Varietal Differences in Biomass Production of Rice Early After Transplanting at Low Temperatures

Akihiro Ohsumi, Masami Furuhata and Osamu Matsumura
(Hokuriku Research Center, National Institute of Agricultural Research Center, NARO, Inada 1-2-1, Joetsu, Niigata 943-0193, Japan)

Abstract: Low temperature decreases biomass production and yield in rice through a number of physiological and morphological changes. We evaluated biomass production in 22 high-yielding genotypes and four commercial japonica cultivars early after transplanting under field conditions for 2 years. The seedlings were transplanted on 30 April or 1 May (early transplanting, ET) and 4 weeks later (late transplanting, LT). The mean air temperature during the 18 days after transplanting in ET was about 4°C lower than that in LT in both years. The seedling length was greater in high-yielding japonica varieties than in indica genotypes, whereas the seedling character index (SCI), which is the product of plant age in leaf number and the ratio of the seedling weight to its length, was the highest in the indica genotypes. Varietal differences in biomass production were greater in ET than in LT in all rice genotypes. The biomass at 18 days after transplanting was largest in the japonica high-yielding varieties Kusayutaka and Beko-aoba in both ET and LT. The biomass production of the indica genotypes was found to decrease severely after transplanting at low temperatures although the indica genotypes with high SCIs showed faster leaf emergence than the high-yielding japonica varieties. There was a strong positive correlation between the varietal differences in biomass production and shoot length at 18 days after transplanting in ET in both years. Our study suggests that superior shoot elongation in the high-yielding japonica varieties with large biomass allocation to the stems may be advantageous in maintaining biomass productivity at low temperatures.

Key words: Biomass production, Early growth after transplanting, Low temperature, Rice.

Rice is cultivated in areas ranging from tropical to cool temperate regions. Rice plants encounter low temperatures soon after transplanting and during the grain filling period in temperate regions and at high altitudes. A change in the temperature around the plants during any period of their development influences both the yield and the yield components, with low temperature at the time of establishment of the plant after transplanting being found to decrease grain yield (Matsushima et al., 1963). Hence, it would be useful to clarify the traits that influence plant growth in response to temperature so as to achieve stable yields of rice.

Changes in temperature affect many traits that are responsible for biomass productivity in rice, including respiration, leaf photosynthesis (Makino et al., 1994; Maruyama and Nakamura, 1997), efficiency of nitrogen (N) use for leaf photosynthesis (Nagai and Makino, 2009), leaf emergence (Hiraoka et al., 1987), leaf elongation (Cutler et al., 1980), and the allocation of biomass and N to leaves (Kanno et al., 2009). However, understanding of the genotypic differences in biomass productivity of rice in response to temperature is limited, especially under field conditions.

Early growth after transplanting is related to seedling characteristics such as leaf number, shoot dry weight and shoot length, which vary with the condition such as temperature and seeding density (Honda and Usuda, 1959; Hayashi and Suzuki, 1960; Yamamoto et al., 1998; Sasaki, 2004). Commercial japonica cultivar seedlings with larger leaf numbers or higher ratios of shoot dry weight to length produce a larger biomass at 12°C during the 21 days after transplanting (Murakami et al., 1982). Kusutani (1986) defined seedling character index (SCI) as the product of the plant age in leaf number and the ratio of shoot dry weight to length, which represents fullness of nutrients in the seedling. Kusutani (1986) showed that rice seedlings with higher SCIs in a japonica cultivar exhibited vigorous growth after transplanting in cool temperate regions. Gui et al. (2000) reported that high-yielding indica varieties showed higher SCIs and greater biomass production than conventional japonica cultivars. However, it is not yet known whether SCI can explain genotypic
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On the other hand, whereas most of the high-yielding indica varieties are the semi-dwarf type, the recently released high-yielding japonica varieties developed not only to provide food for humans but also as livestock forage have long culms (Kato, 2008). Few studies have sought to evaluate the early growth of these high-yielding japonica varieties with different seedling characteristics compared to the semi-dwarf indica genotypes.

The objective of the present study was to evaluate the varietal differences in biomass productivity after transplanting under low temperature conditions. Temperature conditions were changed by changing the transplanting dates in the fields, with earlier transplanting in the spring being associated with cooler temperatures during plant growth.

### Materials and Methods

#### 1. Plant cultivation

Growth characteristics early after transplanting were evaluated in 22 high-yielding rice genotypes and four commercial cultivars after transplanting (Table 1). These test varieties have been bred in different districts of Japan and show large variations in heading date and plant length at maturity.

