Changes in Grain Size and Grain Storage Protein of Rice (Oryza Sativa L.) in Response to Elevated UV-B Radiation under Outdoor Conditions

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Grain size/Grain storage protein/Growth/Rice (Oryza sativa)/Ultraviolet-B Radiation.

Variation in growth, grain size and grain storage protein content of rice (Oryza sativa L.) in response to elevated UV-B radiation under sunlight was examined in a cool rice-growing region of Miyagi Prefecture, Japan, in 1999, 2001 and 2002. Tiller number, dry mass, panicle number, grain yield and grain size significantly decreased under elevated UV-B radiation in 2001 and 2002. The effects of elevated UV-B radiation on the reduction of each growth parameter were greatly enhanced by daily lower temperature during the ripening stage in those two years. On the contrary, total grain nitrogen content and grain storage protein content significantly increased under elevated UV-B radiation in 2001 and 2002. Among grain storage proteins, glutelin content significantly increased but albumin-globulin and prolamin contents did not. It was thus evident that not only grain size but also grain storage protein of rice was markedly influenced due to elevated UV-B radiation.

INTRODUCTION

Ultraviolet-B (UV-B: 280–320 nm) radiation damages plants, resulting in decrease of growth and productivity. Numerous studies on physiological, biochemical, morphological and anatomical changes in plants that are caused by UV-B radiation have been reported.¹⁻³ However, there have been relatively few reports on the effects of elevated UV-B radiation on rice, in spite of its being one of the world’s most important staple food grain. Experiments in greenhouses and growth chambers have indicated that rice cultivars vary widely in their sensitivity to supplementary UV-B radiation in terms of the deleterious effects of elevated UV-B radiation on the production of aerial biomass.⁴⁻⁹ In contrast, there have been few field studies on the effects of elevated UV-B radiation on growth and yield of rice. One such study was conducted for 1 year in 1993 in the temperate climate of Tsukuba, Japan (36°01’ N, 140°07’ N),¹⁰ and the other two were carried out for 2 years (from 1992 to 1993)¹¹ and 4 years (from 1992 to 1995),¹² respectively, in the tropical climate of the International Rice Research Institute (IRRI) in the Philippines (14°10’ N, 121°30’ N). All three studies found rice growth and yield were not likely to be affected by increase in UV-B radiation (the amount of biologically effectiveness of UV-B radiation: 12–14 kJ m⁻² day⁻¹) under field conditions, indicating a need to perform studies on the effects of supplementary UV-B radiation under different climates and latitudes, in which different cultivars are grown and where the UV-B quality is quite different. They also indicated that analyses over multiple seasons are needed because the effects of UV-B radiation on plants are strongly influenced by other seasonal microclimatic conditions. On the other hand, we examined the effects of supplementary UV-B radiation on the growth and yield of Japanese lowland rice cultivars for 5 cropping seasons (from 1993 to 1997)¹³ in a paddy field in a cool rice-growing region at middle latitude in Japan. In those experiments, tiller number, dry mass of aboveground parts and panicle number were reduced by supplementary UV-B radiation. Such reductions were significantly enhanced by unusual climatic conditions such as lower temperature and less sunshine. Also, the sensitivity to the inhibitory effects of supplementary UV-B radiation in a UV-sensitive cultivar was more greatly enhanced by such unusual climatic conditions than in a UV-resistant cultivar. Furthermore, we newly observed that grain size of rice decreased due to supplementary UV-B radiation in all seasons. Such a decrease was most pronounced in the year when the daily middle temperatures were lower throughout most of the cropping season. The reduction in grain size is very important because it would lead to change in grain stor-
age protein, which in turn would result in a change of food taste. To date, however, there have been few reports on the effects of elevated UV-B radiation on grain storage protein content in rice.

Experiments conducted in a real paddy field under natural sunlight are needed to evaluate the effects of supplementary UV-B radiation on growth and grain development such as grain size and grain storage protein content. However, storage protein content was greatly affected by basal fertilizer. In the present study, we conducted experiments using pots under natural sunlight. This paper deals with the changes in grain size and grain quality such as total nitrogen content and storage protein content in grain of rice caused by supplementary UV-B radiation in 1999, 2001 and 2002 in a cool rice-growing region of Miyagi Prefecture, Japan.

