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Morphological Change, Sugar Content, and α-amylase Activity of Rice Seeds under Various Priming Conditions

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ABSTRACT

An experiment was carried out to find out the changes in morphology, sugars, and α-amylase activity during the priming of rice seeds (*Oryza sativa* L. cv. 'Ilpumbyeo'). For priming, seeds were soaked in -0.6 MPa PEG solution at 15℃ for 4 days (properly primed) and at 25℃ for 4 and 10 days (over-primed) and dried at room temperature. The size of coleoptile and differentiated leaves of properly primed seeds were bigger and coleoptile was separated from the other part of embryo compared with non-primed and over-primed seeds. As priming of seeds advanced, compound starch grains in the endosperm disintegrated into tiny starch granules, and small holes were found in the tiny starch granules and a cavities developed between embryo and endosperm. The radicle and plumule of properly primed germinating seeds developed faster than non-primed and overprimed germinating seeds. Sucrose, maltose, and raffinose contents of properly primed seeds decreased, while content of glucose and fructose and α-amylase activity increased. However, sugar content and α-amylase activity of over-primed seeds were lower compared with non-primed seeds or properly primed seeds.

Keywords : priming, rice seed, SEM, embryo, starch granule, α-amylase, sugar.

In recent years a number of papers have been published on the priming of seeds to improve the overall germination rate and uniformity of growth and to reduce the germination time of many vegetable and some field crop seeds (Nath et al., 1991; Khan et al., 1995; Lee et al., 1998).

In rice, the priming of normal seeds enhanced germination speed without improving the germination rate under optimum germination conditions because the germination rate of the seeds was very high. However, the priming of normal seeds improved the germination rate significantly under unfavorable conditions such as suboptimal temperature and too dry or wet soil (Lee et al., 1998b).

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The α-amylase activity of rice seeds primed under optimum priming conditions (in -0.6 MPa PEG solution at 15℃ for 4 days) was higher than that of non-primed seeds, while excessive priming reduced the germination rate and α-amylase activity of the seeds was extremely high (Lee et al., 1998). However, no information was available on the morphological and chemical changes during the priming of rice seeds.

In germinating rice seeds the major reserved food, starch, is broken down by amylases induced or activated in the scutellum. In rice seeds, 100~200 starch granules are accumulated in the form of compound starch grains in the endosperm. At the first stage of germination, these starch grains are disintegrated into tiny starch granules and small holes form in the tiny starch granules as the breakdown of starch advances (Kiribuchi & Nakamura, 1974; Matsuo & Hoshikawa, 1993). The digestion of starch in the endosperm starts from near the embryo because amylases are secreted from the scutellum. However, these events of germination were not visualized during the priming of rice seeds.

Therefore, this experiment intends to show the morphological and physiological changes during the priming and germination of rice seeds.

MATERIALS AND METHODS

Normal seeds of a rice variety, Ilpumbyeo, harvested at the Kyongbuk Provincial Agricultural Technology Administration Farm in Taegu, Korea in 1996 were used in this experiment in 1998.

For priming, seeds were soaked in -0.6 MPa polyethylene glycol (PEG) solution at 15 and 25℃ for 4 days and at 25℃ for 10 days. The primed seeds were washed in running tap water and dried at room temperature for 48 hours.

Scanning electron microscopy

The structural changes in the embryo of primed dry seeds and primed germinating seeds were examined under a scanning electron microscope (SEM). The primed germinating seeds were produced by allowing the primed seeds to germinate in an incubator at 20℃ for 3 days up to immediately before emergence of radicle. The seeds were soaked in liquid nitrogen for 2 minutes (Pallwall et al., 1991). The embryo of the primed dry seeds and frozen primed germinating

seeds was cut longitudinally in half with a razor blade. The frozen primed germinating seeds were dehydrated with a series of 30, 50, 70, 80, 90, 95, 100% ethanol and dried to critical-point in a critical-point drying apparatus (HCP-2, Hitachi, Japan). Both dry and dehydrated primed germinating seeds were coated with gold-palladium for 20 minutes in a coating machine (Eiko IB-5, Hitachi, Japan) and were observed for embryo development in a SEM (S-4100, Hitachi, Japan).

