Effects of Temperature, Sowing Depth and Soil Hardness on Seedling Establishment and Yield of Cambodian Rice Direct-seeded in Flood Paddy Fields

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Abstract: Rice is the most important crop for supporting Cambodian economy, however the cultivated area is limited due to the rice production system. Rice is transplanted by hand and yet the direct-seeding technology and proper cultivars for direct-seeding have not been established. The aim of this study is to find the adaptability of Cambodian cultivars for direct seeding. First, we determined the seedling establishment of rice direct-seeded in a flooded paddy field, and examined their growth and yield. The seedling establishment of Koshihikari and W42 were significantly higher than those of Rumpe and Sen Pidao at both medium and low temperatures, but not significantly different among cultivars at high temperature. Koshihikari and W42 showed significantly higher seedling establishment than Sen Pidao at 0 cm deep sowing. And Koshihikari also showed significantly higher seedling establishment than both Rumpe and Sen Pidao at 1 cm and 2 cm deep sowing. The percentage of seedling establishment from the seeds sown after 4, 1 and 0 day of drainage was higher in Rohat than in the other cultivars. The percentage of seedling establishment in 0 day drainage was 51% in Rohat, but only 28% in Sen Pidao, which was the lowest among the cultivars. Cambodian rice cultivars had a short plant length and short basal low internodes, which contributed to strong lodging resistance. Crossing of Cambodian cultivars with Koshihikari to obtain cultivars adapted to direct-seeding in Cambodia was proposed.

Key words: Growth, Low temperature, Seedling establishment, Sowing depth, Water drainage before sowing, Yield.

Direct-seeding is needed to reduce the cost and labor of rice production in Japan, where the age of the farmers is advancing and the number of farmers is decreasing. Not only in Japan, but also in countries of South East Asia such as Malaysia, Thai and the Philippines, the number of farmers having a large irrigated rice production area is low, and direct-seeding is increasing to replace the traditional transplant system (De Datta and Saaran, 1990), including Cambodia. Cambodia was the third largest rice-exporting country in the world in 1940. However, the civil wars in the past four decades have damaged the industries, which supported the domestic economy. Agriculture was the most important sector at that time. However, agricultural machinery is no longer made domestically. Ninety percent of the population lives in poverty, and only a small number of farmers have access to machinery, all of which is imported. Due to the lack of machinery, labor in agriculture relies on human and animal power. Especially, in the rice production system, transplanting is mostly done by hand. Sixty to 70 people are required per hectare. By direct-seeding, this number can be reduced to 30 to 40 people (Yada, 1975). Generally, the average area cultivated per household is approximately 2 to 5 hectares, which is limited by lack of irrigation facility and low water use efficiency. However, the most important limitation is the labor required for growing rice.

Thus in order to enlarge the area of rice production per household in Cambodia, the establishment of a proper direct-seedling method is the extremely important. There are approximately 2000 local cultivars in Cambodia. However, the data about their agronomic characteristics and productivity are scarce especially under the condition of direct-seeding in flooded paddy fields.

Westcott and Mikkelsen (1983) investigated the effects of sowing depth and CaO₂ coating rate on the emergence of rice seedlings in a flooded soil by changing sowing depth. Ohta et al. (2003) studied the genetic variation of seedling emergence using non-CaO₂-coated seeds by changing the sowing depth in a growth chamber. However, no Cambodian cultivars were used in those studies.

Cambodia has a tropical monsoon climate with mean temperature ranges from 21 to 35°C. According to the climate, it is possible to grow rice three times...
per year. But as the lowest temperature is in January (21–25°C), cultivars which perform high seedling establishment in low temperature are needed. As approximately 86% are cultivated with rainfed lowland in Cambodia, cultivars which perform high seedling establishment under flooded condition are also required. In this study, we paid attention to the genetic resources and attempted to find the cultivars highly adapted to direct-seeding under puddled condition and different temperatures.

In order to select lines from crossing those Cambodian varieties with some Japanese promising cultivars, first we had to focus on seedling establishment under various conditions and some agronomic characters of Cambodian cultivars. Yielding and heading time must be examined to check the response of Cambodian cultivars in different environments, especially in Japan, where we will first select good lines adapted in Cambodia. Thus, it is import to collect the data of agronomic traits of a cultivar under various environments.