MATERIALS AND METHODS

Plant culture

The Japanese lowland rice cultivar Sasanishiki, which is one of a leading variety in Japan, was used as an experimental material. Seeds were surface-sterilized with a 2% (w/v) solution of sodium hypochlorite and then placed on wet filter paper in Petri dishes. The Petri dishes were kept at 30°C for 2 days. The germinated seeds were planted in soil (Gousesibaido No. 3; Mitui-toatu Ltd. Co., Tokyo, Japan) in seedling trays. The trays were transferred to a greenhouse and kept for ca. 4 weeks. The seedling grown to the three-leaf stage was transplanted into a 15-liter pot containing 12-liter paddy field soil. Seven grams of basal fertilizer (N : P : K, at a rate of 1 : 1 : 1), with slow release, was applied to a pot one week before transplantation, and then applied once at the period of seedling development and once at flowering. The soil in the pot was kept submerged under 5–10 cm of water throughout the experiment. Experiments were conducted from late May through early October for 3 years (1999, 2001 and 2002) in field at Kashimadai (37°28' N) in Miyagi Prefecture, Japan.

UV-B radiation was provided by UV-B fluorescent tubes (FL 20 SE; Toshiba Ltd. Co., Tokyo, Japan) with filtered UV-B intensity at the levels of the plants was 1.0 W m⁻² as measured with a spectroradiometer (SS-25; Japan Spectroscope Co., Tokyo, Japan). The dose of biologically effective UV-B radiation (UVBₑ) was calculated from the generalized plant action spectrum of Caldwell (1971), normalized to unity at 300 nm. The amount of supplementary UVBₑ (kJ m⁻² day⁻¹) applied daily was approximately 14.7. Supplementary UV-B radiation was applied from 6:00 a.m. to 5:00 p.m. To keep potted-plants out of the wind, the boundary from 30 cm to 120 cm above the soil was surrounded by polyester film (Shimizu Kagaku Co., Tokyo, Japan).

Measurements and statistical analysis of data

The tiller number of each plant was recorded ten weeks after transplantation. After harvesting, each plant was dried at room temperature to constant weight, and then divided into inflorescence and stem parts; the dry mass of each part was measured. Grains were classified by sifting through different sizes of mesh in an electric sieve (S-type; Ooyotanzu Seisakusho Co., Tokyo, Japan).

Fully ripened grains thicker than 1.9 mm were ground in a motor and pestle. The grain powder was dried at 45°C for one night and was used for measurements of content of nitrogen and protein. The nitrogen content was determined with Nestler’s reagent after Kjeldahl digestion.º Proteins were extracted sequentially by several solvents according to the method of Kumamura et al.º The procedure for extraction of each protein at each step was repeated three times. One milliliter of 50 mM KH₂PO₄-NaOH (pH 6.8) solution containing 0.5 M NaCl for extraction of albumin-globulin was added to 100 mg grain powder in a centrifuge tube. The suspension was sonicated with a ultra-sonicator (Bioruptor UCD-200 T; CosmoBio, Tokyo, Japan) for 3 min on ice and centrifuged at 10,000 × g for 20 min at 4°C. The supernatant was placed in a test tube. One milliliter of 60% n-propanol containing 1 mM EDTA-2Na for extraction of prolamin was added to the precipitate, and the extraction procedure was performed. Finally, glutelin was extracted with 1.0 ml of 1% lactic acid containing 1 mM EDTA-2Na. One hundred microliters of 0.15% deoxycholic acid was added to 1 ml of each albumin-globulin fraction and glutelin fraction. After being allowed to stand at room-temperature for 10 min, 0.1 ml of 15% trichloroacetic acid was added to each sample which was then allowed to stand for 30 min at 0°C. The samples were centrifuged at 1,800 × g for 15 min at 2°C. Each precipitate was washed with 80% acetone and dried. N-propanol in 1 ml of prolamin fraction was evaporated by a vacuum pump and dried. One milliliter of 1 N NaOH was added to each precipitate to solubilize the proteins. The protein content was then determined by the Lowry method using bovine serum albumin as a standard.