Analysis of sugars and α-amylase activity

To analyze the changes in sugars during priming, seeds were ground in a Willey mill to pass through 40 mesh screen. One gram of ground sample was mixed with 10 ml of distilled water, left for 24 hours, and filtered through a Whatman #42 filter paper. Cation, anion, and phenolics in the solution were removed using polyvinylpolypyrrolidone (PVPP), amberite IRA-94, and Dowex-50W, respectively with flow rate of 2 mm/min by a peristaltic pump (Microtube pump, MP-3, EYELA, Japan) (McBee & Maness, 1983). The filtered solution was freeze-dried in a freeze drier (Bondiro, Korea).

The samples were dissolved in 1 ml purified water and filtered through a 45 ㎛ pore size nylon filter.

Sugars were analyzed in a HPLC (Young-Lin, M930). The carrying solvent was 80% acetonitrile (CH_3CN) for fructose and glucose and 70% acetonitrile for sucrose, raffinose, and maltose under flow rate of 1.5 ml/min. The HPLC system consisted of an auto injector with a 20 ㎕ loop (Waters 712 WISP), a carbohydrate analysis column (Waters 84038), and a RI detector (Waters 410).

For the determination of α-amylase activity, 0.25 g of seeds were soaked in water for 3 days and the activity was measured by an iodine method (Reiss & Bernstein, 1994).

Germination rate and speed

Germination was observed daily at 20℃ according to the AOSA method (AOSA, 1990). The time to get 50% germination rate (T_{50}) was calculated according to the Coolbear et al. (1984).

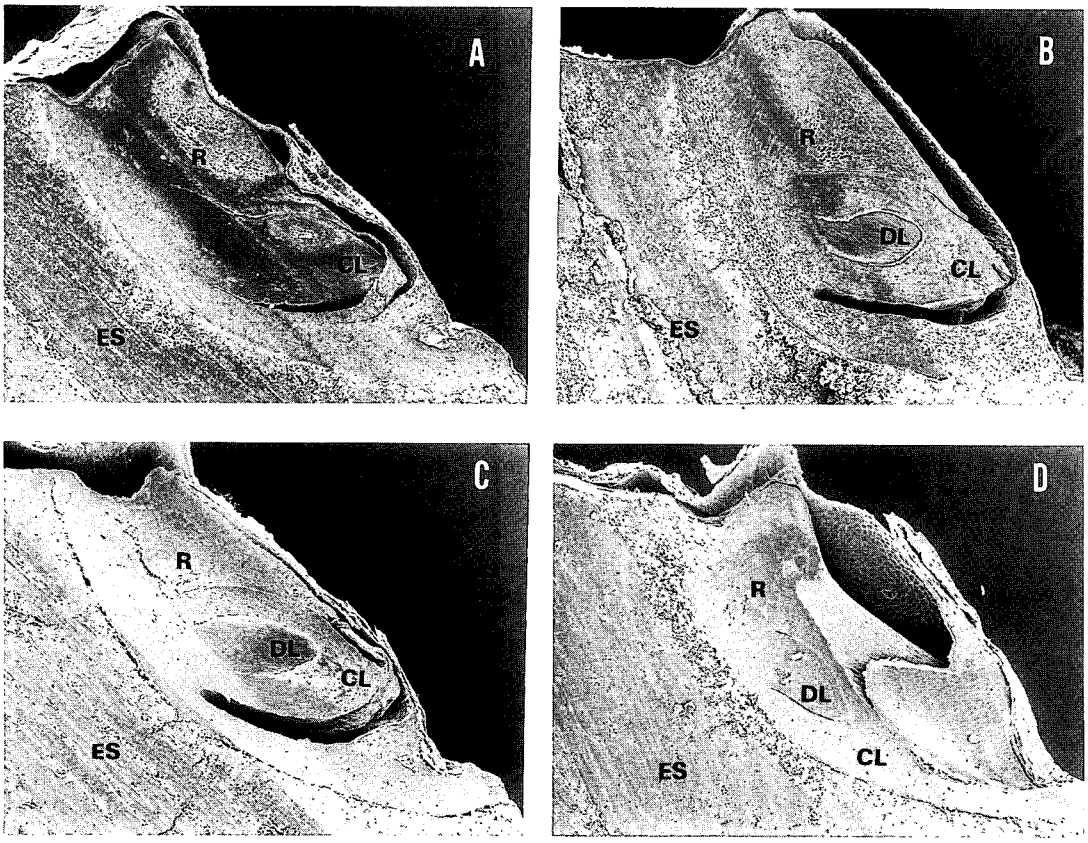

Photo. 1. Embryos of non-primed and primed rice seeds in -0.6 MPa PEG solution at 15℃ for 4 days and at 25℃ for 4 and 10 days (×60 in a SEM). ES; endosperm, CL; coleoptile, R; radicle, DL; differentiated leaves. A: Non-primed seed, B: 15℃ 4days, C: 25℃ 4days, D: 25℃ 10days.