Therefore, using several Cambodian cultivars, we examined the seedling establishment, growth and yield under direct-seeding in flooded paddy fields. Since seeding by machinery using CaO-covered seeds for enhanced germination is not practical in Cambodia, in this experiment, soaked seeds were direct-sown on a puddled soil surface. The purpose of this study is to contribute to breeding new cultivars adapted in Cambodia and broaden their genetic diversity.

Materials and Methods

1. Materials

Seeds of Cambodian cultivars of rice (*Oryza sativa* L.) Sen Pidao, Rohat and Rumpe were obtained from Cambodia. Sen Pidao, Rohat and Rumpe are photoperiod-insensitive rice of *Indica* type, newly released in Cambodia. All three cultivars are cultivated under rain-fed lowland conditions, and classified into early maturity cultivars (less than 120 days). Rohat and Rumpe were released in 1999. Sen Pidao is premium aromatic rice, which was released in 2002. Average yield (t ha⁻¹) of Sen Pidao, Rohat and Rume are 3.5-5.5, 4.0-6.0, 4.0-6.0, respectively, in Cambodia (Cambodian Agricultural Research and Development Institute, 2001). The grain type of all cultivars is slender. Koshihikari and W42 of Japanese cultivars were used as control cultivars. The aim of using of Koshihikari as a control is that Koshihikari has good eating quality and we intend to select lines deriving from crossing between Cambodian cultivars and Koshihikari. W42 is a cultivar selected from the cross between Hinohikari and Koshihikari for direct-seeding in flooded paddy fields (Won et al., 1998, 1999).

2. Effect of temperatures

In Cambodia rice can be grown all year long and the average of temperature ranges from 20°C to 30°C. We wanted to find out how those Cambodia cultivars adapt to such various temperature changing. The experiments were conducted in growth chambers at Utsunomiya University (Utsunomiya, Tochigi, Japan) in 2005, adjusted to 32°C/27°C (high temperature); 27°C/22°C (medium temperature) and 22°C/17°C (low temperature) in 12-hour daytime and 12-hour nighttime. Non coated-seeds were soaked in water for 5 days. Twenty soaked seeds of each cultivar were sown on the surface in a pot (15 cm diameter) filled with Andosol soil of the depth of 3 cm with 5 replications. Soil was puddled and leveled. Water in the pot was kept at a depth of approximately 1 cm. The established plant number was counted one month after the sowing date.

3. Effect of sowing depth

Fifty soaked seeds of each cultivar were sown in a greenhouse of Utsunomiya University in 2005, with 3 replications, in a pot 27 cm in diameter filled with puddled and leveled Andosol soil. Seeds were sown either on the surface, or at a depth of 1 or 2 cm. Plants were grown for 4 weeks after sowing, and the number of plants established was counted.

4. Effect of soil hardness

The experiment was conducted in a paddy field at Utsunomiya University. To change the degree of soil hardness, we drained the puddled field for 0, 1, 2 and 4 days before seeding. For 0 day draining, the field was drained just before seeding, and immediately flooded again. One hundred seeds of each cultivar were sown on the top of the soil. Seeds were sown two times: May 20 and June 2 in 2005 with 3 replications each. Soil penetration resistance was examined by dropping a golf ball (45.93 g) from 1 meter height and the depth of penetration of the ball was measured (Sawamura et al., 1986).

5. Growth and yield trial

The experiment was conducted at the University farm in Moka from 2003 to 2005, where the soil type is Haplic Andosols (Food and Agriculture Organization, 1988). Sowing date was 8 May in 2003, 14 May in 2004 and 12 June in 2005. The field was puddled, leveled and drained just before sowing. Seeds were soaked in water for 5 days and 300 seeds of each cultivar were sown by broadcasting in a 3 m square in 2003 and 2004. In 2005, 300 seeds were drilled in a 1 m square. The row distance was approximately 33 cm. Four weeks after sowing, the number of plants established was counted and plants were reduced to approximately 60 plants per 1 m square. Chemical fertilizer was supplied as a basal dressing at a rate of 4, 7.2 and 6.4 kg per 10 ares for N, P₂O₅ and K₂O, respectively. Herbicide and pesticide for rice water weevil (*Lissorhoptrus oryzophilus*)
were appropriately applied. An area of 50 × 100 cm (2003), 30 × 100 cm (2004), 66 × 100 cm (2005) in each plot was harvested for 1000-grain weight and brown rice weight. After harvesting, the number of stems per plant was counted, and ten plants with the same number of stems per plant were examined for internode length, the number of spikelets per panicle, and the percentage of ripened grains. There is no reference on a method to determine the percentage of ripened grains of Cambodia cultivars. We, therefore, decided to examined the percentage of ripened grains of Koshihikari was examined by submerging in a salt solution with a specific gravity of 1.06. Throughout the growing stage, we determined the leaf stage, plant length and the number of stems per plant for 10 representative plants of each cultivar every two weeks in 2004. Plant length from the ground to the top of the stretched leaf was measured.

