Development of Rice “Seed-Mats” Consisting of Hardened Seeds with a Cover of Soil for the Rice Transplanter

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Abstract: Conventional seedling mats for rice transplanters in Japan are heavy to carry, and much labor is required to collect, wash and store the nursery boxes. In addition, seeding time overlaps with the labor peak in spring. To reduce such labor, we developed a “seed-mat” consisting of hardened rice seeds (Oryza sativa L.) glued onto a molded rice-hull mat with a cover of soil glued on. Seed hardening, which is done by soaking seeds in water at 15°C for 5 d followed by drying, and heating of the seeds at 50°C for 5 or 7 d, reduced the time to 50% germination (T50). In dormant seeds, the heating before the hardening enhanced the effect of the hardening more than the heating after the hardening. Seed hardening increased the dry weight of the shoots from the seed-mats. Covering the mat with soil also increased the percentage of seedling emergence, shoot length, number of leaves and shoot dry weight of the seed-mats. The hardened seeds maintained short T50 and 95% or higher germination for 120 d at room temperature. In the seed-mat, the hardened seeds maintained 95% or higher seedling emergence for 208 d. In conclusion, superior seed-mats could be produced using the procedure involving both seed hardening and cover of soil. The seed-mats can be prepared in winter and stored until seedling-raising period in spring. By the seed-mats, seedling mats can be prepared for the rice transplanter without using nursery boxes, which take up storage space and require much labor.

Key words: Cover of soil, Dormancy, Germination, Hardening, Rice, Seedling, Seed-mat, Storage life.

In Japan, rice (Oryza sativa L.) is usually grown by mechanical transplanting. Conventional seedlings for rice transplanters are grown in nursery boxes filled with soil to make seedling mats (280 mm × 580 mm) with the interwoven roots before transplanting. The problems of the seedling mats are (1) laborious task of seedling transport due to the heavy weight (6 kg per box) and large number (200 boxes per ha) of seedling mats (Tasaka, 1999), (2) overlapping of the time of seed pretreatment and sowing with the labor peak period in spring, and (3) the laborious task of collecting, washing and storing of the boxes after use.

The objective of this study was to develop a “seed-mat” consisting of dry seeds glued onto a molded rice-hull mat (Fig. 1A, B). The seedling mat made from a molded rice-hull mat is lighter than the conventional seedling mat. If the seed-mats can be produced during winter and used in spring, farmers are relieved from seeding work at the labor peak in spring. The seed-mats allow the farmer to raise rice seedlings without nursery boxes because they can keep their own shape without nursery boxes (Fig. 1C). The seedlings grown from the seed-mats are then transplanted with an ordinary rice transplanter (Fig. 2). The seed-mats were developed to replace conventional 20-d-old seedlings because nursery duration is short and less seedling mats are required than older seedlings. Mats of rice seeds have been developed (Nakatani, 1981; Kuwahara et al., 2002). However, they are for direct seeding with a rice transplanter. In addition, the mats cannot be stored at room temperature, because the seeds are pregerminated. There are no conventional rice seed-mats to produce seedlings suitable for a rice transplanter.

For the seed-mats, vigorous seedling growth is desirable and storability under room temperature is required. Various prehydration treatments of rice seeds that improve seed performance have been reported, such as priming, i.e., soaking seeds in an osmotic solution and then drying the seeds (Singh and Chatterjee, 1980; Ochiai et al., 1995; Lee et al., 1998a,b,c; Lee and Kim, 2000), hardening, i.e., soaking seeds in water and then drying the seeds once or repeatedly (Singh and Chatterjee, 1980; Lee et al., 1998a,b; Harris et al., 1999; Andoh and Kobata, 2000, 2002; Lee and Kim, 2000; Yamauchi, 2002), and humidification, i.e., hydration in humid air followed by dehydration (Lee et al., 1998b). We adopted a single cycle of hardening because a
few hundred kilograms of seeds can be treated with a prevalent grain drier. Hardened rice seeds are storable under natural conditions (Yamauchi, 2002) and require less time to 50% germination ($T_{50}$) than untreated seeds (Lee et al., 1998a,b; Harris et al., 1999; Basra et al., 2005). They also show a high percentage of seedling emergence (Andoh and Kobata, 2000, 2002; Basra et al., 2004) and develop shoots with heavier dry weight (Yamauchi, 2002; Basra et al., 2004). We investigated the effects of seed hardening on the germination and seedling growth from the seed-mats. Before hardening, dormancy release by heating is recommended for dormant seeds (Yamauchi, 2002). However, the degree of dormancy which requires heating for effective hardening and interaction between the effects of heating and hardening have not been elucidated. Therefore, the effect of heating and hardening on the seeds under different degrees of dormancy was examined in this study.