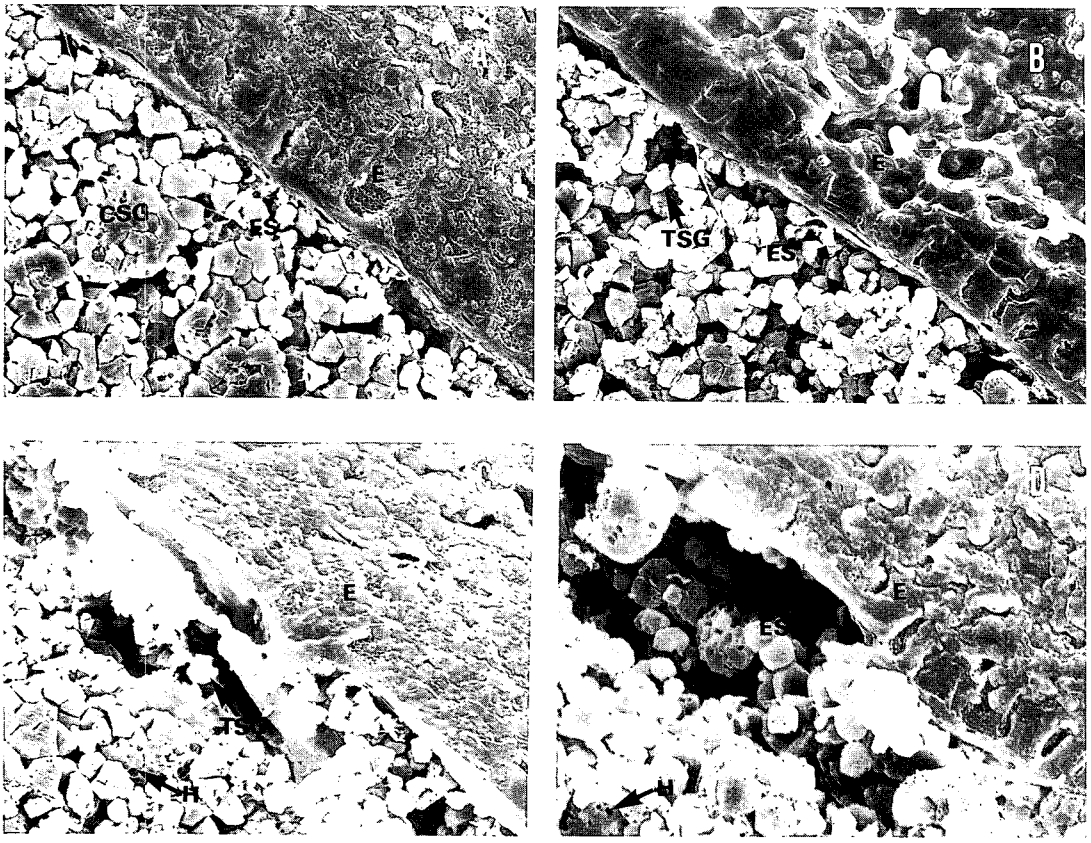

Photo. 2. Starch granules in embryo and endosperm of rice seeds primed in -0.6 MPa PEG solution at 15℃ for 4 days and at 25℃ for 4 and 10 days (× 1500 in a SEM). E; embryo, ES; endosperm, CSG; compound starch grain, TSG; tiny strach granule, H; hole in TSG. A: Non-primed seed, B: 15℃ 4days, C: 25℃ 4days, D: 25℃ 10days.

