Seedling Growth and Dry-Matter Production under Drained Conditions in Rice Direct-Sown into Puddled and Leveled Soil

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Abstract: Drainage after sowing promotes plant growth and enhances seedling establishment in rice direct-sown into puddled and leveled soil. We studied the effect of drainage on seedling growth and on dry weight of the plant parts. We also examined carbohydrate, chlorophyll, and protein content of the seedlings grown under flooded and drained conditions to see which factors control plant growth during seedling establishment. Drainage for 10 days after sowing enhanced root elongation but inhibited shoot growth until seedlings emerged above ground. Drainage promoted leaf development and shoot elongation after seedlings emerged but affected root growth only slightly at this stage. Although the dry weight of grain decreased and that of shoot and root increased more rapidly in the drained plot than in the flooded one, the utilization efficiency of grain reserve for shoot and root growth was similar in both plots. Growth analysis indicated that the difference in growth rates between the drained and flooded plots was attributable to the amount of carbohydrates supplied from grain reserve until seedlings emerged, but to the photosynthate after seedlings emerged. Sugar contents of shoot and root in the drained plot were rather lower than those in the flooded plot as seedlings emerged. In contrast, chlorophyll and protein contents of shoot in the drained plot were markedly higher than those in the flooded one after seedlings emerged. These results suggest that drainage promotes leaf development, increases chlorophyll and protein contents of shoot, accelerates photosynthesis, and enhances dry-matter production after seedlings emerged.

Key words: Carbohydrates, Chlorophyll, Direct sowing, Drainage, Dry-matter production, Oryza sativa L., Proteins, Rice.

Direct sowing, which eliminates the raising and transplanting of seedlings, is regarded as the most effective way to reduce cost and labor in rice cultivation (Washio, 1984). Direct sowing, however, has constraints such as unstable seedling emergence, weed infestation and frequent rice plant lodging, and thus the yield of direct-sown rice remains lower and less stable than that of transplanted rice.

Unstable seedling establishment is a major constraint in rice direct-sown in puddled and leveled soil (Tanaka, 2000). Seed coating with calcium peroxide (CaO₂) was developed to improve plant growth during seedling establishment (Mitsuishi and Nakamura, 1977), but seedling establishment remains unstable because local soil is reduced when fields are flooded (Hagiwara et al., 1990).

Oba (1997) found that drainage after sowing effectively improves seedling emergence when CaO₂-coated seeds were direct-sown into puddled and leveled soil. Our previous study (Sato and Maruyama, 2002) confirmed this finding, showing that drainage enhances seedling emergence and establishment independent of seed coating with CaO₂ and sowing depth. Our subsequent study (Tsuchiya et al., 2004) showed that drainage after sowing promotes plant growth mainly after seedling emergence and thereby enhances seedling establishment. Since seed coating with CaO₂ accelerates coleoptile elongation and seedling emergence (Mitsuishi, 1975), the physiological mechanism of growth enhancement by drainage differs from that by seed coating with CaO₂ (Tsuchiya et al., 2004). It remains to be clarified, however, how drainage enhances plant growth after seedlings emerged.

Plant growth is sustained both by grain reserve and photosynthate during seedling establishment (Whalley et al., 1966). Growth analysis of seedlings thus requires evaluation of the contribution of grain reserve to the growth. Carbohydrates, the major components of grain reserve, move to new organs and are used for their respiration and growth. The levels of carbohydrates in the shoot and root together with changes in grain...
dry weight indicate how grain reserve supports seedling growth when photosynthesis is negligible. Seedling growth gradually depend on photosynthate as green leaves develop. The levels of chlorophyll and soluble proteins in shoot reflect cellular functions, in particular photosynthesis.

In the present study, we studied the effect of drainage on growth and dry-matter production of seedlings during seedling establishment. We first compared the growth and dry weight of seedlings in flooded and drained plots. We then estimated the utilization efficiency of grain reserve for shoot and root growth when the seedling emerged, and evaluated the contribution of grain reserve and photosynthate to dry-matter production in the seedlings grown under a flooded and drained condition. We finally analyzed the difference in sugar, starch, chlorophyll and protein contents between the seedlings in flooded and drained plots to see which factors control the growth of seedlings after emergence.