The effect of covering soil on seedling growth was also examined. Seedlings can grow on the seed-mats without being covered with soil because seeds are fixed on the mats which allows roots to enter the mats without harmful movement of the seeds. If covering soil proves unnecessary, the seed-mats could be produced at a lower cost and be lighter.

The storage life of the seed-mats with the hardened seeds was also examined under room temperature conditions.

**Materials and Methods**

1. **Seed materials**

Japanese *japonica* lowland rice variety “Koshihikari” was used in all experiments. Dormancy of the seeds of Koshihikari is ranked high among Japanese rice varieties. Seeds were harvested in Ibaraki Prefecture, Japan in autumn of 2002 (Experiment 4, 5), 2003 (Experiment 1–3) and 2004 (Experiment 6, 7). The seed lots were different in Experiment 1–3.

2. **Germination test (Experiment 1, 2 and 3)**

A germination test was conducted three times to examine the effect of seed hardening and heating on the germination of seeds under various levels of dormancy, which were determined by the results of these tests. The seed lots were different from each other in these experiments. The seeds were hardened by soaking in water at 15ºC for 5 d and drying at about 35ºC for 2 d in Experiment 1 and 2 and at about 45ºC for a day in Experiment 3 (hardening treatment) (Table 1). Heating at 50ºC for 7 d in Experiment 1 and 2 and for 5 d in Experiment 3 was given alone (heating treatment) or before the hardening treatment (heating-hardening treatment). The hardening followed by the heating (hardening-heating) was also conducted in Experiment 1. Untreated seeds were used as a control.

A hundred seeds per replication were placed on moist filter paper in a 9 cm diameter Petri dish. The Petri dishes were placed in a dark incubator maintained at 30ºC. Germinated seeds with radicles or plumules longer than 2 mm were counted and removed daily for 9 d. $T_{50}$ on total germinated seeds basis was calculated according to Farooq et al. (2005).
The experimental design was a completely randomized block design with five blocks in Experiment 1 and four blocks in Experiment 2 and 3.

### 3. Effect of hardening on seedling growth (Experiment 4)

Seedlings were raised in a plastic greenhouse at the National Agricultural Research Center in Tsukuba, Japan (36°1’N, 140°6’E) to examine the effect of hardening on the seedling growth of the seed-mats. The seed-mats were made by gluing onto the molded rice-hull mats (450 g) containing 1 g N, 1 g P$_2$O$_5$, and 1 g K$_2$O with polyvinyl alcohol, 150 g of the hardened seeds which were soaked in water at 15ºC for 5 d and dried at 35ºC for 2 d or 150 g untreated seeds (Table 1) with a cover of 380 g soil containing 0.18 g N, 0.36 g P$_2$O$_5$, and 0.32 g K$_2$O glued onto the seeds. On March 28, 2004, the seed-mats without nursery boxes were placed on polyethylene film, watered and covered with silver polyethylene film containing aluminum with nonwoven cloth (Silver-love #90, Tokankosan, Tokyo, Japan) to promote seedling emergence. The film was removed when shoot length reached about 4 cm. A hundred individuals per seed-mat were sampled on April 5 and emerged seedlings were counted. On April 18, 1 g N, 1 g P$_2$O$_5$, and 1 g K$_2$O per seed-mat was top-dressed. Twenty seedlings per seed-mat were sampled and shoot length and number of leaves were measured on April 24. The coleoptile was not included in the number of leaves. The roots and seeds were removed from the seedlings; then the seedlings were dried at 80ºC for 3 d and weighed. The experimental design was a completely randomized block design with four blocks.