RESULTS AND DISCUSSION

Structural changes in primed seeds

Structural changes in embryos and starch granules in the endosperm during the priming at 15 and 25℃ are presented in Photo. 1. In non-primed seeds, the coleoptile and differentiated leaves were not obvious and the coleoptile was separated a little from the other parts of the embryo (Photo. 1-A), while those of primed seeds in -0.6 MPa PEG solution at 15 and 25℃ for 4 days were obvious and increased in size and the coleoptile was separated from the other parts of the embryo (Photo. 1-B, 1-C). However, the coleoptile and differentiated leaves of primed seeds in -0.6 MPa PEG solution at 25℃ for 10 days were not obvious like non-primed seeds, but the coleoptile was separated from the other parts of the embryo (Photo. 1-D). During the priming, the radicle was not obvious in all priming treatments. Under the aerobic conditions the radicle emerges earlier than the plumule.

However, seeds primed in PEG solution developed the plumule faster than the radicle in this experiment. Although air was bubbled during the priming, oxygen might not be sufficient for normal germination of rice seeds.

The anatomical differences around the scutellum of seeds primed at different temperatures and durations are shown in Photo. 2. In non-primed seed, starch was accumulated as compound starch grains in the endosperm and no cavity was observed between the embryo and endosperm (Photo. 2-A). However, in seeds primed at 15 and 25℃ for 4 days and at 25℃ for 10 days (Photo. 2-B, C, and D) the compound starch grains disintegrated into tiny starch granules, small holes developed in the segregated starch granules, and the cavities developed between the embryo and endosperm probably due to the consumption of starch during the development of the embryo as priming advanced (Photo. 2-C and D).

Structure of primed germinating seeds

The morphological changes in the primed seeds germinated at 20℃ for 3 days are presented in Photo.