Results were analyzed separately for each season. Statistical comparisons were made between mean values per plot for UV-B treated and control plants using an unpaired t-test, with means separated by least significant differences (LSD). Significance was recognized at p < 0.05. The data were ana-
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The developmental stage of rice can be divided into three stages: tillering, panicle differentiation and ripening. In normal years in a cool region of Japan, the period of each stage approximately corresponds to from the beginning of June to the middle of July (tillering stage), from the middle of July to the middle of August (panicle differentiation stage), and from the middle of August to the end of September (ripening stage) in that order. The heading date of the cultivar Sasanishiki is around 5–10 August. Table 1 shows the mean daily middle temperatures and mean daily hours of sunshine of each 10-day period during the rice-cropping season in 1999, 2001 and 2002 and their average values for the last 30 years (1966–1996) according to the Annual Report of Miyagi Prefecture Meteorological Station. In the table, the mean daily middle temperatures lower than the average by 1°C of the last 30 years and the daily hours of sunshine <50% of the average values of the last 30 years are italicized. There were many days with higher mean daily temperatures throughout most of the cropping season in 1999, while there were many days with lower mean daily temperatures during the ripening stages in 2001 and 2002. On the other hand, there were relatively more days with less sunshine in 1999 throughout most of the cropping season in comparison with such days in 2001 and 2002.

Table 2 shows the effects of supplementary UV-B radiation on the growth parameters, namely, tiller number, dry mass, culm length, panicle number and grain yield under natural sunlight in 1999, 2001 and 2002. The number of tillers was counted 10 weeks after transplantation, while the other parameters were measured after harvest. In Table 2, percent change for each growth parameter was based on the following equation: (the value of sample – the value of control)/the value of control × 100%. The growth parameters except for culm length of plants grown with supplementary UV-B radiation were similar to those of plants grown without supplementary UV-B radiation in 1999. In 2001 and 2002, however, the growth parameters except for culm length were significantly reduced by supplementary UV-B radiation. These results showed that the reduction in each growth parameter was more evident in 2001 than in 2002.

Table 1. Mean daily middle temperatures and mean daily hours of sunshine during the rice cropping seasons in 1999, 2001 and 2002.

| Period | Mean daily middle temperature (°C) | Mean daily hours of sunshine (h) |
|--------|----------------------------------|--------------------------------|
|        | Average | 1999 | 2001 | 2002 | Average | 1999 | 2001 | 2002 |
| May    |         |      |      |      |         |      |      |      |
| Last 10 days |         |      |      |      |         |      |      |      |
| June   |         |      |      |      |         |      |      |      |
| First 10 days | 17.5 | 18.4 | 18.7 | 18.9 | 5.6 | 4.6 | 4.4 | 9.0 |
| Middle 10 days | 18.2 | 19.0 | 16.8 | 17.8 | 4.5 | 1.3 | 3.0 | 2.2 |
| Last 10 days | 19.2 | 19.2 | 20.5 | 15.5 | 3.7 | 1.7 | 1.6 | 0.8 |
| July   |         |      |      |      |         |      |      |      |
| First 10 days | 20.0 | 19.0 | 22.4 | 20.1 | 3.2 | 1.9 | 5.7 | 0.7 |
| August |         |      |      |      |         |      |      |      |
| First 10 days | 24.5 | 25.8 | 20.4 | 25.4 | 5.7 | 6.1 | 1.5 | 3.8 |
| III    |         |      |      |      |         |      |      |      |
| Middle 10 days | 24.5 | 25.6 | 20.9 | 22.2 | 4.9 | 2.1 | 3.9 | 1.4 |
| Last 10 days | 23.5 | 23.1 | 22.3 | 22.3 | 4.5 | 2.3 | 3.0 | 5.9 |
| September |         |      |      |      |         |      |      |      |
| First 10 days | 22.0 | 23.1 | 21.3 | 22.9 | 3.9 | 4.0 | 4.1 | 5.1 |
| Middle 10 days | 20.1 | 21.5 | 21.0 | 18.4 | 3.9 | 1.2 | 2.0 | 4.7 |
| Last 10 days | 18.1 | 19.4 | 14.7 | 16.5 | 4.3 | 3.2 | 6.7 | 4.6 |

*This table was constructed from the data of the Annual Report of the Miyagi Prefecture Meteorological Station (1999, 2001, and 2002). Italicized letters in daily middle temperature and daily hours of sunshine indicate values that are lower by more than 1°C and lower by more than half, respectively, than the mean values for the last 30 years.

Period I (from 1 June to 10 July): the tillering stage in both cultivars. Period II (from 11 July to 10 August): the panicle differentiation stage in both cultivars. Period III (from 11 August to 21 September): the ripening stage of seeds in both cultivars.

