Early identification of pre-harvest sprouting in rice seeds by using ^{1}H-NMR

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Abstract

Preharvest sproutiong (PHS) is a serious problem in economically important yield and grade losses for producers. Unreleased test lines of cereals should be screened for resistance to PHS. However, screening large numbers of test lines is relatively time-consuming or expensive. Nuclear Magnetic Resonance (NMR) which provides a non-destructive method can be effective in characterizing seed water status. Spin-spin NMR relaxation time (T_2) in rice indicated that cellular water in the dry seeds mainly consisted of tightly bound water. The objective of this study was to evaluate whether the ^{1}H-NMR relaxation times can be applied for screening test for resistance to PHS. T_2s in non-germinating seeds of 28 days after-pollination (DAP) were constant. While, T_2s in germinating seeds of mature seeds linearly increased as germination advanced. Therefore, it was suggested that the T_2 reflected the germination process. Thus, measurement of ^{1}H-NMR relaxation times were applied for screening test of resistance to PHS.

Media summary

Spin-spin NMR relaxation times (T_2) reflected the germination process, and were applied as a screening test for resistance to pre-harvest sprouting.

Keywords

^{1}H-NMR relaxation times, Germination process, Near-isogenic lines

Introduction

Precocious germination of cereal grain is a serious problem in crop production. Pre-harvest sprouting (PHS) is closely associated with agronomic difficulties and loss in functional quality which equate to economic losses for producer, marketing agency and end-user. Sprouted grain can be difficult to thresh resulting in harvest losses and it is a downgrading factor resulting in reduced economic return for the producer (Derera 1989). The Falling Number method is applied for estimating PHS damage in wheat and rice, (Imabayashi et al. 1998; AACC 2000). However, it is necessary to grind the seed for this method.

Nuclear Magnetic Resonance (NMR) which provides a non-destructive method can be effective in characterizing seed water status. It has been shown that NMR relaxation time, indicating water status, is greatly enhanced in tumour tissues in comparison with the corresponding healthy ones (Damadian 1971; Williams et al. 1980). Water plays an important role not only as a solvent for biochemical reactions, but also as a stabilizer of macromolecular structure. Recently, dynamic contrast-enhanced NMR imaging (MRI) has been widely used, and is one of the best and most precise methods for tracing water movement in plant tissues (Pietzak et al. 2002). Using pulsed ^{1}H-NMR it has been demonstrated that water status of woody plant seeds can be efficiently monitored by T_1 and T_2 during seed maturation (Iwaya-Inoue et al. 2001). The dynamic states of water in biological tissues was considered to reflect their physiological changes. The components of T_1 and T_2 have been shown to arise from distinct populations of water in plant tissues (Gusta et al. 1979; Belton et al. 1985; Isobe et al. 1999; Iwaya-Inoue and Nonami. 2003). The objective of this study was to evaluate whether the ^{1}H-NMR relaxation times can be applied as a screening test of resistance to PHS.

Method

Oryza sativa L. 'Taichung 65' (TC65) and 'Notched' were used for the experiments. 'Notched' which has the with notched kernel phenotype was obtained as BC_4F_2 seeds through the reciprocal crosses between *O.glumaepatula* and 'TC65' and is homozygous for *O.glumaepatula* allele at *R1607* on long arm of chromosome 5 (*nk2 nk2*) (Sobrizal et al. 1999; Sobrizal et al. 2002). Fresh weights of the rice seeds collected at days after-pollination (DAP) were measured. Ratios of PHS were measured as germination ratio of seeds for ears which incubated at 25?C for 7 days. The samples measured by NMR were dried at 90?C for 20 h. Water content was expressed as the ratio of the amount of water vs. dry weight basis. In seeds of 28 DAP and mature seeds, ^{1}H-NMR spin-spin relaxation time (T_2) was measured everyday until 7 days, at 10 and 14 days after the imbibition. A ^{1}H-NMR spectrometer with a magnet operating at 25 MHz for ^{1}H (JNM Mμ25A, JEOL Ltd., Tokyo, Japan) was used for the measurements of T_2. The T_2 was measured by the Carr-Purcell-Meiboon-Gill (CPMG) method. T_2 is determined from $M_{2\mu\tau} = M_0 \exp(-2\mu\tau / T_2)$, where M_0 in the magnetization amplitude of the proton signal occurring at time 2τ after the initial 90? pulse in CPMG (90?x－τ－180?y－2τ－180?y－2τ…) pulse sequence. The T_2s were calculated based on 500 echo signals acquired by accumulation of 32 scans. The probe temperature was 25?C controlled by connected thermostat to the sample chamber of the spectrometer. The solid-echo (90?－τ－90?) method was also applied for T_2 measurements when T_2 values were below 1 ms. The solid echo signal was obtained by accumulation of 32 scans. In the T_2 measurement, repetition time of the pulse sequence was also kept more than five times of T_1. A decay curve of echo signal was decomposed for components of $M(t) = \Sigma\, ai \cdot \exp[-(t / T_2i)^{mi}]$ where *mi* is Weibull coefficient, 1 or 2, and $fi = ai / \Sigma\, ai$, where *fi* is fraction ratio (Sato 1994).