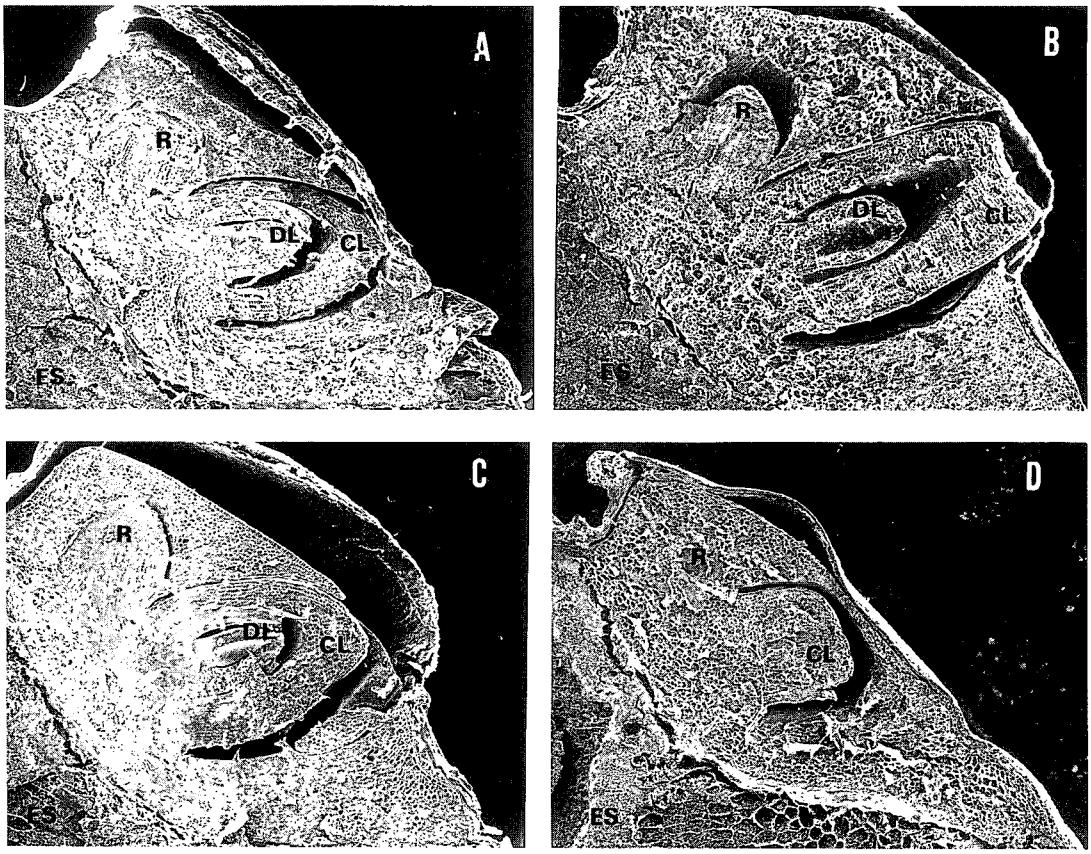

Photo. 3. Embryos of rice seeds germinated at 20℃ for 3 days. Prior to germination the seeds were primed in -0.6 MPa PEG solution at 15℃ 4 days and at 25℃ for 4 and 10 days (× 60 in a SEM). ES; endosperm, CL; coleoptile, R; radicle, DL; differentiated leaves. A: Non-primed seed, B: 15℃ 4days, C: 25℃ 4days, D: 25℃ 10days.

3. The radicle and plumule of seeds primed at 15℃ for 4 days (Photo. 3-B) developed faster than those of non-primed seeds (Photo. 3-A) and this would cause earlier germination of primed seeds.

However, the development of the radicle and plumule of seeds primed at 25℃ for 4 and 10 days (Photo. 3-C and D) was less than that of seeds primed at 15℃ for 4 days. Especially the development of the radicle and plumule of seeds primed at 25℃ for 10 days were not normal (Photo. 3-D).

Generally, the radicle emerges earlier than the plumule under aerobic conditions. However, in this experiment all seeds developed the plumule faster than the radicle probably due to germinating in a water saturated paper towel which could limit the supply of oxygen for germination.

Changes in sugars and α-amylase activity during priming and seed germination

Sugar content, α-amylase activity, and germination rate of non-primed and primed seeds at 15 and 25℃ for 4 days are shown in Table 1. In non-primed seeds, the content of glucose, maltose, sucrose, raffinose, and fructose was 4.0, 1.9, 1.5, 0.6 and 0.4 mg/g seed, respectively. After priming at 15℃ for 4 days, sucrose decreased significantly, maltose and raffinose decreased slightly, while glucose and fructose increased. However, in seeds primed at 25℃ for 4 days, content of all the sugars was lower than that of non-primed seeds or primed seeds at 15℃ for 4 days.

The α-amylase activity of non-primed seeds was 2.1 unit, that of primed seeds at 15℃ for 4 days was 2.8 unit, and primed seeds for 4 days at 25℃ was 2.0 unit. The higher α-amylase activity of primed seeds at 15℃ compared with non-primed seeds may be related to the high sugar content in seeds and low T_{50} (fast germination) in Table 1. However, the low sugar content and low α-amylase activity in seeds primed at 25℃ for 4 days compared with seeds primed at 15℃ for 4 days may be related to some detrimental effects of seed priming at 25℃. Also, in the seeds primed at 25℃ for 4 days the development of the radicle and plumule was slower than that of seeds primed at 15℃ in Photo. 3.

Similar results are reported by other research wor-

Table 1. Sugar content, α-amylase activity, germination rate, and time to 50% germination (T_{50}) of rice seeds primed at 15 and 25℃ for 4 days.

Priming	Sugar (mg/g seed)						α-amylase activity(Unit)	Germination rate(%)	T_{50} (days)
	Fructose	Glucose	Sucrose	Maltose	Raffinose	Total			
No priming	0.4 b†	4.0 ab	1.5 a	1.9 a	0.6 a	8.5 b	2.1 b	94 ns	6.4 c
15℃ 4 days	1.1 a	6.2 a	0.6 b	1.6 ab	0.5 b	10.0 a	2.8 a	97	5.7 a
25℃ 4 days	0 c	2.1 b	0.5 b	0.8 b	0 c	3.4 c	2.0 b	95	6.1 b

† Means within a column followed by the same letter are not different by the Duncan's New Multiple Range Test at the 5% level.

kers. As the germination of barley progressed, the total amount of glucose in both embryo and endosperm increased, while sucrose decreased (Abdul-Baki & Anderson, 1970).

Lee at al. (1998) reported that the priming effects (germination rate, T_{50}, and uniformity) of seeds primed at 15 and 25℃ for 4 days were similar. However, this result indicated that priming of rice seeds at 25℃ for more than 4 days may deteriorate the seeds during priming such as changes in morphology of embryo, decrease in α-amylase activity, and loss of sugars, etc. Thus, this experiment confirms that the optimum priming conditions of rice is 15℃ for 4 days.

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