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# Total Sugars, α-amylase Activity, and Germination after Priming of Normal and Aged Rice Seeds

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**ABSTRACT: Osmoconditioning and hardening effects for the seed germination of normal and naturally aged rice seeds were studied through analyzing the total sugars and α-amylase activity. The normal seeds which used to have high germination rate accelerated germination with the osmoconditioning at the suboptimal temperature of 17°C. On the other hand, the aged seeds did not affect germination rate at 25°C, while increased germination rate and accelerated germination a little at 17°C. Hardening of aged seeds increased germination rate by 10~15% compared with control seeds at both 17 and 25°C and accelerated germination. Total sugar content and α-amylase activity of normal seeds were higher than did aged seeds. The aged seeds with treatment of osmoconditioning and hardening increased total sugar content and α-amylase activity, but hardening was more effective than osmoconditioning. The α-amylase activity was positively correlated with the total sugar content and germination rate.**

*Keywords :* osmoconditioning, hardening, total sugars, α-amylase, rice, aged seed, germination rate, $T_{50}$.

Preplant treatments of seeds or seed priming such as osmoconditioning, matriconditioning, hardening, humidification, and growth regulator can be employed to reduce the duration of germination, to synchronize germination, and to improve germination rate in the laboratory and field emergence rate of low quality seeds (Kahn, 1992).

Osmoconditioning, hardening, and humidification of normal rice seeds of which germination rate was very high did not increase germination rate, but they accelerated the seed germination, especially under the unfavorable soil conditions and suboptimal temperatures (Lee, 1998; Lee *et al.*, 1998b; Lee *et al.*, 1998c). Optimum osmoconditioning of rice seeds helped to develope the embryo before germination with increasing α-amylase activity, and the content of fructose and glucose, and with reducing sucrose content (Lee and Kim, 1999). As osmoconditioning of rice seeds was advanced, large compound starch grains in the endosperm were disintegrated into tiny starch granules, and small holes were found in the tiny starch granules and many of cavities were developed between embryo and endosperm. The radicle and plumule of seeds treated with properly osmoconditioning were developed faster than those of the control seeds (Lee and Kim, 1999). However, excess osmoconditioning, even in a optimum PEG solution (-0.6 MPa), caused to reduce seed germination rate with disturbing α-amylase activity (Lee *et al.*, 1998a), and it made abnormal growth of radicle and plumule of seeds (Lee and Kim, 1999).

In aged rice seeds, the treatments of osmoconditioning and humidification reduced germination rate and delayed germination (Lee, 1998) although the osmoconditioning improved significantly germination rate for the artificially and naturally aged soybean seeds (Park *et al.*, 1999). In contrast, the hardening with treatment of 1~4 times of water soaking/drying increased germination rate of artificially aged rice seeds to about 10~15% and reduced germination duration by 1.4~2.0 days (Lee *et al.*, 1998b). In the aged rice seeds, it seems that the hardening, called wetting/drying or hydration/dehydration, is most beneficial to improve germination rate of aged seeds among the preplant seed treatments (Gray & Steckel, 1977).

Although the mechanism of the preplant seed treatments is not fully understood, it has been observed that the physiological and biochemical changes are occurred during the seed treatments and could be allowed for seeds to develop the germination sequences immediately before germination. This would be the basis for germination improvement or better sprout emergence potentials of the primed seeds due to pre-enlargement of embryo (Lee and Kim, 1999), enzyme activation in seeds of rice (Lee *et al.*, 1998a), DNA and RNA synthesis in tomato and lettuce (Coorbear & Grierson, 1979). However, the effects of osmoconditioning and hardening treatments on the germination of rice seeds were not fully studied on the biochemical basis.

In this study some biochemical changes of both normal and aged seeds on the treated conditions of osmoconditioning and hardening such as total sugar content and α-amylase activity were studied and discussed their roles related to the germination of the seeds.

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## MATERIALS AND METHODS

### Seed materials

Normal and two years old naturally aged seeds of a rice variety, Ilpumbyeo, were used in this experiment. Before seed treatment, the germination rate of the normal and aged seeds was 95.3 and 72.0%, respectively.