Materials and Methods

1. Plants and growth conditions

The experiment was conducted in a greenhouse at the National Agriculture Research Center (Tsukuba, Ibaraki) in October, 1999. Rice (Oryza sativa L., cv. Koshihikari) seeds, harvested at the paddy field of the Niigata Agricultural Research Institute Crop Research Center in 1998, were presoaked in tap water at 20°C for 5 days and coated with CaO2 using the method of Mitsuishi and Nakamura (1977), as described elsewhere (Sato and Maruyama, 2002). After CaO2-coating, seeds were dried for 5 hours at room temperature and stored in plastic bags at 4 ºC until sowing.

Coated seeds were sown in a 3.8-litter plastic pot (16 cm diameter, 19 cm tall) filled with Gray Lowland soil that was basally fertilized with 0.5 g each of nitrogen, phosphate, and potash. Soil was puddled and leveled, then drained a day before sowing. Ten coated seeds were sown in individual pots on October 4. Seeds were placed 10 mm below the soil surface with a thin rod. We selected this sowing depth referring to previous reports (Hagiwara et al., 1990; Yoshinaga et al., 2000; Sato and Maruyama, 2002; Tsuchiya et al., 2004) and direct-sowing culture practice standards in Japan. Water management was started just after sowing. In the drainage plot, water was not added after sowing, and water was introduced up to 3 cm above the soil surface 10 days after sowing (DAS). Water was maintained at 3 cm above the soil surface throughout the experiment in the flooded plot. Air temperature in the greenhouse was controlled between 15 and 27°C.

2. Seedling emergence

Seedling emergence above ground was followed daily from sowing to 10 DAS. To determine the percentage of seedling emergence, twelve pots were used as replicates in each plot with 10 seeds in each replication.

3. Growth and dry weight of seedlings

Seedling growth and dry weight of shoot, root and grain were measured at 2- to 6-day intervals from sowing to 25 DAS. Five seedlings were randomly selected from 2 pots at each sampling, and the leaf age, plant length, the length of the longest root, total root length and the number of roots recorded. Total root length included the lengths of a seminal root and primary crown roots in a plant. Seedlings were washed with water and separated into shoot, root and grain. Separated plant parts were dried at 80°C for 48 hr, then the dry weight of each part was determined.

The growth rate of shoot plus root (GR) was calculated and growth rates attributable to grain reserve (GRg) and to photosynthate (GRp) were estimated as follows:

$$GR = \frac{(S_2 - S_1) + (R_2 - R_1)}{(t_2 - t_1)}$$

$$GR_g = E \times \frac{(G_2 - G_1)}{(t_2 - t_1)}$$

$$GR_p = GR - GR_g$$

where $S_1$ and $S_2$ are shoot dry weight, $R_1$ and $R_2$ are root dry weight, and $G_1$ and $G_2$ are grain dry weight at times $t_1$ and $t_2$, respectively. E is the utilization efficiency of grain reserve for shoot and root growth, estimated by the ratio of dry weight increase in shoot plus root to the dry weight decrease in grain (Tanaka and Yamaguchi, 1968).

4. Analysis of sugars and starch

Ten seedlings were taken from the 2 pots at each sampling time, and separated into shoot and root. After the fresh weight was quickly measured, samples were stored at –85ºC until further analysis. Sugars and starch in seedlings were measured as described elsewhere (Ding and Maruyama, 2004). Shoot or root tissues were placed in 20 mM 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid buffer (pH 7.4) and heated at 90ºC for 5 min. The heated sample was homogenized on ice with fine quartz sand, then centrifuged at 18,000 g for 10 min at 4°C. The supernatant was used to analyze soluble sugars. Glucose, fructose and sucrose in the supernatant were determined by coupled enzymatic assay (Bergmeyer and Bernt, 1974).