6. Statistical analysis
The data were analyzed by analysis of variance (ANOVA) for the number of spikelets per panicle, the percentage of ripened grains, 1000-grain weight and brown-rice weight. Years were treated as replications.

Results
1. Effect of temperature
The percentage of seedlings established was scarcely influenced by temperature in Koshihikari and W42 (Fig. 1). However, it was extremely decreased by low temperature in Cambodian rice, especially in Sen Pidao. In Sen Pidao, the percentage of seedling establishment was 82, 26 and 8 % at high, medium and low temperatures, respectively, and in Rumpe it was 92, 49 and 25 %, respectively. The seedling establishment of Koshihikari and W42 were significantly higher.
than those of Rumpe and Sen Pidao at both medium and low temperatures, but not significantly different among cultivars at high temperature.

2. Effect of sowing depth

Both Cambodian rice cultivars sown deep in the soil showed an extremely low seedling establishment compared with Koshihikari (Fig. 2). The percentage of seedling establishment from the seeds sown at a depth of 0, 1 and 2 cm was 82, 33 and 0%, respectively, in Rumpe, and 67, 3 and 0%, respectively in Sen Pidao. Koshihikari and W42 showed significantly higher seedling establishment than Sen Pidao at 0 cm deep sowing. And Koshihikari also showed significantly higher seedling establishment than both Rumpe and Sen Pidao at 1 cm and 2 cm deep sowing.

3. Effect of soil hardness

Fig. 3 shows the soil penetration resistance after drainage for various periods. The longer the drainage period, the harder the soil surface. The percentage of seedling establishment from the seeds sown after 4, 1 and 0 day of drainage was higher in Rohat than in the other cultivars (Fig. 4). The percentage of seedling establishment in 0 day drainage was 51% in Rohat, but only 28% in Sen Pidao, which was the lowest among the cultivars. The value in Sen Pidao was 29, 33, 37 and 28% at 4, 2, 1 and 0 day-drainage, respectively.

4. Growth and yield

No lodging was observed in any of the cultivars. Fig. 5 shows the change in the number of leaves on the main stem through the growing stage. All Cambodian cultivars had a larger number of leaves than Koshihikari. The number of leaves in Koshihikari and Cambodian cultivars at the ripened stage was 14 and 16, respectively.

Fig. 6 shows the change in plant length during the growing stage. The plant length of Cambodian cultivars was shorter than that of the Koshihikari. The plant length of Koshihikari was 106 ± 1 cm, and that of Cambodian cultivars 100 ± 1 cm on the average at the ripened stage.

Fig. 7 shows the change in the number of stems per plant. At the maximum tiller number stage (10 weeks after sowing), Rumpe, Sen Pidao, Rohat and Koshihikari had 22 ± 1, 18 ± 1, 14 ± 1 and 11 ± 1 stems per plant, respectively. However, tillers of Cambodian cultivars decreased rapidly thereafter, and the average number of productive tillers at the maturation stage
was $8 \pm 1$ per plant in Cambodian cultivars, and $6 \pm 1$ in Koshihikari at the maturation stage.

Fig. 8 shows the panicle and internode lengths of Cambodian cultivars and Koshihikari. The average panicle length of Cambodian cultivars was $24.1 \pm 0.8$ cm, which was longer than that of Koshihikari of $18.6 \pm 0.3$ cm. The lengths of third and fourth internodes of Cambodian cultivars were $8.1 \pm 1.3$ cm and $4.6 \pm 0.2$ cm, respectively, which were shorter than those of Koshihikari, $15.1 \pm 0.3$ cm and $8.9 \pm 0.6$ cm, respectively.