### 4. Effect of cover of soil (Experiment 5)

Seedlings were raised in a plastic greenhouse as in Experiment 4 to examine the effect of covering the seed-mats with soil on seedling growth. The seed-mats with the hardened seeds as in Experiment 4 (Table 1) and the seed-mats not covered with soil were used. Those seed-mats were placed on polyethylene film without nursery boxes, watered to hydrate the seeds at low temperature without being covered with film on March 27, 2004 and then covered with the same film as in Experiment 4 on April 1. The film was removed when the shoot length reached about 4 cm. From April 8, the seed-mats were placed in a water pool or watered from the top.

Emerged seedlings were counted on April 13 and seedling characteristics were examined on April 27 in the same way as Experiment 4. The experimental design was a split-plot with four blocks, in which water management was the main plot and cover of soil was the subplot.

### 5. Storage life test (Experiment 6 and 7)

In Experiment 6, the germination percentage and

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| Exp. | Seed treatments | Heating before hardening | Hardening | Heating after hardening |
|------|-----------------|---------------------------|-----------|-------------------------|
|      |                 | Heating 50ºC, 7 d         | Soaking   | Drying 35ºC, 2 d        |                        |
| 1    | Heating         | 50ºC, 5 d                 | 15ºC, 5 d | 35ºC, 2 d               | −                      |
|      | Heating-hardening | 50ºC, 7 d                | −         | 35ºC, 2 d               | −                      |
|      | Hardening       | −                         | 15ºC, 5 d | 35ºC, 2 d               | −                      |
|      | Hardening-hardening | −                     | 15ºC, 5 d | 35ºC, 2 d               | 50ºC, 7 d             |
|      | Control         | −                         | −         | −                       | −                      |
| 2    | Heating         | 50ºC, 5 d                 | −         | −                       | −                      |
|      | Heating-hardening | 50ºC, 7 d                | 15ºC, 5 d | 35ºC, 2 d               | −                      |
|      | Hardening       | −                         | 15ºC, 5 d | 35ºC, 2 d               | −                      |
|      | Control         | −                         | −         | −                       | −                      |
| 3    | Heating         | 50ºC, 5 d                 | −         | −                       | −                      |
|      | Heating-hardening | 50ºC, 7 d                | 15ºC, 5 d | 45ºC, 1 d               | −                      |
|      | Hardening       | −                         | 15ºC, 5 d | 45ºC, 1 d               | −                      |
|      | Control         | −                         | −         | −                       | −                      |
| 4    | Hardening       | −                         | 15ºC, 5 d | 35ºC, 2 d               | −                      |
|      | Control         | −                         | −         | −                       | −                      |
| 5    | Hardening       | −                         | 15ºC, 5 d | 35ºC, 2 d               | −                      |
| 6, 7 | Hardening       | −                         | 15ºC, 5 d | 40ºC, 2 d               | −                      |
|      | Control         | −                         | −         | −                       | −                      |

− shows untreated.
T50 of the hardened and untreated seeds stored for various periods were examined to elucidate storage life. The seeds were hardened by soaking in water at 15°C for 5 d from March 3, 2005 and drying them at 40°C for 2 d (Table 1). The hardened and untreated seeds were stored at about 7°C until March 23 and then placed in mesh bags in a cardboard box in a non-air-conditioned room and in plastic bottles in a refrigerator until February 16, 2006 (for 300 d). The daily mean temperature in the room was 22.9 ± 5.9°C (standard deviation) during the storage, daily mean relative humidity was 58 ± 5%, and the daily mean temperature in the refrigerator was 7.2 ± 0.5°C (Fig. 3). The germination percentage and T50 were examined under a 24-h lit condition for 10 d at about 30-day intervals until 300 d after the start of the storage.