The precipitate was washed 3 times with 80% methanol to remove soluble sugars. The washed pellet was suspended in distilled water, and the suspension was heated at 90ºC for 2 hr. Gelatinized starch was digested with amyloglucosidase at 55ºC for 30 min and resultant glucose determined based on the method mentioned above (Bergmeyer and Bernt, 1974). Starch content was expressed as the amount of glucose per unit fresh weight of shoot or root.
5. Analysis of chlorophyll and proteins

Chlorophyll and protein contents of seedlings were measured as described elsewhere (Maruyama et al., 1990). Shoot tissues were homogenized in a prechilled extraction buffer that contained 50 mM of Tris(hydroxymethyl)aminomethane hydrochloride buffer (pH 7.5), 1 mM of disodium ethylenediaminetetraacetate, 8 mM of magnesium chloride and 2% (v/v) 2-mercaptoethanol. An aliquot of the homogenate was extracted with acetone to determine chlorophyll content by the method of Arnon (1949).

To measure soluble protein content, we homogenized the sample of shoot or root in the same buffer and the homogenate centrifuged at 18,000 g for 20 min at 4°C. The supernatant was mixed with trichloroacetic acid and left to stand for 30 min. After centrifugation at 18,000 g for 20 min at 4°C, the pellet was reextracted with 0.1 M of sodium hydroxide containing 2% (w/v) sodium dodecyl sulfate at 40°C for 60 min. The protein content of the fraction was determined by the method of Lowry et al. (1951), with bovine serum albumin as the standard.

For measurement of insoluble proteins, the precipitate was washed 3 times with the same buffer without 2-mercaptoethanol to remove soluble proteins. Insoluble proteins were extracted from the washed precipitate after suspension in 0.1 M of sodium hydroxide containing 2% (w/v) sodium dodecyl sulfate. The suspension was kept at 40°C for 60 min, then centrifuged at 18,000 g for 20 min at 25°C. The supernatant was subjected to protein measurement, as for the soluble protein fraction. The amount of total proteins was calculated as soluble proteins plus insoluble proteins.

6. Air temperature, solar radiation and soil water content during drainage

Air temperature in the greenhouse was measured every 5 min with a thermorecorder (Model TR-71S, T&D Corporation, Nagano, Japan), then daily mean air temperature was calculated. Temperature at 10 mm below the soil surface in the flooded and drained plots was also measured as described above, and mean soil temperature during 10 days after sowing was calculated. The amount of solar radiation was obtained from the meteorological observatory at the National Institute for Agro-Environmental Sciences, about 1000 m away from the greenhouse.

Soil water content (SWC) was measured as described elsewhere (Sato and Maruyama, 2002). Soil was sampled from 1 to 3 cm deep in 3 pots in the drained plot at 2- to 3-day intervals from sowing to 10 DAS. Soil weight was quickly determined and the soil dried at 105°C for 24 hr. Dry soil weight was measured and SWC calculated as the percentage of water in oven-dry soil.

Results

1. Air temperature, solar radiation and soil water content during drainage

Fig. 1 shows the air temperature, solar radiation and soil water content during the experiment. Soil in pots was drained during 10 days after sowing. Mean air temperature in the greenhouse during treatment was 23.6°C and daily solar radiation was 12.5 MJ m⁻². During drainage, SWC decreased from 71.8 to 52.3%. Mean soil temperature 10 mm below the soil surface at this period in the drainage plot was 21.6°C, which was 0.2°C lower than that in the flooded plot.

In the subsequent period, mean air temperature fell to 18.5°C from 11 to 20 DAS, then rose to 22.0°C from 21 to 25 DAS. Daily solar radiation largely fluctuated during the experimental period.

![Fig. 1. Weather conditions and soil water content during the experiment.](image1)

Soil water contents in the drained plot are means ± SE of results from 3 replicates.

![Fig. 2. Effect of drainage on seedling emergence in rice direct-sown into puddled and leveled soil.](image2)

The vertical line indicates standard error of results from 12 replicates.
2. Seedling emergence

Fig. 2 shows the effect of drainage on seedling emergence. Seedlings began emerging 5 DAS, percent of emergence rapidly increased from 5 to 8 DAS, and increased slightly thereafter. Drainage hardly affected seedling emergence at an early stage, but slightly increased it at a late stage. Finally, 83% of the seedling emerged in the flooded plot and 88% in the drained plot, although the difference was not significant at the 5% level, by Student’s t-test.