Table 1 shows the percentage of seedling establishment in the field in 2003, 2004 and 2005. Sen Pidao showed the lowest percentage of seedling establishment except in 2003. Rohat showed the highest percentage of seedling establishment among the Cambodian cultivars in all the years. On the average, Sen Pidao and Rumpe showed nearly the same percentage of seedling establishment. Sen Pidao showed a significantly lower percentage of seedling establishment than Rohat and Koshihikari. Rohat and Koshihikari showed a similar percentage of seedling establishment.

Table 2 shows the heading date in the field. The heading date of Sen Pidao and Rumpe were almost the same, and were approximately 2 weeks to 3 weeks later than that of Koshihikari. Rohat headed earlier than Sen Pidao and Rumpe except in 2004, and later than Koshihikari in all the years.

Table 3 shows the average yield and yield components. Sen Pidao and Rumpe had a significantly larger number of spikelets per panicle than either Rohat or Koshihikari. Rohat had a significantly larger number of spikelets per panicle than Koshihikari. The percentage of ripened grains in Sen Pidao was significantly lower than that in Rohat and Koshihikari, but not significantly different from that in Rumpe. The percentage of ripened grains in Rohat and Rumpe was not significantly different from that in Koshihikari. Sen Pidao and Rumpe had a significantly lower 1000-grain weight than Rohat and Koshihikari. Between Rohat and Koshihikari, there was no significant difference in the 1000-grain weight. Because of the high variation of brown rice weight among the cultivars, there was no significant difference in the 1000-grain weight between the Cambodian cultivars and Koshihikari.

### Discussion

Cambodian cultivars showed a low seedling establishment rate at a low temperature (Fig. 1). Akita et al. (1998) reported that most of the indica cultivars did not reach the 25% emergence rate in submerged soil at 18°C. Uchimura et al. (2001) showed that the emergence rate of IR lines was low (1%~2%) when direct-seeded without the coating of oxygen-generator on the soil surface of flooded paddy fields. The seedling establishment rate of Sen Pidao was low (26% and 8% at medium and low temperatures, respectively). This cultivar is considered to have low adaptability to direct-seeding in flooded paddy fields in some parts of Cambodia where the maximum and minimum temperatures from October to December are lower than 30°C and 22°C, respectively, (IRRI, 1997). Cambodian cultivars also showed a low seedling establishment rate at deep sowing (Fig. 2). Yamauchi and Chuong (1995) reported that the seedling establishment rate was decreased by increasing the sowing depth in flooded soil. In the present experiment, the Cambodian cultivars sown at the

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**Table 1.** Seedling establishment (%) in a field of 2003 to 2005.

| Cultivar    | 2003 | 2004 | 2005 | Average |
|-------------|------|------|------|---------|
| Sen Pidao   | 31   | 52   | 60   | 48b     |
| Rohat       | 39   | 61   | 81   | 60a     |
| Rumpe       | 26   | 59   | 61   | 49b     |
| Koshihikari | 41   | 69   | 75   | 62a     |

Average values followed by the same letter are not significantly different at 5% level according to Duncan's multiple range test.

**Table 2.** The heading date in a field.

| Cultivar    | 2003  | 2004  | 2005  |
|-------------|-------|-------|-------|
| Sen Pidao   | 3rd Sep. | 30th Aug. | 18th Sep. |
| Rohat       | 29th Aug. | 30th Aug. | 12th Sep. |
| Rumpe       | 3rd Sep. | 30th Aug. | 20th Sep. |
| Koshihikari | 19th Aug. | 13th Aug. | 27th Aug. |

**Table 3.** Yield and yield components in the field in 2003, 2004 and 2005.

| Cultivar    | Number of spikelets per panicle | Percentage of ripened grains (%) | 1000-grain weight (g) | Brown rice weight (g m⁻²) |
|-------------|---------------------------------|----------------------------------|-----------------------|--------------------------|
| Sen Pidao   | 132 a                           | 53 b                             | 19 b                  | 421                      |
| Rohat       | 99 b                            | 72 a                             | 21 a                  | 508                      |
| Rumpe       | 134 a                           | 65 ab                            | 18 c                  | 491                      |
| Koshihikari | 88 c                            | 78 a                             | 21 a                  | 506                      |

