Seed Injuries in Rice Plants Exposed to Temperature Stresses during Ripening Process Monitored by $^1$H-NMR Technique

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Dynamic states of water in seeds of rice (Oryza sativa L., cv. Hinohikari) plants grown at low/high temperatures during seed maturation were examined by NMR relaxation times ($T_1$ and $T_2$). Dry weight of grain grown at 20°C gradually increased while both 25 and 30°C enhanced growth rate at early- to mid-stage. $T_1s$ in the seeds treated by three different temperatures related to their water content until 36 DAF (days after flowering). $T_2$ values of grain treated both at 25 and 30°C sharply dropped to less than 1ms after 22 DAF, while those treated at 20°C remained more or less unchanged until 29 DAF. Therefore, the low temperature maintained free water in grain one week longer than the high temperature treatment. The 20°C-treatment produced thin rice kernels which were lower than 1.8 mm in thickness, while 30°C-treatment enhanced the kernel size over 2.0 mm in thickness. The present results indicated that the $^1$H-NMR spectroscopy was useful for early diagnosis of temperature stresses on rice kernel development. (Received Oct. 27, 2005: Accepted Dec. 24, 2005)

INTRODUCTION

Plants are subjected to a constantly fluctuating environment. Temperature is one of the most important factors which have tremendous influence on plant growth, development and propagation. Every plant requires optimum temperature for its proper growth, but it may face stresses and injured when it fails or exceeds the range of the optimum growth temperature.

Cultivation area of rice in Japan is spread from warm-temperate to cool-temperate regions. Quality of rice kernel is strongly affected by temperature at ripening period. Temperature stresses deteriorate quality of rice. It has been reported that temperature, especially the mean daily air temperature, during grain filling is one of the dominant climatic factors affecting rice quality. Low temperature causes failure of fertilization, suppression of the development of embryo and endosperm of kernel, and increase in the rate of green immature rice kernel. It was suggested that specifically the low temperature accelerated the expression of the bound starch synthase gene in rice endosperm, which resulted in elevated amylose biosynthesis in the...
endosperm\textsuperscript{9}. On the other hand, high temperature enhances the occurrence of sterility, deterioration of grain weight and increase in appearance of white-back and white-based rice kernels \textsuperscript{7,8,10}. The high temperature may significantly accelerate the grain filling rate, but correspondingly shorten its duration. It induces loosely packed starch granules, thus resulted in decreased grain weight. Also it enhanced occurrence of abnormal and chalky rice \textsuperscript{9,10}.

Water plays a key role in the physical and thermal properties of rice grains \textsuperscript{11}. It has been demonstrated that water status of woody plant seeds can be efficiently monitored by $T_1$ and $T_2$ during seed maturation using pulsed $^1$H-NMR (nuclear magnetic resonance)\textsuperscript{12}. NMR is a nondestructive method of studying the state of water in many biological systems\textsuperscript{13}. The dynamic states of water in biological tissues were considered to reflect the physiological and metabolic activities in living cells. Several studies reported the changes in NMR relaxation times of plant tissues exposed to chilling and heat stresses\textsuperscript{14-17}.

Therefore, the NMR technique is expected to provide a sensitive and direct method of characterizing water status in seeds exposed to different temperatures during ripening process. The objective of this study was to evaluate the feasibility of $^1$H-NMR relaxation times for monitoring early diagnosis of rice kernel injuries caused by temperature stresses at the time of ripening.

**MATERIALS AND METHODS**

**Plant materials**

Rice (Oryza sativa L. cv. Hinohikari) seeds were sown at 4th June 2004. Seedlings were transplanted to the plastic pots at 25th June. The size of a plastic pot was 1/5000 a, and two seedlings were planted in each pot. Rice plants were grown at the experimental field of Kyusyu University from planting to heading stage. At heading stage on 27 August, all the pots were transferred to phytotron and plants were allowed to grow there until maturation of grain under three temperature treatments such as 20, 25 and 30°C. Each 7-8 days interval after flowering, 4 plants was randomly selected from per temperature treatment for necessary assessment. Twenty to twenty four grains were collected from upper part of primary and secondary rachis branches from two rice ears for the following experiments.

**NMR relaxation times of rice seeds**

NMR spin-lattice relaxation time ($T_1$) and spin-spin relaxation time ($T_2$) of the grain were measured based on the method described in detail\textsuperscript{13}. The probe temperature was controlled by a thermostat connected to the sample chamber of the spectrometer at 30°C. For $T_1$ measurements, the saturation recovery method (90° · τ · 90° pulse sequence) was used. The $T_2$ was measured by the Carr-Purcell-Meiboom-Gill (CPMG) method and solid echo method.

After determination of the NMR relaxation times, the grains were oven-dried at 90°C for 20h. Fresh weight, dry weight and amount of water in each grain during ripening process were measured.

**Thickness of rice kernels**

Ears were harvested at 51 days after flowering (DAF) for each treatment and left it for air-drying. Grains were threshed to kernels for determination of ratio of ripened grains. Then thickness and quality of kernels were determined based on Funaba et al. \textsuperscript{18}.

