ^{15}N analysis in the precipitate obtained by centrifuging at 33,000 x g was isolated as follows. The precipitate was dissolved in 50 mM sodium phosphate buffer (pH 7.2) which contained 5% (v/v) glycerol, 0.7% (v/v) 2-mercaptoethanol, 4 mM monoiodeacetic acid and SDS [3% (w/v), final concentration] at 100ºC for 3 min and centrifuged at 10,000 x g at 4ºC for 15 min. The supernatant was applied to electrophoresis. LHCII was purified by SDS-PAGE using continuous-elution electrophoresis system (Bio-Rad). The ^{15}N in Rubisco and LHCII were determined by emission spectrography (Yoneyama et al. 1975) 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 in Mae et al. (1983).

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 supplemental UV-B radiation. The amounts of total leaf N and Rubisco were lowered by supplemental UV-B radiation at any different leaf stages after the emergence in the two rice cultivars, UV-resistant Sasanishiki and UV-sensitive Norin 1 (Fig. 1). The degree of lowering the amounts of any types of nitrogen was greater in Norin 1 than in Sasanishiki. The ratio of Rubisco to total leaf N was dramatically decreased during the early stages of leaf development in Norin 1 by supplemental UV-B radiation. On the other hand, the LHCII content gradually increased during the early stages of leaf development when the two cultivars were grown with or without supplemental UV-B radiation, and almost similar levels were kept with leaf age but not decrease so much as the Rubisco content (Fig. 1). It was noticed that only the Rubisco content was significantly reduced by supplemental UV-B radiation in both cultivars while the LHCII content was not lowered. In this experiment, our interest was focused on the following two possibilities: one is due to lack of nitrogen supply for protein biosynthesis, the other being due to suppression of protein biosynthesis and/or enhancement of protein degradation. The former possibility was considered reasonable because the pattern of changes in the Rubisco content with leaf age was very similar to that in the total leaf N content in both cultivars grown with or without supplemental UV-B radiation. This consideration was supported by the reports that the

amounts of soluble protein and Rubisco reduced in response to the total leaf N content in C3 plants (Makino et al. 1983). However, when Norin 1 was grown with supplemental UV-B radiation, the Rubisco content alone drastically dropped in the early stages of leaf development and kept on lowering at a faster rate with leaf age. We therefore examined the latter possibility 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 supplemental UV-B radiation, meaning that the Rubisco was actively synthesized when the leaf was vigorously expanding. However, when Norin 1 was grown with supplemental UV-B radiation, the level of ^{15}N incorporated into Rubisco was significantly dropped at the periods of 3-6 and 6-9 days after leaf emergence. This was not the case in Sasanishiki. On the other hand, at the period of 6-9 days grown with or without supplemental UV-B radiation in both cultivars, ^{15}N once had incorporated in Rubisco began to flow out of ^{15}N-Rubisco. This result implies that the degradation of Rubisco began to occur around this period in both cultures grown with or without supplemental UV-B radiation. The degradation of Rubisco was transiently enhanced by supplemental 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. With regard to the degradation of Rubisco, it has been reported that active oxygen species directly degrade Rubisco in isolated chloroplast (Ishida et al. 1997). Mackerness et al. (1998) reported that elevated UV-B-induced injury might be associated with active oxygen species. In our experiment, it is as yet unknown whether or not supplemental UV-B radiation may generate active oxygen species resulting in the degradation of Rubisco. However, it should be noted that the great degradation of Rubisco occurred when the leaf developed to mature stage. Around that time, active growth of leaf drew near the end and the stage for senescence began. Thus, it is speculated that formation of some proteases may be enhanced around that time, resulting in enhancement of Rubisco degradation (see Callis 1995).

As for LHCII, we could not observe the great difference between cultivars and the strong effect of supplemental UV-B radiation as compared with that of Rubisco, which might be due to the small content of this protein in the leaf. Except, there was a little higher increase in the degree of degradation at the period of 9-12 days after leaf emergence in both cultivars grown with supplemental UV-B. This might be correlated with progress of leaf age. At any rate, it proved that the synthesis of Rubisco, but not LHCII, was remarkably suppressed by supplemental 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.

We next examined the changes in the mRNA levels for *rbc*S, *rbc*L 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 supplemental 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 during early stages of leaf development in Norin 1 grown with supplemental UV-B radiation. It was thus suggested a possibility that the supplemental 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 supplemental UV-B radiation in rice. On the other hand, the level of *cab* drastically reduced in 3 days after leaf emergence in Norin 1 grown with supplemental UV-B. As described previously, the LHCII content in Norin 1 grown with supplemental UV-B increased until 9th days after leaf emergence, and then gradually lowered with age. Furthermore, there were not so greater changes in the levels of ^{15}N-LHCII. 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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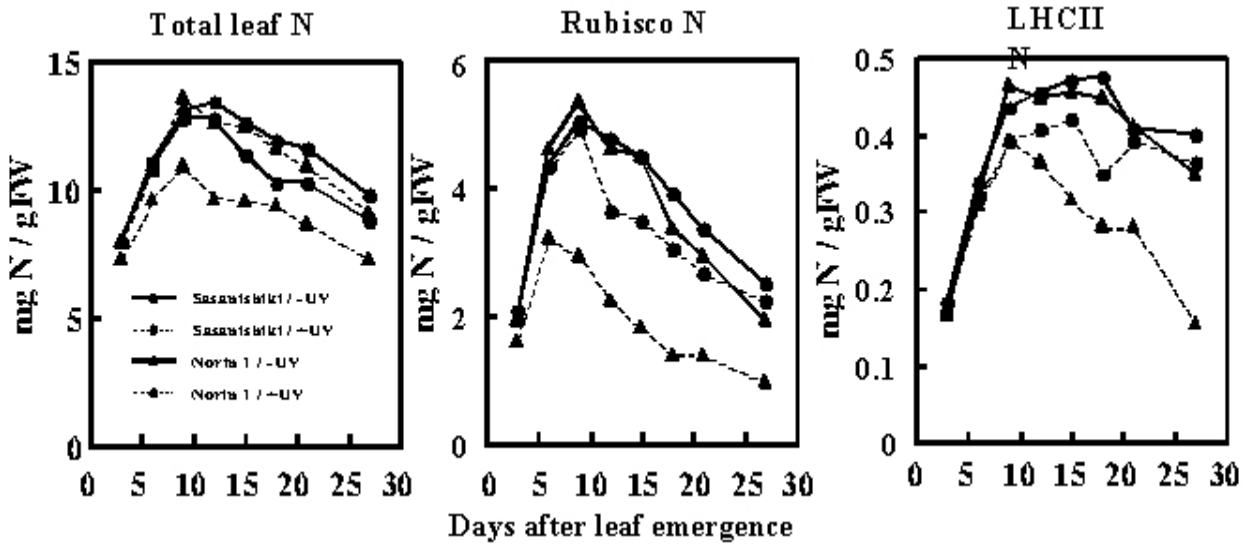

Fig. 1 Changes in the amounts of total leaf N, Rubisco N and LHCII N in the 8th leaf of Sasanishiki and Norin 1 grown with or without supplemental UV-B radiation with leaf age.