Daily middle temperature: (daily maximum temperature+daily minimum temperature)/2.

Average: mean values of daily middle temperatures and daily hours of sunshine for the last 30 years.
parameter due to supplementary UV-B radiation was greatly enhanced by unusual climatic conditions, especially by daily lower temperatures. Table 3 shows the relative distribution of weight percentage of grain with grain size thicker than 1.9 mm or 2.0 mm in plants grown with and without supplementary UV-B radiation in 1999, 2001 and 2002. As usual, fully ripened grain of this cultivar thicker than 1.9 mm is commercially used for human consumption in Japan. The weight percentage of grain thicker than 1.9 mm and 2.0 mm in plants grown without supplementary UV-B radiation was larger than that in plants grown with supplementary UV-B radiation for the three cropping seasons, although the values varied seasonally, namely, the size of fully ripened grain became smaller due to supplementary UV-B radiation. These results also showed that the effects of supplementary UV-B radiation on the reduction of the size of fully ripened grain were enhanced when experimental plants were grown for a longer period at lower mean daily temperatures in the ripening stages as seen in 2001 and 2002. These results are consistent with our previous findings.13)

We imagined that the reduction in grain size would lead to an increase in the weight percentage of grain with smaller size. To investigate this possibility, we measured the weight percentage of grains classified in terms of grain size for plants grown under control conditions and those grown with supplemental UV-B radiation for three cropping seasons.

| Season | Grain size | Control (%) | +UV-B | Change (%) | p* |
|--------|------------|-------------|-------|------------|----|
| 1999   | >1.9 mm    | 91.1        | 86.2  | -5.4       | ns |
|        | >2.0 mm    | 79.8        | 71.9  | -7.9       |    |
| 2001   | >1.9 mm    | 86.1        | 75.7  | -12.1      | *  |
|        | >2.0 mm    | 63.1        | 47.1  | -25.4      | ***|
| 2002   | >1.9 mm    | 88.2        | 77.0  | -12.7      | **|
|        | >2.0 mm    | 68.9        | 46.2  | -22.9      | **|

*Each relative value (%) is the ratio of the grain weight for each grain size to the total grain weight for plants grown under control conditions and with supplemental UV-B radiation.
*The percent change is based on the following equation: ((The value of sample - the value of control)/the value of control) × 100%.
to change in grain storage protein content, which is very important for taste. During ripening, nitrogen accumulates in the grain mainly as protein, and the protein is stored in protein bodies in the endosperm. We therefore examined the total nitrogen content and the storage protein content of grain thicker than 1.9 mm in plants grown with or without supplementary UV-B radiation under natural sunlight in 2001 and 2002. Table 4 shows the change in total nitrogen content of fully ripened grain (mg per 100 mg grain) in response to supplementary UV-B radiation. The total grain nitrogen content significantly increased due to supplementary UV-B radiation; the percent changes in 2001 and 2002 were 12.5% and 14.7%, respectively. Here, there was no significant difference between the dry weights per one grain (grain thicker than 1.9 mm) in plants grown with and without UV-B radiation (data not shown).

Table 5 shows the changes in storage protein of grain (mg protein was collected as water soluble, salt-water soluble, alcohol soluble and distilled acid soluble fractions in this order. In the experiment, albumin and globulin were treated together (see Material and Methods). As shown in the table, total protein content (sum of each storage protein content) increased due to supplementary UV-B radiation in the two years studied; the percent changes in 2001 and 2002 were 12.5% and 13.2%, respectively. Among proteins, glutelin content alone markedly increased by 10.9% in 2001 and 13.1% in 2002. The other proteins were not changed. It was thus evident that both total grain nitrogen content and grain storage protein content, especially glutelin content, were significantly increased by supplementary UV-B radiation.