3. Seedling growth

Table 1 shows the effect of drainage on plant growth during seedling establishment. Drainage somewhat inhibited shoot elongation until seedlings emerged (5-6 DAS), but accelerated leaf development as shown by leaf age and increased plant length after seedlings emerged. In contrast, drainage markedly promoted root elongation at the time of seedling emergence but scarcely affected the length of the longest root, total root length, or the number of roots after seedlings emerged.

Table 1. Effect of drainage on plant growth during seedling establishment.

| Growth parameter | Treatment | Days after sowing |
|------------------|-----------|------------------|
| Leaf age (cm)    | Flooding  | 0.0 0.0 1.6 2.2 2.7 2.2 |
|                  | Drainage  | 0.0 1.0** 2.0** 2.7** 3.0** 3.4 |
| Plant length (cm) | Flooding  | 1.4 1.8 5.7 8.2 13.0 14.8 |
|                  | Drainage  | 0.6* 2.1 6.5 12.4** 17.7** 16.4** |
| Length of the longest root (cm) | Flooding  | 0.1 1.0 7.1 10.0 13.5 13.8 |
|                  | Drainage  | 1.6** 3.1** 9.3 10.0 13.4 18.4** |
| Total root length (cm) | Flooding  | 0.1 1.0 11.9 26.3 39.7 56.2 |
|                  | Drainage  | 1.6** 2.1** 15.4 28.7 38.5 66.7 |
| Number of roots   | Flooding  | 1.0 1.0 4.0 4.4 4.8 6.2 |
|                  | Drainage  | 1.0 1.0 3.4 4.4 5.4 7.6 |

Values are the mean of 5 seedlings. *, ** Difference between treatments was significant at the 5 and 1% level, respectively, by Student’s t-test.

4. Changes in dry weight of plant parts

Fig. 3 shows the effect of drainage on the dry weight of shoot, root and grain during seedling establishment. The grain dry weight in the drained plot decreased more rapidly than that in the flooded plot at the time of seedling emergence. The dry weight of shoot and root after seedling emergence increased more rapidly in the drained plot than in the flooded plot. Drainage increased shoot dry weight more rapidly than root dry weight.

Fig. 4 shows the relationship between the decrease in grain dry weight and the increase in dry weight of shoot plus root after sowing in flooded and drained plots. ** Correlation coefficient was significant at the 1% level.

5. Dry-matter production

Fig. 5 shows the effect of drainage on dry-matter production during seedling establishment. Growth rate of shoot plus root (GR) in the drainage plot was higher than that in the flooded plot throughout the experiment. Growth rate attributable to the grain reserve (GR<sub>G</sub>) in the drained plot was higher than that in the flooded plot at the time of seedling emergence, but the difference became negligible thereafter. In contrast, growth rate attributable to photosynthetic
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6. Changes in sugar and starch contents

Fig. 6 shows the effects of drainage on sugar and starch contents of shoot and root during seedling establishment. Data at 5 DAS is not shown in Fig. 6B, because roots were too few to measure. Sugar contents of shoot and root in the drained plot were lower than those in the flooded plot at the time of seedling emergence. Starch contents of shoot and root were lower than the sugar contents, and little difference was seen between the flooded and drained plots.

7. Changes in chlorophyll and protein contents

Fig. 7 shows the effect of drainage on chlorophyll content of shoot during seedling establishment. The levels of chlorophyll in the drained plot increased more rapidly than those in the flooded plot after seedlings emerged.