Average values followed by the same letter are not significantly different at 5% level according to Duncan’s multiple range test.
depth of 1 cm and 2 cm showed less than 50% and 0% seedling establishment rate, respectively. This means that Cambodian cultivars have low adaptability to deep sowing or thick soil covering in direct sowing in flooded paddy fields. Due to the result, the percentage of seedling establishment of W42 and Koshihikari showed almost the same tendency, we decided to discuss the seedling establishment of Cambodian cultivars comparing with Koshihikari. Koshihikari has high adaptability to direct seeding in flooded paddy fields showing a high seedling establishment rate even at a low temperature or deep-sowing condition (Fig. 1, 2). Won and Yoshida (2000a, b) reported varietal differences of establishment rate in rice direct seeded in flooded paddy fields at a low dissolved oxygen level. In order to improve the seedling establishment of Cambodian cultivars, we need to cross them with Koshihikari and to select progenies which have a high ability to germinate in both low temperature and deep-sowing conditions.

Rice seeds sown on puddled soil, may penetrate into the soft and reduced zone of soil, which is lacking in oxygen necessary for seed germination. Rice seeds can germinate even in anoxia, but O_2 is required for seedling establishment. The elongation of coleoptile is promoted, but that of leaf and roots are reduced at a low O_2 (Alpi and Beevers, 1983; Ishizawa and Esashi, 1984). Rice seedlings can be established in flooded paddy fields because O_2 is dissolved in water (Chapman and Peterson, 1962). When seeds are sown deep in the soil lacking in O_2, seedling establishment becomes erratic (Jones, 1933). In the present experiment, water was drained before sowing to avoid seed penetration into soil. However, all of the cultivars except Sen Pidao showed a high seedling establishment rate even after 0-day drainage, and Sen Pidao showed a high establishment rate after 1-day drainage (Fig. 4). Sen Pidao also showed low establishment rate in other experiment (data not shown). We concluded that seedling establishment of Sen Pidao was unstable in water direct-seeding and the cultivar might be less adapted to the condition of low oxygen in water because it showed low seedling establishment rate at after 0-day drainage plot. The depth of seed penetration might be less than 1 cm even without drainage and drainage before sowing might not be necessary. Long drainage caused soil surface harder and the plant roots were hard to anchor to the soil surface. We observed that there were many floated seedling at the 2-day and 4-day drainage plots. That might have caused low seedling establishment (data not shown).

Plant length of Cambodian cultivars was shorter than that of Koshihikari (Fig. 6). In particular, the lengths of the lower internodes (third and fourth internodes) of Cambodian cultivars were shorter than those of Koshihikari, which may contribute to the high resistance to lodging of Cambodian cultivars. Cambodian cultivars had a high number of stems per plant at the maximum tiller number stage, but the number of tillers decreased almost to half of the maximum at the maturation stage (Fig. 7). The growing period between Koshihikari and Cambodian cultivars were not quite different, suggesting that Cambodian cultivars are ear-number type. In order to improve yield of Cambodian cultivars by increasing the number of productive tillers, it is necessary to decrease the planting density and to apply topdressing. Although Sen Pidao and Rumpe showed a low percentage of ripened grains, they had a higher number of spikelets per panicle than either Rohat or Koshihikari and exhibited a high yield (Table 3). Topdressing is needed to increase the percentage of ripened grains, which contributes to the increase in filled grains per panicle.

In order to establish direct-seeding in flooded paddy fields in Cambodia, we need to breed cultivars which show a high germination rate and high establishment rate under a low temperature and deep sowing, high ratio of productive tillers to total tillers, high percentage of ripened grains, heavy 1000-grain weight and early maturing. Though Koshihikari is sensitive to lodging, it performs high yield by proper growing technique. And it has good eating quality by having low amylose content. Cambodian people tend to prefer low amylose content cultivars. Thus, the crossing of Cambodian cultivars with Koshihikari, which has not only good eating quality but also adaptability for direct-sowing, might be effective for these purposes and results obtained in this study must contribute to breeding new cultivars adapted in Cambodia. The data of Cambodian cultivars evaluated in this study might also contribute to rice breeders who want to broaden their genetic diversity.

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