The panicle differentiation stage, during which the young panicle is formed on the stem, is very important for determining panicle number per plant and grain number per one panicle of rice. Ripening of rice is a developmental process in which endosperm is formed accompanied by embryo formation and accumulation of reserve substances in the endosperm tissue. Storage protein is formed from photosynthates which are translocated from leaves (mainly flag leaves) and reserved in the endosperm tissue. Accumulation of storage protein in rice proceeds around the 5th day after flowering, reaches a maximum around the 20th day after flowering, and then remains at almost constant level throughout ripening. This means that the climatic conditions towards the end of the panicle differentiation stage and during the ripening stage play an important role in determining grain yield, grain size and grain storage protein content. Many researchers have shown that elevated UV-B radiation suppresses photosynthesis in leaves of higher plants, i.e., reductions in contents of some chloroplast proteins, total leaf nitrogen and soluble protein, and gene expression of photosynthetic genes. Besides, UV-B-induced DNA damages, especially cyclobutane pyrimidine dimer (CPD), occur in rice leaves as the result of solar UV-B radiation and are enhanced by elevated UV-B radiation. Accumulation of CPD may result in the inhibition of growth and development of rice. We thus speculate that the impairment of photosynthesis and the CPD accumulation in flag leaves due to supplementary UV-B radiation would also result in a reduction of grain yield and grain size. It is well known that the low temperatures suppress photosynthesis and reduce grain yield of rice. However, it is unclear why the effects of elevated UV-B radiation on the reductions of growth, grain yield and grain size were enhanced by daily lower temperatures. This problem should be solved in the future. On the contrary, the total grain nitrogen content and grain storage protein content significantly increased in response to supplementary UV-B radiation. Among proteins, only glutelin specifically increased. The production of grain storage protein

| Table 4. Grain nitrogen contents in plants grown under control conditions and with supplemental UV-B radiation for two cropping seasonsa. |
| Season | Control | +UV-B | Changeb (%) |
|--------|--------|-------|-----------|
|        | (mg N/100 mg grain) |          |           |
| 2001   | 1.36 ± 0.02  | 1.53 ± 0.03 | +12.5     |
| 2002   | 1.16 ± 0.01  | 1.33 ± 0.01  | +14.7     |

*Each value is the average of three experiments.
^The percent change is based on the following equation: ((the value of sample – the value of control)/the value of control) × 100%.

Table 5. Protein content of grains in plants grown under control conditions and with supplemental UV-B radiation for two cropping seasonsa.

| Season | Protein | Control | +UV-B | Changeb (%) |
|--------|---------|--------|-------|------------|
|        | (mg/100 mg grain) |          |       |            |
| 2001   | Total proteinb | 6.4 | 7.2 | +12.5 |
|        | Albumin-globulin | 2.3 | 2.4 | +0.02 |
|        | Glutelin | 3.4 | 4.1 | +10.9 |
|        | Prolamin | 0.7 | 0.7 | 0 |
| 2001   | Total protein | 7.6 | 7.2 | +13.2 |
|        | Albumin-globulin | 2.6 | 2.6 | 0 |
|        | Glutelin | 4.2 | 5.2 | +13.2 |
|        | Prolamin | 0.8 | 0.8 | 0 |

*Each value is average of three experiments. Error of each value was below 0.1%.
^Total protein is sum of albumin-globulin, glutelin and prolamin.
^The percent change is based on the following equation: ((the value of sample – the value of control)/the value of control) × 100%.

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of rice is well known. Nevertheless, the mechanism of biosynthesis is still unclear although a few studies have been reported as mentioned below. Glutelin is a major storage protein accounting for 60% of total grain storage protein in rice and is synthesized as a 57-kD precursor and then cleaved into a 37- to 39-kD acidic subunit and a 22- to 23-kD basic subunit.27) It is encoded by a multigene family that consists of the GluA and GluB subfamily.28) Furthermore, Kumamaru et al.18) reported the selection of rice mutants which revealed changes in the content of glutelin and improvement of grain storage protein. Recently, a few studies on the regulation mechanism of biosynthesis of grain storage protein in developing rice grain endosperm have been reported.29–30) However, there have been few reports on the effects of environmental factors, including elevated UV-B radiation, on the accumulation of storage protein and on the expression of genes related to biosynthesis of each storage protein. On the other hand, regarding the effects of elevated UV-B radiation on higher plants, elevated UV-B radiation regulates gene expression of photosynthetic genes and defense genes (relating to biosynthesis of flavonoids and anthocyanins) in an up- or down-direction depending on the kind of gene,31–34) and the photosynthetic proteins.35) Not only the biosynthesis of grain storage protein of rice but the mechanism of regulation due to elevated UV-B radiation are extremely complicated. Consequently, more time is necessary to resolve the mechanism of the increase in grain storage protein of rice due to elevated UV-B radiation. At any rate, this study demonstrated for the first time that grain storage protein content, especially glutelin content, of rice is greatly influenced by elevated UV-B radiation.

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