Results and Discussion

Ratio of PHS of ears in rice lines during maturation are shown in Fig.1. At 21 DAP, 'TC65' did not germinate, but 'Notched' germinated. At 28 DAP, the germination rate was about 20% in 'TC65', and about 70% in 'Notched'. From these results, it was indicated that these rice lines have different characteristics for PHS.

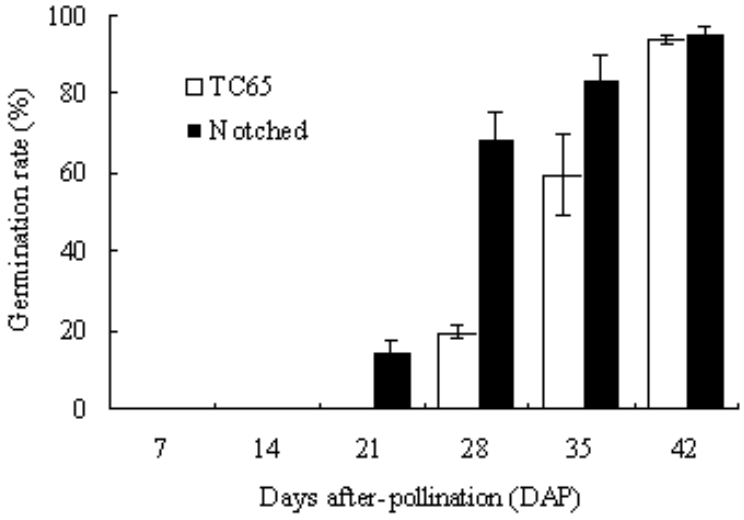

Figure 1. Ratio of pre-harvest sprouting (PHS) of seeds in ears of 'TC65' and 'Notched' during seed development. n = 5, 80 seeds / ear

Seeds of 'TC65' during maturation are shown in Fig.2. At 21 DAP, seeds have grown up to the same size as harvest stage and at 28 DAP they showed loss of green color. Similar growth stage was observed in 'Notched' seeds.

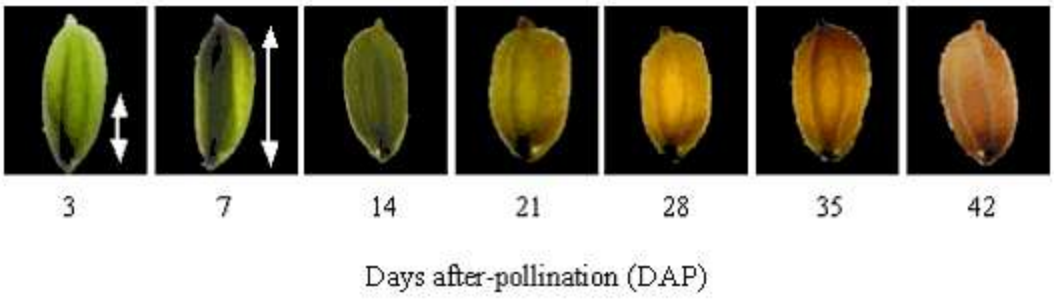

Figure 2. Development of seeds in *Oryza sativa* L. 'TC65'. Numerals indicate days after-pollination (DAP). Arrows in 3 and 7 DAPs indicate ovary length in a seed picture taken by transmitted light.

Fresh weight and dry weight in a seed markedly increased at 14 DAP (Fig.3 A). Water content was the highest at 7 DAP, and markedly decreased until 21 DAP (Fig.3 B). Rice seeds at 28 DAP when the water content declined to low level indicating their physiological maturity stage. It has been suggested that raffinose family oligosaccharides may form a viscous glassy state (a thermodynamically unstable solid state with an extremely high viscosity) during seed dehydration (Minorsky 2003). Although the rice seeds are protected against drought stress, there was no significant difference between 'TC65' and 'Notched' line in relation to vivipary.

