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Physiological and Biochemical Analyses of Rice Sensitivities to UVB Radiation

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Rice is widely cultivated in various regions throughout Asia. Over a five-year period, we investigated the effects of supplemental UVB radiation on the growth and yield of Japanese rice cultivars in the field. The findings of that study indicated that supplemental UVB radiation has inhibitory effects on the growth and grain development. Furthermore, we investigated the sensitivity to UVB radiation of rice cultivars of 5 Asian rice ecotypes, and found that rice cultivars vary widely in UVB sensitivity. The aim of our study is improving UVB resistance in plants by bioengineering or breeding programs. In order to make it, there is need to find the molecular origin of the sensitivity to UVB. Cyclobutane pyrimidine dimer (CPD) is major UV-induced DNA lesions. Plants possess two mechanisms to cope with such DNA damage. The first is the accumulation of UV-absorbing compounds. Our previous data showed 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 UV-absorbing compounds content, although there was a significant positive correlation between the CPD levels induced by challenge UVB exposure and the UV-absorbing compounds content. The other is the repair of DNA damage. Photorepair is the major pathway in plants for repairing CPD. We found that the sensitivity to UVB could seriously correlate with the low ability in CPD photorepair in rice plants. These results suggest that photolyase might be an excellent candidate for restoration by way of selective breeding or engineering in rice.

Key words : ultraviolet B radiation, rice, cyclobutane pyrimidine dimer, photolyase, UV-absorbing compounds

INTRODUCTION

UVB radiation can damage plants, decreasing growth and productivity. UVB-augmentation studies have identified many UV-sensitive cultivars of higher plants of economic importance, including rice. However, there are no definitive answers for the mechanisms of UVB-resistance in higher plants, and the idea of bioengineering design and development of plants, which would be resistant to predicted future environments.

We have been studying fundamentally physiological and biochemical aspects of effects of UVB radiation on growth and yield of rice plants (*Oryza sativa*), regarding the mechanisms of resistance to UVB radiation in rice. This paper shows the followings: (1) cultivar differences in the sensitivity to UVB radiation among rice cultivars of Asian rice ecotypes; (2) the origins of cultivar differences in the

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UVB sensitivity between UV-resistant and -sensitive rice.

Effects of UVB Radiation on the Growth and Yield of Japanese Rice Cultivars in the Field

We have been investigating the effects of supplemental UVB radiation on the growth and yield of Japanese rice cultivars in the field in a cool rice-growing region of Japan (Miyagi Prefecture) since 1993. This study indicates that supplemental UVB radiation has inhibitory effects on the growth and yield of rice [9]. Furthermore, we found that grain size tends to be smaller with supplemental UVB radiation. This observation is commercially very important in Japan. It is assumed that the reduction in grain size might result in a change in protein content in grain, which is very important for taste. In addition, these inhibitory effects of supplemental UVB radiation might be markedly promoted by less sunshine and lower temperature during cropping seasons. Multiple-year experiments under different climates and latitudes should be conducted in the future.

Differences in the Sensitivity to UVB Radiation Among Rice Cultivars of Asian Rice Ecotypes

We previously investigated the sensitivity to UVB radiation of 198 rice cultivars of five Asian rice ecotypes (*aus*, *aman*, *boro*, *bulu*, *tjereh*) and Japanese lowlands and uplands [12]. The *aus*, *aman*, *boro* ecotypes from Bengal region and the *tjereh* ecotype from Indonesia belong to *indica*. The *bulu* ecotype from Indonesia belongs to the tropical *javanica*, and Japanese lowlands and uplands rice groups belong to the temperate *japonica*. UVB radiation caused the inhibitory effects on growth, biomass, photosynthetic rate and chlorophyll content in all the cultivars, and rice cultivars vary widely in their sensitivity to UVB radiation. We found that various cultivars which belonged to the same ecotype or the same group showed different sensitivities to UVB radiation and, moreover, that the Japanese lowland rice group and the *boro* ecotype were more resistant to UVB radiation than others; rice cultivars cultivated in southern regions, where the amount of UVB in sunlight is larger, are not always more resistant to UVB. Furthermore, among the Japanese lowland rice group, Sasanishiki, which is one of the most popular varieties in Japan, exhibited elevated resistance to UVB radiation, while Norin 1 was less resistant, although these two cultivars are closely related [2, 10]. However, it is unclear the origin of the differences in UVB sensitivity among rice cultivars. Thus, we should determine the molecular origins of the defective UVB resistance in UVB sensitive cultivars.