Fig. 8 shows the effects of drainage on the soluble and total protein contents of shoot and root during seedling establishment. Soluble and total protein contents of shoot in the drained plot were greater than those in the flooded plot throughout the experiment. Soluble and total protein contents of root were less than those of shoot, although the difference was scarcely detected between plots.
Rice coleoptile elongates more slowly in air than under water, but seminal root grows more rapidly in air than under water (Yamada, 1954). Takahashi et al. (1998) reported that drainage after sowing suppressed shoot elongation but promoted root growth at an early stage of seedling establishment. Our results confirmed these facts, indicating that drainage inhibits shoot elongation but accelerates root growth at the time of seedlings emergence. Drainage, however, enhances leaf development and shoot elongation after seedling emergence (Furuhata et al., 1998; Tsuchiya et al., 2004), and thus increases the dry weight of shoot and root during seedling establishment (Sato and Maruyama, 2002). Our results confirmed these effects of drainage on the dry weight of shoot and root after seedlings emerged, but also showed that drainage increased the dry weight of shoot more rapidly than that of root. Drainage scarcely affected root development and elongation after seedlings emerged in our experiment, indicating that drainage enhances shoot growth markedly but promotes root growth only slightly after seedlings emerged. These results suggest that drainage after sowing accelerates root growth at the time of seedling emergence but promotes shoot growth after seedlings emerged.

Cereal seedlings grow heterotrophically at an early stage, depending entirely on the reserve in endosperm, and then enter a transition during which growth is sustained by both the reserve in endosperm and photosynthesis (Whalley et al., 1966). Seedlings eventually become autotrophic, depending totally on photosynthesis. Yoshida (1973) reported that the grain reserve mainly supported the growth of rice seedlings in the early stage after sowing, and seedlings became autotrophic at a leaf age of 3.7. The growth of rice seedlings should therefore be analyzed in relation to both grain reserve and photosynthate during seedling establishment. In our study, drainage enhanced the decrease in the dry weight of grain and accelerated the increase in the dry weight of shoot and root as seedlings emerged, although drainage scarcely affected the efficiency of grain reserve in forming new organs. This efficiency estimated in our study was 0.586, irrespective of water management, which was similar to reported values (0.56 to 0.58) for rice seedlings grown at 21, 26, and 32°C in the dark (Tanaka and Yamaguchi, 1968). These results suggest that the utilization efficiency of rice grain for seedling growth is approximately 0.6 and is hardly affected by environmental conditions. Drainage enhanced the decrease in the dry weight of grain, probably because accelerated respiration and seedling growth under high oxidation-reduction state in top soil required more carbohydrates from grain reserve.

In our study, seedling growth attributable to grain reserve was estimated assuming that the utilization efficiency of grain reserve for seedling growth was 0.586. Our results indicated that plant growth depended entirely on grain reserve at the time of seedling emergence, but was sustained by both grain reserve and photosynthate after seedlings emerged. Growth analysis showed that the difference in growth between the plants in flooded and drained plots at the time of seedling emergence was ascribable to grain reserve, but that after seedlings emerged to photosynthesis. Carbohydrates from grain reserve, however, may not necessarily control plant growth at the time of seedlings emergence, since sugar levels in shoot and root were lower in the drained plot than in the flooded plot. Higher sugar content of seedlings...
under flooded conditions indicates that carbohydrate supply from grain reserve exceeded the amount of substrate required for seedling respiration and growth.

Drainage after sowing enhances leaf development after seedlings emerged (Furuhata et al., 1998; Tsuchiya et al., 2004). Our results indicate that drainage increased chlorophyll and soluble protein contents of shoot after seedlings emerged, while it scarcely affected carbohydrate content at this stage. Our study also showed that photosynthetic growth increased earlier in the drained plot than in the flooded plot after seedlings emerged, although growth attributable to grain reserve was similar regardless of water management. These results suggest that drainage promotes leaf development, accelerates chlorophyll and protein synthesis in shoot, and enhances photosynthetic growth after seedlings emerged.

In conclusion, our results indicate that drainage after sowing enhances root growth at the time of seedling emergence, but it promotes shoot growth after seedlings emerged. Growth enhancement by drainage appears to be ascribable to grain reserve at the time of seedling emergence but to photosynthetic growth after seedlings emerged. Our results suggest that drainage promotes leaf development, increases chlorophyll and protein contents of shoot, accelerates photosynthesis, and enhances dry-matter production after seedlings emerged. Further study is required, however, to clarify whether photosynthesis is actually involved in growth enhancement by drainage after seedlings emerged.

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