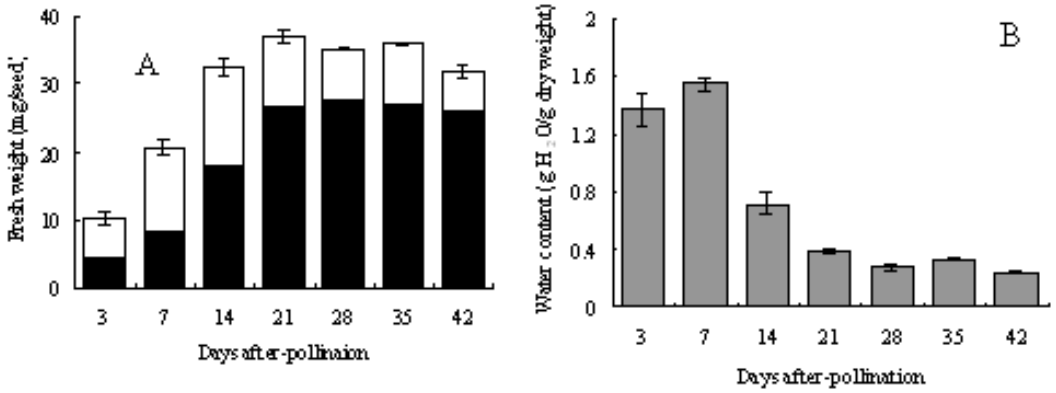

Figure 3. Weights of dry matter and amount of water per seed (A), and seed water content (B) in rice 'TC65' during seed development. White columns and black ones indicate amount of water and dry weight, respectively. Gray columns indicate seed water content (g H_2O/g dry weight).

Fig.4. shows NMR spin-spin relaxation time (T_2) in 'TC65' at days after germination treatment. T_1 and T_2 reflects dynamic states of water such as free water and bound water binding to macromolecules (Iwaya-Inoue et al. 2004). Especially, T_2 reflects compartment size of crosslinked polymer gel and is strongly affected by the concentration of crystalline water (Murase and Watanabe 1989). The T_2 value in the dry seeds indicated that the cellular water mainly consisted of loosely bound water and tightly bound water. T_2s in non-germinating seeds at 28 DAP were about 20ms and they maintained constant values until 10 days. However, T_2s in germinating seeds at mature stage in 'TC65' linearly increased as germination advanced. Additionally, there was no significant difference in T_2 values of non-germinating seeds but significant difference was observed in T_2 of germinating seeds at mature stage between 'TC65' and 'Notched' (data not shown).

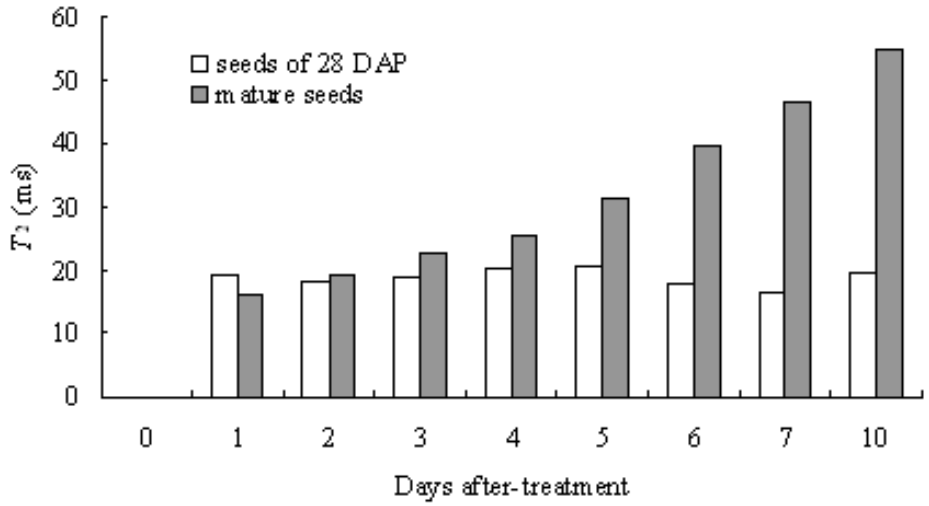

Figure 4. NMR spin-spin relaxation time (T_2) in seeds of 'TC65' during germination treatment. Non-germinating seeds were measured at 28 DAP. Germinating seeds were measured at mature seed stage. (*Ref*. Figs. 1 and 2).

Conclusion

T_2s for non-germinating seeds of 28 DAP indicated constant values during imbibition treatment. On the other hand, T_2s for germinating seeds at mature stage linearly increased as germination process advanced. There was a significant difference not in water content of developing seeds but in T_2 of germinating seeds between 'TC65' and 'Notched' in relation to their characteristics of vivipary. Therefore, it was suggested that the T_2 reflected the germination process and measurement of ^{1}H-NMR relaxation times (T_2) were applied for screening test for resistance to pre-harvest sprouting.

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