Ascorbic Acid Suppresses Germination and Dynamic States of Water in Wheat Seeds

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NMR relaxation time ($T_2$) indicating dynamic states of water denotes degree of degradation of endosperm in germinating crop seeds (Ishibashi et al., 2005). Pre-harvest sprouting (PHS) in wheat causes devastating damage to the quality of wheat flour due to the degradation of seed starch during germination. Thus far, it has not been possible to establish a technique for suppressing PHS.

Exogenously applied hydrogen peroxide (H$_2$O$_2$) ameliorates seed germination in many plants (Chien et al., 1994; Fontaine et al., 1994). H$_2$O$_2$ is produced during the early imbibition period in several seeds. The plant tissues contain ascorbic acid which acts as an antioxidant that scavenges reactive oxygen species such as hydrogen peroxide.

Mobile and less mobile water molecules are distinguished from each other by their different NMR relaxation times ($T_1$, $T_2$), and their relative amounts have been calculated in ryegrass leaves and roots (Iwaya-Inoue et al., 2004) and rice seeds (Ishibashi et al., 2004). The aims of this study were (1) to find a technique for suppressing germination, and (2) to investigate the effect of ascorbic acid on the germination process and dynamic states of water in seeds by using NMR. Here we clearly show that wheat seed germination is inhibited by exogenously applied ascorbic acid and the treatment will be effective for inhibition of PHS.

Materials and Method

1. Plant materials

*Triticum aestivum* L. cultivar Norin61 was used as the material. This cultivar was grown in an experimental field of Kyushu University in 2003-2004 in Fukuoka on 30m$^2$ plots. Irrigation, fertilization and pesticide were performed to ensure optimal plant growth. Ripe kernels were harvested on July 1, 2004, and stored at 4º C for six months before use.

2. Germination test

Twenty seeds of wheat were placed on filter paper in a Petri dish (diameter 9 cm). Six milliliters of distilled water, ABA at 100 µM, ascorbic acid at 10, 50 and 100 mM, succinic acid at 10, 50, and 100 mM and mannitol at 10, 50, and 100 mM were applied to each dish. The Petri dishes were incubated at 14ºC in the dark, and the number of germinating seeds was counted daily for 7 days. The weighted germination index (GI), in which the maximum weight was given to the seeds that germinated first and less weight to those that germinated subsequently (Walker-Simmons et al., 1990), was calculated as follows:

$$GI = (7 \times n_1 + 6 \times n_2 + \cdots + 1 \times n_7) / (\text{Total days} \times \text{Total grains})$$

Where, $n_1$, $n_2$,···,$n_7$ are the number of germinating seeds on the first, second, and subsequent days until the seventh day, respectively, and 7, 6,···, are the weights given to the number germinated on the first, second, and subsequent days. The maximum GI is 1.0 and the minimum is 0.

3. Water content

The fresh weights of the wheat seeds used in the NMR determinations were measured. The samples applied by NMR spectroscopy were dried for 20 h at 90ºC and weighed. The water content was expressed as a percentage of fresh weight.

4. Measurements of $^1$H-NMR relaxation time, $T_2$

A $^1$H-NMR spectrometer with a magnet operating at 25 MHz for $^1$H (JNM Mz25A, JEOL Ltd., Tokyo, Japan) was used to measure the $^1$H-NMR relaxation times,
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the spin-spin relaxation time ($T_2$). Fifteen grains were packed into an NMR tube 10 mm in diameter. $T_2$ was determined more than five times.

$T_2$ was measured by the Carr-Purcell-Meiboom-Gill (CPMG) method. $T_2$ is determined from $M_2n = M_o \exp(-2n\tau/T_2)$, where $M_o$ is the magnetization amplitude of the proton signal occurring at time $2\tau$ after the initial 90° pulse in the CPMG (90°x-τ-180°y-2τ-180°y-2τ--τ) pulse sequence (Iwaya-Inoue et al., 2004). The $T_2$s were calculated based on 500 echo signals acquired by the accumulation of 32 scans. The probe temperature was adjusted to 30°C by a thermostat connected to the sample chamber of the spectrometer.

The decay curve of the echo signal was analyzed by using a non-linear least-square method on semi-log plots of signal intensity (Braga et al., 1997; Kumamoto et al., 1998).

Results and Discussion

1. Suppression of seed germination by ascorbic acid

Ascorbic acid, which acts as an antioxidant, suppressed seed germination. The inhibition by 50 mM ascorbic acid was similar to that by 100 µM ABA. The GI of wheat seeds treated with 100 mM ascorbic acid was lower than that treated with 100 µM ABA. The seeds treated with succinic acid solutions had a higher GI than the seeds treated with ascorbic acid solutions, suggesting that suppression by the ascorbic acid was not effective due to pH, because the pH of ascorbic acid solutions was almost the same as that of succinic acid solutions. Indeed, the GI in ascorbic acid solutions adjusted to pH 6.8 also showed the same tendency (data not shown). The GI of seeds treated with mannitol solutions was almost the same as that of the control, suggesting that the suppressive effect was not due to their osmolality.
The relationship between germination and ROS has been reported in various plant seeds. \( \text{H}_2\text{O}_2 \) enhanced the germination rate in wheat, rice and barley (Neredo et al., 1998). \( \text{H}_2\text{O}_2 \) is produced in the early imbibition period of wheat seeds (Caliskan et al., 1998). Antioxidants in the pericarp and/or seed coat have been reported to function as a germination inhibitor (Qi et al., 1993). Therefore, the suppression of germination affected by ascorbate solutions suggests that \( \text{H}_2\text{O}_2 \) produced in the early imbibition period was scavenged by exogenous ascorbic acid. In contrast, ascorbate is required from the beginning of germination for cell division in pea roots (Citterio et al., 1994) and cell expansion in *Lupinus albus* seedlings (Arrigoni et al., 1997). Wheat seeds at the end of their development are devoid of both ascorbic acid and ascorbate peroxidase (De Gara et al., 2003). It is interesting to note that ascorbic acid suppressed germination, but is required after germination had started.

2. Shortening of \( T_2 \) in wheat seeds treated with ascorbic acid

The falling number and amylograph of grain methods have been used to estimate PHS damage in wheat and rice (Imabayashi et al., 1998). Recently, we reported that \( T_2 \) is a suitable indicator for screening seeds for their resistance to PHS and for examining the degree of endosperm degradation in germinating seeds of rice (Ishibashi et al., 2005). Water contents of germinating seeds treated with distilled water, ABA, mannitol and ascorbic acid were nearly the same (Fig. 2), whereas \( T_2 \) values of long and short fractions of germinating seeds were markedly shortened by both 100µM ABA and 100mM ascorbic acid solutions (Fig. 3). \( T_2 \) values in the wheat seeds treated with ascorbic acid solutions were shorter than that of the control treated with distilled water. This indicates that the degradation of endosperm was significantly suppressed by ascorbic acid. From these results, the NMR relaxation time (\( T_2 \)), especially that of the long fraction, is considered to be effective as a predictive indicator of wheat germination. Therefore, our results suggested that ascorbic acid suppresses the wheat seeds.
Ascorbic acid suppresses wheat germination.

**Conclusion**

Ascorbic acid, which acts as an antioxidant, suppressed seed germination and reduced NMR relaxation time ($T_2$) that reflects the degree of degradation of endosperm. Because ascorbic acid is a harmless substance, it may be useful as a suppresser of pre-harvest sprouting in wheat. On the other hand, NMR relaxation time ($T_2$) may be useful as a predictive indicator of wheat germination.

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