### Osmoconditioning and hardening

For the osmoconditioning, both normal and aged seeds were soaked into the solution of -0.6 MPa polyethylene glycol (PEG) 8000 at 15°C for four days with air-bubbling (Lee *et al.*, 1998a). For the hardening, 150 g of aged seeds were soaked into 500 ml water for 18 and 24 hours at 25°C and dried at room temperature for two days. This soaking/drying cycles of seeds were repeated three times (Lee *et al.*, 1998b).

### Seed germination

Germination was observed daily at 20°C according to the AOSA method (AOSA, 1990). The time to get 50% germination rate (T50) were calculated according to the Coolbear *et al.* (1984).

$$T_{50} = t_i + \frac{(N+1)/2 + n_i}{(n_j - n_j)}(t_j - t_j)$$

where $N$ is the final number of germination and $ni$, $nj$ cumulative numbers of seeds germinated by adjacent counts at times $t_i$ and $t_j$ when $n_i < (N+1)/2 < n_j$.

### Total sugars

To analyze the total sugars in the osmoconditioning and hardening treated seeds, brown rice were ground in a cyclone sample mill (UDY Corp., U.S.A.) to pass a 100-mesh screen. One gram of ground sample was mixed with 10 ml distilled water and left for 24 hours at 25°C. The mixture was filtered through a Whatman filter paper 42 and then the distilled water was added to get the final volume of 10 ml. Total sugars were determined using a phenol-sulfuric method (Dubois *et al.*, 1956).

### α-amylase activity measured by an iodine method

For the determination of α-amylase activity, 0.25 g of brown rice were soaked into distilled water for three days and enzyme activity was measured by an iodine method (Reiss and Bernstein, 1994).

### α-amylase activity measured by an electrophoresis

The brown rice were germinated in a dark growth chamber for three days at 25°C. The 0.5 g of germinating seeds was ground with 1.5 ml of 50 mM Tris-HCl buffer solution (pH 7.0) and centrifuged at 10,000 rpm for five minutes. An aliquot of the supernatant was used for electrophoresis. Electrophoresis was carried out at 30 mA for 90 minutes at 4°C. After electrophoresis, the gel was incubated at 30°C for 30 minutes in 1% starch solution and buffered to pH 5.5. The starch solution was removed and the gel was soaked in KI-$I_2$ solution. The α-amylase bands were decolorized on the blue-brown background (RDA, 1993).

## RESULTS AND DISCUSSION

### Seed germination

The effects of osmoconditioning and hardening on seed germination rate and duration of seed germination were observed at 17 and 25°C with both normal and two year-old seeds (Fig. 1). The final germination rate of normal rice seeds with two treatments of osmoconditioning and control was similar (over 95%) at both 17 and 25°C, although osmoconditioning accelerated seed germination. However, the effects of osmoconditioning and hardening treatments on the germination of aged seeds were quite different from those of normal seeds as shown in Fig. 2. The germination rate of aged seeds with osmoconditioning condition was similar to that of non-treated control seeds at 25°C, while it with osmoconditioning was slightly higher than that of control at 17°C. In contrast with the seed germination in osmoconditioning, hardening treatment of aged seeds increased germination rate by 10~15% at the both temperatures.

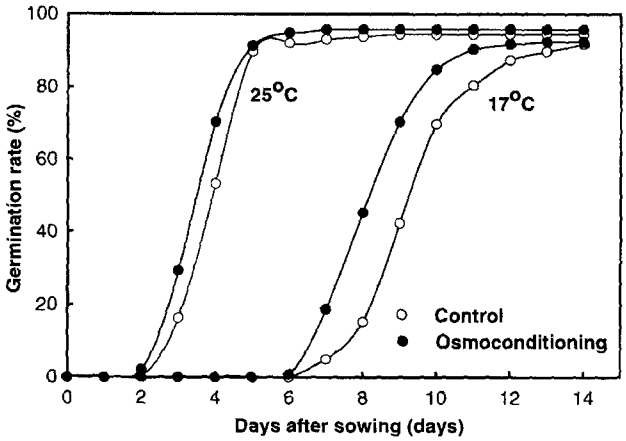


**Fig. 1.** Germination rate of normal rice seeds at 17 and 25°C (Osmoconditioning : Soaked into -0.6 MPa PEG solution for 4 days at 15°C).

