Water Status of Thermal Stress-resistant Rice Seeds

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Dry weight, water content, water status in relation to kernel quality of ripening seeds of rice cultivars against thermal stresses were examined. Although a temperature of 20 °C suppressed the growth rate of developing seeds of cvs. Nikomaru and Hinohikari, the dry weight of cv. Nikomaru was entirely recovered on 21 DAF. Additionally, the change in dry weight of cv. Nikomaru seeds grown at higher temperature did not differ from that of control during maturation. The water contents and $T_l$ values of cv. Nikomaru grown at 20 °C between 14 and 21 DAF markedly decreased in seeds indicating that the free water disappeared under low temperature stress during early ripening stage. After harvesting, thermal stresses did not severely suppress the ratio of ripened seeds and its quality of cv. Nikomaru. These results suggested that the diminish of free water in developing seeds is a reason that the quality in seeds can be maintained under low temperature stress.

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INTRODUCTION

Thermal stress during the grain-filling stage usually causes deleterious effects on the yield and quality of crop products. Lower temperature reduces the growth rate of grain, extends duration of the grain filling period but delays grain maturation, while moderately cool temperature sometimes benefits grain yield. On the other hand, high temperature restricts the grain growth of rice, wheat and barley. The previous studies consider that this is because of disappearance of enzyme activity, which relates to starch synthesis of grains. Thermal stress also influences in the period of rice grain maturation by changing photosynthetic rate as well as dehydration process.

Seed maturation is associated with biochemical and physiological changes in tissues along with dehydration. The dynamic states of water compartments in seed tissues correlate with the organic properties of macromolecular structures related to seed development. Recently, water status of rice (Oryza sativa L. cv. Hinohikari) grains exposed to thermal stress was
evaluated by NMR relaxation times. During early ripening stage, $T_1$ values of rice grains grown at 20°C slightly increased but those grown at 30°C decreased markedly\(^9\). This revealed that the changes in $T_1$ of rice grains closely related to the quantity of water until the mid-mature stage, influenced by low/high temperature stress.

Therefore, the present study was undertaken to evaluate the changing pattern of $T_1$ of seeds of sensitive and insensitive rice cultivars against thermal stress during seed maturation. We will discuss the profiles of NMR relaxation times of seeds in relation to kernel quality during the development and maturation periods.

**MATERIALS AND METHODS**

**Plant materials**

Rice seeds (*Oryza sativa* L.) of cvs. Hinohikari and Nikomaru were sown in seed bed on May 25th, 2006. Two cultivar seedlings were transplanted on June 19th in each plastic pots, sized 1/5000 a, and contained 2.5 kg soils. Plants were allowed to grow until heading stage at the experimental field of Kyushu University. Irrigation and pesticide were applied to ensure optimal plant growth. Compound fertilizer (N-P$_2$O$_5$-K$_2$O:16-16-16) and ammonium sulfate (N:21%), 0.6 g and 0.5 g per pot, respectively were applied, as basal dressing. Additionally, 0.3 g of ammonium sulfate (N:21%) was topdressed at panicle formation and booting stages. Cultivar Hinohikari on August 21st and cv. Nikomaru on August 24th both at heading stage, all the pots were transferred to phytotron growth cabinet, and the plants were started to grow there under three temperature treatments 20, 25 and 30°C until maturity. After flowering, at seven-days interval four pots were randomly selected from each treatment for analyzing necessary parameters. Seeds (rough rice) of primary branches were used for the required studies. Each experiment was replicated four times. All parameters were examined at seven days intervals until 49 days after flowering (DAF).

**1H-NMR spin-lattice relaxation time ($T_1$)**

Spin-lattice relaxation time ($T_1$) of the samples were measured based on the procedure described by Iwaya-Inoue et al.\(^9\). A 1H-NMR spectrometer with a magnet operating at 25MHz for 1H (Mu25A, JEOL Ltd., Tokyo, Japan) was used for the measurements of $T_1$. Twenty to 24 seeds (rough rice) were prepared for the measurements of NMR relaxation times. The sample was put into an NMR tube (10 mm in diameter) set in the NMR spectrometer. The probe temperature was controlled at 30°C thermostat connected to the sample chamber of the spectrometer.

For $T_1$ measurement, the saturation recovery method (90°-τ-90° pulse sequence) was used. In this method, $T_1$ is determined from $M_\tau=M_0[1-\exp(-\tau/T_1)]$, where $M_\tau$ is the magnetization amplitude of proton at interval time $\tau$, and $M_0$ is the magnetization amplitude of proton in the equilibrium state. For detailed analysis, the two-component analysis was applied\(^9\). For each treatment, four replications was applied\(^9\).

**Water content and rice quality**

Rice seeds were oven-dried at 90°C for 20 h to determine the water content and dry weight. Twenty to 24 seeds were used for NMR at each sampling time. Rice ears grown at each temperature were harvested at 49 DAF and left for air drying. One hundred kernels of the husked seeds were evaluated for their quality\(^9\).

**RESULTS AND DISCUSSION**

**Dry matter accumulation and water content of rice seeds exposed to thermal stresses during maturation**

Dry weight and water content of cv.
Nikomaru seeds were compared to cv. Hinohikari. They were exposed to three temperature treatments, 20, 25 and 30 °C, during rice seed maturation (Fig. 1). The changes in dry weight and water content of seeds cv. Hinohikari grown at each temperature (Fig. 1A, C) indicated a similar tendency observed by Funaba et al.8.