The Origins of Cultivar Differences in the UVB Sensitivity Between UV-Resistant Sasanishiki and UV-Sensitive Norin 1

UVB radiation is capable of directly altering the structure of DNA. The two main photoproducts known to be formed between adjacent pyrimidines on the same DNA strand by UVB are cyclobutane pyrimidine dimer (CPD) and (6-4) photoproducts [1]. Such DNA damages can block transcription, and produce lethality, mutation. Plants possess mechanisms to cope with UVB-induced CPD. One of the mechanisms is DNA repair, which includes photorepair and excision repair to reduce the levels of CPDs. In photorepair, a single enzyme, photolyase, uses light as energy in the 300 to 600 nm range to monomerize dimers. In excision repair, dimers are replaced by *de novo* synthesis in which the undamaged complementary strand is employed as a template. Photorepair and/or excision repair of CPDs have been reported in a number of plant species. In generally, in most plants CPDs are repaired principally by photorepair. We examined the susceptibility to CPD induction by UVB radiation and the ability to photorepair CPD in UV-resistant Sasanishiki and UV-sensitive Norin 1.

The susceptibilities to CPD induction at various leaf stages of UV-resistant Sasanishiki and UV-sensitive Norin 1 were examined. The dimer levels produced by challenge UVB radiation in leaves of various stages changed dramatically. UVB radiation produced more CPDs in second, third or fourth leaves than that in fifth or sixth: the CPD induction in the fourth leaf was approximately four times of that in the sixth leaf. Here, it should be noted that there was no significant difference in the susceptibility to CPD induction between both cultivars [4, 6].

We determined the ability to photorepair in the leaves of these two cultivars. The CPD levels in the leaves of Sasanishiki were greatly diminished by blue radiation. By contrast, the degree of photorepair of CPD in Norin 1 was lower than that in Sasanishiki in all the leaf stages [4, 6]. Similar tendency was observed for the photorepair ability between UVB-resistant Marich-bati and UVB-sensitive Surjamkhi, which are *Indica* rice cultivars belonging to *aus* ecotype from Bengal region [7]. Therefore, these results suggest that the sensitivity to UVB could seriously correlate with low ability to CPD photorepair. Furthermore, we tested the origin of the low ability to CPD photorepair in Norin 1 using a photoflash approach. Consequently, this deficiency results from a structure/function alteration of photolyase [5].

Other Mechanisms to Cope With UV-Induced DNA Damages

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 UVB filters, play very important rules in coping with UV-induced DNA damages [11]. Previous our data show that there was a significant positive correlation between the CPD levels induced by challenge UVB exposure and the amount of UV-absorbing compounds in leaves of rice grown under various light environmental conditions [8].

By contrast, we examined the relationship between the steady-state CPD levels (the initial CPD levels found in harvested leaves prior to exposure challenge UVB radiation) and the amounts of UV-absorbing compounds in leaves of normal varieties of rice grown indoors or outdoors under various light environmental conditions [3, 8]. The CPD levels in leaf of rice grown under natural sunlight remain between 3 and 6 CPD Mb^{-1} throughout the day, and are elevated with UVB supplementation. However, the steady-state CPD levels under chronic radiation in leaves of rice grown in any culture were not so greatly influenced by the increased amounts of UV-absorbing compounds. Thus, it has been questioned whether the accumulation of UV-absorbing compounds plays important rules in coping with the inhibitory effects caused by UVB radiation.

CONCLUSION

We have been studying physiological and biochemical aspects of effects of UVB radiation on growth and yield of rice plants (*Oryza sativa*), regarding the mechanisms of resistance to UVB radiation in rice. We investigated the sensitivity to UVB radiation of rice cultivars of 5 Asian rice ecotypes, and found following results: (1) rice cultivars vary widely in UVB sensitivity; (2) among Japanese rice cultivars, Sasanishiki is more resistant to UVB, while Norin 1 is less resistant, although these cultivars are closely related; (3) UV-sensitive cultivar is deficient in photorepair of CPD, and the sensitivity to UVB radiation could seriously with deficient CPD photorepair. (4) the deficiency in Norin 1 results from a functionally altered photolyase.

Many other UV-sensitive rice cultivars or other important plant species have been found in field and laboratory tests. In most cases the origin of the sensitivity is unknown, making the design of bioengineering or breeding programs difficult for improving UVB resistance. Thus, determination of the susceptibility of such plants to UV-induced CPD and their ability to photorepair CPD should allow complementation of deficiencies to increase UVB resistance to increase crop yields.

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