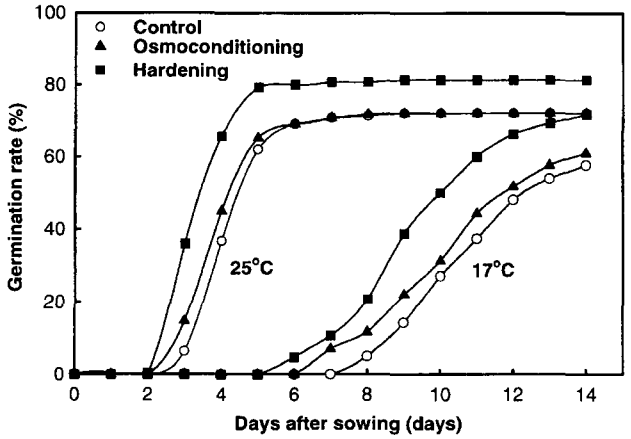


**Fig. 2.** Germination rate of naturally aged rice seeds at 17 and 25°C (Osmoconditioning : Soaked into -0.6 MPa PEG solution for 4 days at 15°C. Hardening : Three times of soaking in water for 24 hours and drying).

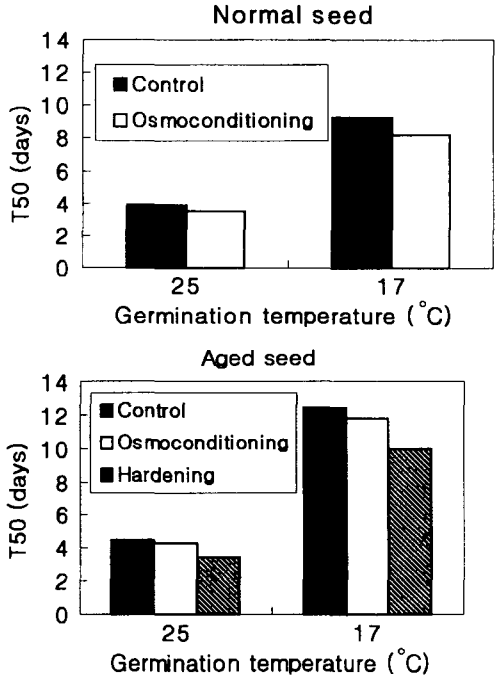


**Fig. 3.** Days to get 50% germination rate ($T_{50}$) of normal and aged rice seeds with treatment of osmoconditioning and hardening at 17 and 25°C (Osmoconditioning : Soaked into -0.6 MPa PEG solution for 4 days at 15°C. Hardening : Three times of soaking into water for 24 hours and drying).

The time to get 50% germination rate ($T_{50}$) of normal and aged seeds is shown in Fig. 3. Generally normal seeds germinated faster than that of the aged seeds (lower $T_{50}$ value), especially at 17°C. In both normal and aged seeds, osmoconditioning did not affect $T_{50}$ at 25°C, while it reduced $T_{50}$ by 0.6-1.1 days at 17°C. However, hardening of the aged seeds reduced $T_{50}$ by 1.0 and 2.4 days at 25 and 17°C, respectively. These results are similar to the other research results (Lee, 1998; Lee *et al.*, 1998b; Lee *et al.*, 1998c).

**Table 1.** Total sugar content and α-amylase activity of differently primed rice seeds.

| Seed | Priming | Total sugars (mg/g seeds) | α-amylase activity (Unit) |
|---|---|---|---|
| Normal | Control | 20.7 b$^{+++}$ | 9.5 ab |
| | Osmoconditioning$^{+}$ | 23.1 a | 10.4 a |
| Aged | Control | 16.9 d | 5.6 d |
| | Osmoconditioning$^{+}$ | 18.3 cd | 7.2 cd |
| | Hardening$^{++}$ | 18.9 c | 8.9 abc |

$^{+}$ Osmonconditioning; Primed into -0.6 MPa PEG 8000 solution for 4 days at 15°C.
$^{++}$ Hardening; Three times of the water soaking for 24 hours and drying.
$^{+++}$ Means within a column followed by the same letter are not significantly different by the Duncan's New Multiple Range Test at the 5% level.