**RESULTS AND DISCUSSION**

**Influence of temperatures on dry weight and water content in grain during ripening process**

Growth in grains exposed to temperature
stresses during ripening process are presented in Fig. 1. Dry weights of grain grown at 20°C gradually increased during ripening process and it was the lowest until 36 DAF among three temperature treatments. However, it was attained to approximately equal level to others at 44 DAF (Fig. 1). On the contrary, dry weight of grain treated both at 25 and 30°C rapidly increased until 28 and 22 DAF respectively (Fig. 1). Subsequently, weight of seeds treated by both temperatures gradually increased and did not present such a difference after 28 DAF until 51 DAF.

![Graph showing dry weight and water content in rice grain during maturation at different temperature treatments.]

**Fig. 1.** Dry weight and water content in rice grain during maturation at different temperature treatments.

Previously, Nagato²⁰ had reported that kernel weight of rice plants grown at 30°C rapidly increased, but it stopped at early stage while rice plants grown at 23°C slowly increased in kernel weight, and it exceeded those grown at 30°C. Almost similar results were noticed in the present experiment. The high temperature throughout the ripening period accelerated the starch accumulation into kernel and the kernel development during the early period of ripening, but depressed it in late period ²⁰. They reported that embryo grew faster than the endosperm and reached maximum stage around 10 days after anthesis. These processes continued until grain maturation.

**Changes in **$T_1$** and **$T_2$** of grain during ripening process**

Changes in $T_1$ are shown in Fig. 2A. The $T_1$ of grains treated at 20°C was lower than other treatments at 7 DAF, but it was higher than other two temperature treatments from 15 to 44 DAF. The $T_1$ value at 7 DAF was about 220 ms, and it gradually decreased to about 70 ms at 36 DAF.

On the other hand, $T_1$s of grain treated both at 25 and 30°C rapidly decreased until 22 DAF, and $T_1$s were constant thereafter. Water content of grain grown at 20°C treatment was the lowest among temperature treatments at 7 DAF, but it increased remarkably at 15 DAF, and next linearly decreased until 36 DAF (Fig. 1). On the contrary, water in seeds treated both at 25 and 30°C decreased rapidly from 7 to 22 DAF, but it gradually decreased until 51 DAF. $T_1$s in the seeds treated by three different temperatures related to their water content until 36 DAF. Previously, it was reported that seasonal changes in $T_1$ were closely correlated with water content of dormant buds of azalea florets ²¹.

Changes in $T_2$ during ripening process are presented in Fig. 2B. The $T_2$ of grain treated by 20°C at 7 DAF was lower than other treatments, similarly it was the lowest as in $T_1$ (Fig. 2). After 22 DAF, both $T_2$ values of grain treated at 25 and 30°C sharply dropped to less than 1ms. Whereas, $T_2$ values of grain treated at 20°C remained more or less unchanged until 29 DAF. These results implied that the low temperature maintained free water one week longer than the high temperature treatment.

Towards the ripening stage, the $T_2$ of all treatments were less than 1ms. During grain
Fig. 2. Influence of temperatures (20°C, 25°C and 30°C) on (A) $T_1$ values and (B) $T_2$ values in rice grain maturation at three temperature treatments.

filling at high temperature (daily air temperature, 32°C) the component and crystalline structure of starch are resulted in deterioration of eating and cooking quality of indica rice. It is known that $T_2$ reflects compartment size of crosslinked polymer gel and is strongly affected by the concentration of crystalline water. The present results were diagnosed the $T_2$ sensitively related to decrease of water content of grain at 29 DAF. Similar facts were also observed in rice grown at outdoor.

**Influence of temperatures on thickness and quality of rice kernels**

Rice ears were harvested at 51 DAF for each treatment and left for air-drying. Grains were threshed to kernels for determination of thickness and rice quality. The temperature, 20°C treatment produced thin rice kernels indicating less than 1.8 mm in thickness, while 30°C treatment enhanced the kernel size over 2.0 mm in thickness (Fig.3). The percentage of ripened kernels increased at temperature enhanced: about 60, 74 and 82% at 20, 25 and 30°C treatment, respectively. Low temperature decreased ratio of ripened kernel while it increased kernel weight of ripened grains. It was considered that the milky stage was sensitive to temperature and critical for determining the final size of endosperm.

Additionally, at 20°C treatment, many notched-belly kernels were observed while 70% of both perfect and green kernels were observed at 25°C. It was reported that notched-belly rice kernel occurred when the development of ventral radius was partially stopped at 23°C treatment.
Contrary, at 30°C treatment, white-back kernels occurred more than 80% of kernels harvested. It has been reported that over 27°C of mean air temperature at flowering stage to 20 DAF caused many white-back kernels in rice plants 7). Indeed, high temperature resulted in loosely packed starch granules, decreased kernel weight, and thus it enhanced occurrence of abnormal and chalky rice kernels 9, 10. It was also shown that rice caryopsis was most sensitive to environmental stresses, such as high temperature at early development, i.e. milky stage 10).

Mentioned above of these facts, it was clear that 1H-NMR spectroscopy was useful for early diagnosis of temperature stresses on rice kernels.

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イネ種子の登熟過程における温度障害の $^1$H-NMR モニタリング：松場 貢 12, 岩浪 賢司 1，アプル フセイン モラ 13，石橋 勇志 1，井上 眞理 1(九州大学大学院生物資源環境科学府，2長崎県庁，3BSMR 農業大学) [キーワード：温度障害，イネ種子，$^1$H-NMR，登熟過程]