In Experiment 7, the seed-mats were prepared using hardened seeds and cover of 500 g of soil on March 14, 2005, and stored in the room or refrigerator, as in Experiment 6. The soil contained 0.24 g N, 0.48 g P2O5, and 0.42 g K2O and the molded rice-hull mats contained 1.2 g N, 1.2 g P2O5, and 1.2 g K2O. Four pieces of seed-mat (about 6 cm × 7 cm) were sampled at about 30-d intervals. Each piece of seed-mat was placed in a plastic box (12.5 cm × 7 cm × 5 cm) and watered. The seedlings were grown for 7 d under the same conditions as in Experiment 6. One hundred seeds were sampled and emerged seedlings were counted. The experimental design was a completely randomized block design with four blocks.

6. Statistical analysis
Tukey’s test (p<0.05) was conducted on transformed percentage of germination by square-root transformation in Experiment 1, 2, and 3 between the treatments using the GLM procedure in SAS (ver. 9.1, SAS Institute Inc, Cary, NC). Analysis of variance (ANOVA) was conducted on transformed percentage of seedling emergence by square-root transformation, shoot length, number of leaves and shoot dry weight in Experiment 4 using the GLM procedure in SAS. In Experiment 5, ANOVA was conducted on transformed percentage of seedling emergence by square-root transformation, shoot length, number of leaves and shoot dry weight with a split-plot design.

Results and Discussion
1. Effect of heating and hardening of seed on germination
Experiments 1, 2 and 3 were conducted to investigate the effects of heating and hardening of seed on germination. The seeds in Experiment 1−3 were classified into three classes of dormancy, i.e., dormant, moderately dormant, and slightly dormant, respectively, on the basis of T50 (Table 2). The seeds with longer T50 were considered to be more deeply dormant. The percentage of germination was increased by all treatments (heating, heating-hardening, hardening and hardening-heating) in dormant and moderately dormant seeds, but was lowered by heating-hardening in slightly dormant seeds (Table 2). On the other hand, T50 was reduced by all treatments in all kinds of seeds irrespective of dormancy. Hardening was more effective than heating in reducing T50 in slightly dormant seeds but the effect of hardening was similar to that of heating in dormant and moderately dormant seeds. However, the effect of hardening on T50 was significantly increased by preceding heating (heating-hardening) in dormant and moderately dormant seeds, but not in slightly dormant seeds. In addition, in dormant seeds, hardening-heating was less effective than heating-hardening. These results suggest that the effects of the hardening and dormancy release by the heating are not independent and that dormancy release is necessary for an effective hardening. Similar results were reported previously. Dormancy release by stratification prior to priming decreased median germination time more than stratification or priming alone in loblolly pine (Hallgren, 1989). Dormancy release was required for the effective priming of true potato seeds (Pallais, 1989). However, in these studies, the dormancy-releasing treatments were not conducted after priming, and consequently whether the effects of the dormancy release and the prehydration treatments like hardening and priming are independent of each other or interactive was not clear.

The result that the dormancy release enhances the effect of the following prehydration treatment suggests that germinative metabolism more active in less dormant seeds than in dormant seeds causes the effect of the prehydration treatments. The effects of priming are considered to be related to germinative metabolism during priming treatments (Khan, 1992; Bradford, 1995; Bray, 1995; Welbaum et al., 1998), embryo growth (Bray, 1995; Welbaum et al., 1998) and repair of damaged membrane (Welbaum et al., 1998).
Similar physiological changes seem to occur in seeds with other prehydration treatments like hardening (Khan, 1992). However, germinative metabolism which occurs only in non-dormant seeds has not been reported yet (Bewley, 1997).

2. Effect of the hardening treatment on the seedling growth

Seedling growth of the hardened seeds was investigated in Experiment 4. The hardened seeds are preferable for seed mats in view of seedling growth. The film cover for promoting seedling emergence on the seed-mat with hardened seeds, was removed 1 d earlier than that on the seed-mat with non-hardened seeds (control, covered for 8 d) (Table 3). This suggests that seedling emergence was hastened by hardening treatment. The percentage of seedling emergence was comparable in the two seed-mats. The dry weight of shoots from the seed-mats with hardened seed was heavier than that of the shoots from the control seed-mats, while there was no difference in the shoot length or the number of leaves between the hardened and control seed-mats.