A temperature of 20 °C suppressed the growth rate of developing seeds of cv. Nikomaru and cv. Hinohikari as previously known that the lower temperature reduces the rate of dry matter accumulation and delays seed maturation8. However, the dry weight of cv. Nikomaru seeds exposed to low temperature was entirely recovered on 21DAF. Water content of the two cultivars grown at 20 °C slightly decreased during the early ripening period while that of the seeds grown over 25 °C markedly decreased (Fig. 1C, D). Although low temperature prolongs seed maturation period of rice by delaying dehydration process8, the water contents of cv. Nikomaru seeds grown at 20 °C from 14 to 21 DAF were changes in dry weight and water contents of cv. Nikomaru seeds grown at 30 °C did not differ from that of 25 °C treated seeds during maturation (Fig. 1B, D).

Water status of rice seeds exposed to thermal stresses during maturation

The changes in NMR spin-lattice relaxation time (T1) in rice seeds are closely related to the quantity of water until mid-mature stage8. Therefore, the changes in T1 were observed in seeds for both cultivars during the developing and maturing stages. Throughout the measurement, the T1 values of long and short fractions of cv. Nikomaru seeds indicated similar change in all temperature treatments (Fig. 2B, D). However, those of cv. Hinohikari grown at 20 °C were longer and those grown at 30 °C were shorter compared with the control grown at 25 °C (Fig. 2A, C). T1 values between 100 ms and 3 s are considered to show the existence of free water that mainly originates from vacuolar water8.10. These results

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Fig. 1. Dry weight (A, B) and water content per dry weight (C, D) in ripening seeds of two rice cultivars exposed to thermal stresses. A and C, cv. Hinohikari; B and D, cv. Nikomaru. Open rhombuses, 20 °C; closed squares, 25 °C; open triangles, 30 °C treatment, respectively.
suggested that cv. Hinohikari seeds grown at 20 °C maintained free water for over seven days longer than those grown at 25 and 30 °C. The temperature of 20 °C delayed dry matter accumulation and thus might maintain the water mobility for a long duration in Hinohikari (Figs. 1A, 2A). These results suggest that the marked prolongation of T1 value on 14 DAF in the seeds grown at 20 °C was due to immaturity of endosperm and its higher water content. Close relationship between T1 and water content in rice seeds was observed in early ripening stage. Water status of seed correlated with the change in storage substances associated with seed development and maturation. Kano et al. stated that low molecular compounds reduced NMR relaxation times not so significantly at low concentrations but strongly at a higher concentration.

The present study indicated that cv. Hinohikari seeds grown at 20 °C maintained longer T1 values than those at 25 °C and 30 °C until late ripening stage. However, T1 values of cv. Nikomaru decreased from about 200 ms to less than 100 ms between 14 and 21DAF. Since T1 values indicating over 100 ms are considered to show the existence of free water, the fact indicates that free water disappeared in seeds of cv. Nikomaru exposed to a temperature of 20 °C during early ripening stage.

The decrease in the NMR signal intensity in the endosperm of rice seeds during the development of the caryopsis corresponded with the change in the physical property of the starch storage tissue from fluid to doughy, in appearance from milky white color to translucent. Horigane et al. indicated that water in caryopsis was mostly distributed along the peripheral layer and in the pericarp vascular bundle in seed before 15DAF with NMR microimaging. The starch in the endosperm of rice seeds slowly changed from fluid to doughy along with decrease of water content and dry matter accumulation during seed development. This change led to the solidification of endosperm and formation of translucent area.

Fig. 2. T1 values of long fraction (A, B) and short fraction (C, D) in ripening seeds of two rice cultivars exposed to thermal stresses. A and C, cv. Hinohikari; B and D, cv. Nikomaru. Open rhombuses, 20 °C; closed squares, 25 °C; open triangles, 30 °C treatment, respectively.
These observations are in agreement with the results that water with high fluidity in the seeds grown at either 25 or 30 °C disappeared at late maturing stage, and that free water in the seeds grown at 20 °C is maintained for a longer period than in the seeds grown at a higher temperature (Fig. 2).

Maturation and quality of kernels in relation to water status of rice cultivars exposed to thermal stresses

The ratio of perfect kernels of cv. Nikomaru was 64, 84 and 70% while that of cv. Hinohikari was 13, 61 and 4% at 20, 25 and 30 °C treatment, respectively (Fig. 3).

![Fig. 3. Influence of thermal stresses on distribution of rice kernel quality. A, cv. Hinohikari; B, cv. Nikomaru.](image)

On the contrary, the percentage of the kernels with a white-back or a white-ridged was over 83% in cv. Hinohikari seeds grown at 30 °C while that of cv. Nikomaru was around 13%. Seeds with white opaque parts in their cores or ventral sides of the endosperm, called white core rice kernel or white belly rice kernel, have an unusual loose cell arrangement. In addition, the percentage of ripened seeds of cv. Nikomaru increased as the temperature rose; it was 41, 75, and 87% while that of cv. Hinohikari was 34, 78, 76% with 20, 25 and 30 °C treatment, respectively (data not shown).

These results indicated that low and high temperatures did not severely suppress the ratio of ripened seeds and its quality of cv. Nikomaru.

The present study suggested that the early reduction of free water in cv. Hinohikari seeds at a higher temperature caused the formation of a higher number of white-back or chalky seeds during maturation (Figs. 2A and 3A). It was indicated that high temperature stress enhanced cell development of endosperm when kernel entered the linear phase of starch accumulation, and thus enhanced occurrence of abnormal and chalky rice kernels (Fig. 3).

In the thermal stress-resistant cultivar, cv. Nikomaru, starch accumulation in seeds grown at low/high temperature normally changed from a fluid state to a doughy state accompanied by a decrease in water content. Especially, the free water in the developing seeds diminished as similarly in seeds grown at 25 °C. This caused the production of higher quality seeds under thermal stress.

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