## Total sugars and α-amylase activity

Total sugar content and α-amylase activity of the germinating seeds treated the osmoconditioning and hardening are shown in Table 1. Total sugar content of normal seeds was much higher than that of aged seeds. In normal seeds, the total sugar content of germinating seeds with the osmoconditioning was significantly higher than that of the control and α-amylase activity of it was slightly higher compared with the control seeds. Also, in the aged seeds, total sugar content of germinating seeds with osmoconditioning and hardening was higher compared with the control seeds. However, α-amylase activity was greater in the order of hardening treatment, osmoconditioning treatment, and the control seeds. The higher α-amylase activity and total sugar content of seeds with properly osmoconditioning have been reported with previous researches (Lee and Kim, 1999).

## Relationships between α-amylase activity and total sugars or germination rate

Relationships between α-amylase activity and total sugars or germination rate are shown in Fig. 4. There were highly significant positive correlations between α-amylase activity and total sugars or germination rate at both 17 and 25°C.

## α-amylase activity measured by electrophoresis

The α-amylase band of gel electrophoresis of germinating seed with the osmoconditioning and hardening is shown in Fig. 5. The α-amylase bands of normal seeds (E & F) were stronger compared with the aged seeds (A, B, C, & D). Among the normal seeds, the α-amylase band with osmo-

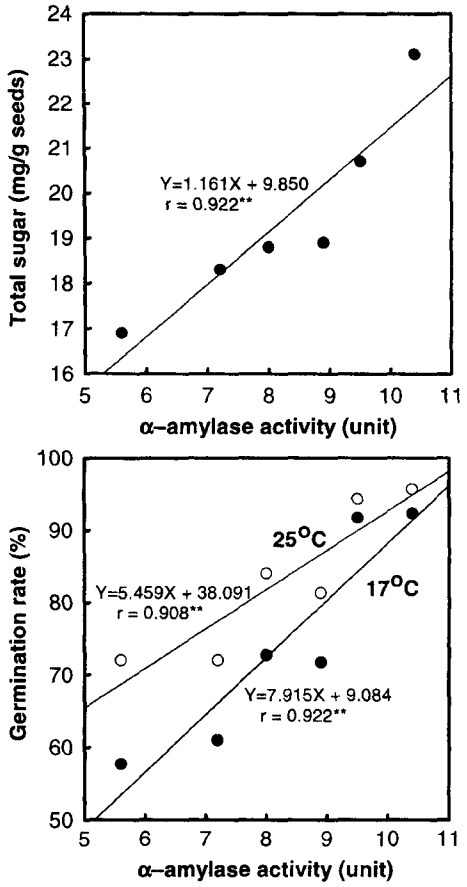


**Fig. 4.** Relationships between α-amylase activity and total sugar content or germination rate of rice seeds at 17 and 25°C.


**Fig. 5.** The α-amylase bands on gel electrophoresis of germinating rice seeds with osmoconditioning and hardening. A : Control (aged seed), B : Osmoconditioning for 4 days at 15 (aged seed), C : Hardening with 3 times of 18 hr water soaking/drying (aged seed), D : Hardening with 3 times of 24hr water soaking/drying (aged seed), E : Control (normal seed), F : Osmoconditioning for 4 days at 15 (normal seed).

conditioning (F) was wider than the control seeds (E). Among the aged seeds, the α-amylase band was greater in the order of hardening with three times of water soaking for 24 hours and drying (D), hardening with three times of water soaking for 18 hours and drying (C), osmoconditioning (B), and the control seeds (A). These results were coincidence with the α-amylase activity measured by an iodine method shown in Table 1.

In conclusion, the hardening of naturally aged rice seeds was more effective to recover seed viability partly due to the higher α-amylase activity and higher sugar mobilization than that with osmoconditioning. Although osmoconditioning treatment to the aged seeds increased α-amylase activity and total sugar content, they may not high enough to recover the lowered seed viability.

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