3. Effect of cover of soil on seedling growth

Effect of cover of soil on seedling growth was investigated in Experiment 5. The film cover for promoting seedling emergence on the seed-mat with the cover of soil could be removed one day earlier than that on the seed-mat without the cover of soil (Table 4), suggesting that the seedling emergence from the seed-mats was promoted by the cover of soil. The cover of soil also increased shoot length, number of leaves and shoot dry weight even on the seed-mats watered in a pool where seed-mats were not dry as well as on the seed-mats watered from the top. This suggests that cover of soil not only maintains moisture but also another effects. The amount of fertilizer nitrogen in the covering soil was only 0.18 g, which is much less than that in the seed-mat (1.00 g), suggesting that the cover of soil could have some functions other than the fertilizing and moisture retention. The weight of a seedling mat obtained from the seed-mat covered with soil was about 3 kg, which is about half of the weight of a conventional seedling mat (unpublished data).

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**Table 2. Effect of seed treatments on percentage of germination and time to 50% germination (T50) in rice.**

| Experiment | Seed dormancy | Treatment          | Percentage of germination (%) | T50 (d) |
|------------|---------------|--------------------|-------------------------------|---------|
| 1          | Dormant       | Heating            | 99 ± 0 a 1                   | 2.7 ± 0.1 b |
|            |               | Heating-hardening  | 96 ± 1 a                      | 1.7 ± 0.0 d |
|            |               | Hardening          | 96 ± 1 a                      | 2.7 ± 0.1 b |
|            |               | Hardening-hardening| 98 ± 1 a                      | 2.3 ± 0.0 c |
|            |               | Control            | 86 ± 2 b                      | 4.3 ± 0.1 a |
| 2          | Moderately    | Heating            | 99 ± 0 a                      | 1.9 ± 0.0 b |
|            |               | Heating-hardening  | 99 ± 1 a                      | 1.0 ± 0.1 c |
|            |               | Hardening          | 99 ± 0 a                      | 1.7 ± 0.0 b |
|            |               | Control            | 96 ± 0 b                      | 3.6 ± 0.2 a |
| 3          | Slightly      | Heating            | 98 ± 0 a                      | 2.2 ± 0.0 b |
|            |               | Heating-hardening  | 95 ± 0 b                      | 1.5 ± 0.0 c |
|            |               | Hardening          | 99 ± 0 a                      | 1.5 ± 0.0 c |
|            |               | Control            | 99 ± 0 a                      | 2.7 ± 0.0 a |

Data are means ± standard errors.  
1) Means followed by the same letter in each experiment are not significantly different at P < 0.05 (Tukey’s test).

**Table 3. Effect of seed hardening on seedling growth from the rice seed-mats (Experiment 4).**

| Treatment   | Covered duration (d) | Seedling emergence (%) | Shoot length (cm) | Number of leaves | Shoot dry weight (mg) |
|-------------|----------------------|------------------------|-------------------|------------------|-----------------------|
| Hardening   | 7                    | 96 ± 2                 | 12.1 ± 2.3        | 4.1 ± 0.0        | 13.6 ± 0.1*           |
| Control     | 8                    | 94 ± 3                 | 12.5 ± 1.0        | 4.0 ± 0.0        | 12.8 ± 0.3            |

Data are means ± standard errors.  
*indicates significant difference between the hardening treatment and the control at P < 0.05.
Storage life of the hardened seeds

Storage life of the hardened seeds was investigated in the experiment 6. The percentage of germination of the hardened seeds under the room temperature condition was above 94.8% up to 186 d but decreased thereafter, while that of the non-hardened seeds (control) remained higher than 95% for at least 300 d (Fig. 4). Under the cool condition, the percentage of germination of the hardened seeds remained higher than 95% for at least 300 d.

$T_{50}$ of the hardened seeds under the room temperature condition remained low until 120 d of storage and then increased slightly (Fig. 5). The $T_{50}$ of the hardened seeds was shorter than that of the control until 120 d, while after 208 d $T_{50}$ of the hardened seeds became longer than that of the non-hardened seed (control). These results suggest that the hardened seeds can maintain the advantage of the hardening treatment for at least 120 d at room temperature. How long the hardened rice seeds can maintain a short $T_{50}$ and a germination percentage above 95% at room temperature has not been reported. Our results are consistent with the previous reports that primed seeds can deteriorate faster than untreated seeds during storage (Khan, 1992; Bray, 1995) and the priming effect can diminish rapidly during the aging process (Bray, 1995). By contrast, Basu and Pal (1980) reported that the percentage of germination of the rice seeds which were hardened 1 mo after harvest showed a greater decrease than that of the control seeds at room temperature, while that of the rice seeds which were hardened 12 mo after harvest showed a smaller decrease than that of untreated 12-mo-old seeds. The hardening effect on storability of rice seeds could be different between indica and japonica varieties and be dependent on seed conditions, since the priming

| Water management | Covered soil (g) | Covered duration (d) | Seedling emergence (%) | Shoot length (cm) | Number of leaves | Shoot dry weight (mg) |
|------------------|------------------|----------------------|------------------------|-------------------|-----------------|----------------------|
| From top         | 380              | 6                    | 100 ± 0                | 10.5 ± 0.1        | 4.1 ± 0.1       | 12.5 ± 0.2           |
|                  | 0                | 7                    | 92 ± 3                | 7.8 ± 0.2         | 3.9 ± 0.0       | 8.9 ± 0.2            |
| In a pool        | 380              | 6                    | 99 ± 1                | 13.0 ± 0.7        | 4.1 ± 0.0       | 14.0 ± 0.5           |
|                  | 0                | 7                    | 97 ± 1                | 11.0 ± 0.4        | 3.9 ± 0.0       | 11.5 ± 0.2           |

ANOVA

|                     | Water management | Covering soil | Water management × Covering soil |
|---------------------|------------------|---------------|---------------------------------|
|                     | **               | *             | ***                             |

Data are means ± standard errors.

*, ** and *** indicate significance at $P < 0.05$, 0.01 and 0.001, respectively.

Fig. 4. Percentage of germination of the hardened and non-hardened (control) rice seeds during storage under the room-temperature and cool conditions (Experiment 6). Vertical bars indicate standard errors.

Fig. 5. Time to 50% germination ($T_{50}$) of the hardened and non-hardened (control) rice seeds during storage under the room-temperature and cool conditions (Experiment 6). Vertical bars indicate standard errors.
effect on seed storability seems to vary with the species (Bray, 1995) and depend on seed quality (Hilhorst and Toorop, 1997). Under the cool condition, $T_{50}$ of the hardened seeds did not increase for 300 d (Fig. 5).

5. Storage life of the seed-mats

Experiment 7 was conducted to investigate the percentage of seedling emergence from the seed-mats with the hardened seeds during storage. The percentage of seedling emergence from the seed-mats with the hardened seeds remained higher than 95% for 208 d under the room temperature condition and declined to 90% at 240 d (Fig. 6). The decline in the percentage of seedling emergence was slower than the decline in percentage of germination of the hardened seeds, suggesting that the production process of the seed-mats and storage of the seed-mats do not adversely affect the quality of the hardened seeds. Under the cool storage, the percentage of seedling emergence from the seed-mats with the hardened seeds remained higher than 95% for at least 300 d. Considering that the hardened seeds maintain a high germination performance for 300 d under the cool condition and for 120 d under the room temperature condition, the seed-mats can be prepared in winter (from December to March) and stored until the seedling-raising period (from April to May in Japan) with a high germination performance. The laborsaving effects of the seed-mats will be investigated in future.

6. Conclusions

The hardening of seeds and covering them with soil to prepare the seed-mats is suitable for seedling growth. In dormant seeds with $T_{50}$ longer than 3.6 d, the effect of hardening is increased by preceding heating treatment. The seed-mats will maintain high seed performance for at least 120 d under the room temperature condition, which means that the seed-mats produced in winter can be used in spring and consequently eliminate seeding at labor peak in spring in Japan. The seedling mats for the rice transplanter can be prepared from the seed-mats without using nursery boxes, which take up storage space, and which need to be washed after they are collected, both laborious tasks.

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