Rice seeds were sown on two different dates both in 2009 and 2010. The rice seeds for early transplanting (ET), were sown in nursery boxes on 1 and 2 April and transplanted on 1 May and 30 April 2009 and 2010 respectively. An indica variety, Hokuriku 193, was not evaluated for ET in 2009. For late transplanting (LT), rice seeds were sown on 7 May both 2009 and 2010 and transplanted on 29 and 27 May 2009 and 2010, respectively. The seedling period was designed to be longer in ET than LT.

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**Table 1. List of plant materials and their characteristics.**

| Variety / cultivar | Heading | Plant length | Genotype | Site of origin (prefecture) |
|--------------------|---------|--------------|----------|----------------------------|
| **Multiple-use variety** |         |              |          |                            |
| Kita-aoba          | 18 July | 92           | japonica | Hokkaido                   |
| Bekogonomi         | 26 July | 100          | japonica | Akita                      |
| Fukuhibiki         | 4 August| 99           | japonica | Akita                      |
| Beko-aoba          | 10 August| 98          | japonica | Akita                      |
| Yume-aoba          | 10 August| 116         | japonica | Niigata                    |
| Hoshiaoba          | 17 August| 125         | japonica | Hiroshima                  |
| Kasaiyatake        | 19 August| 116         | japonica | Niigata                    |
| Akembohoshi        | 24 August| 117         | japonica | Hiroshima                  |
| Nishiaoba          | 2 September| 125        | japonica | Fukuoka                    |
| Kasahonoumi        | 3 September| 136        | japonica | Ibaraki                    |
| Kasunohoshi        | 5 September| 138        | japonica | Hiroshima                  |
| Leafstar           | 5 September| 139        | japonica | Ibaraki                    |
| Hama-sari          | 6 September| 117        | japonica | Saitama                    |
| Tachiaoba          | 13 September| 135       | japonica | Fukuoka                    |
| Tachisugata        | 18 August| 134         | mixed    | Ibaraki                    |
| Momiromani         | 25 August| 128         | mixed    | Ibaraki                    |
| Hohatake           | 11 August| 105         | indica   | Niigata                    |
| Hokusoku218        | 18 August| 118         | indica   | Niigata                    |
| Takanari           | 19 August| 107         | indica   | Ibaraki                    |
| Hokusuku193        | 21 August| 114         | indica   | Niigata                    |
| Hokusuku147        | 22 August| 111         | indica   | Niigata                    |
| Mohretsu           | 31 August| 140         | indica   | –                          |
| **Commercial cultivar** |         |              |          |                            |
| Kirara397          | 18 July | 85           | japonica | Hokkaido                   |
| Sasanishiki        | 8 August| 113          | japonica | Miyagi                     |
| Koshihikari        | 13 August| 120         | japonica | Fukui                      |
| Nipponbare         | 21 August| 120         | japonica | Aichi                      |

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a) heading date in the late transplanting treatment in 2009.
b) measured at maturity in the late transplanting treatment in 2009.
c) from Yamamoto et al. (2010).
d) estimated from genotypes of their parents.
in LT so as to provide seedlings that were similar in plant age, because of the lower temperature in ET. After sowing the seeds in the nursery boxes, they were incubated at 30ºC for 2 days to ensure emergence of the seedlings. Seedlings were grown in a plastic greenhouse and transplanted into the paddy field of the Hokuriku Research Center, Joetsu, Japan (37º6’ N, 138º16’ E). Air temperature and solar radiation in the fields were recorded in both years. The air temperature inside the greenhouse and the water temperature at the surface of the soil in the fields were recorded in the second year of the study (2010). All varieties were grown in three separate rows, in a random block design, with one seedling per hill and more than 12 hills per row. Spacing was kept at 15 cm between plants and 30 cm between rows. ET and LT plants were grown in separate plots. The fields were irrigated to a depth of 5 cm when the water level decreased to about 1 cm in depth. Environmental interferences by the different neighboring varieties were considered to be negligible because the rice plants were small until the time of harvest. A slow-release coated urea, LP140, and ammonium sulfate were each applied at 4 g N m⁻² to make the total amount 8 g N m⁻² and applied to all the plots before transplanting.

2. Plant harvesting

Plants were harvested twice: at the time of transplanting (n=20) and 18 days after transplanting (18 DAT, n=6) for each replicate. We evaluated the genotypic difference in early growth after transplanting at 18 DAT, as in the previous studies (Murakami et al., 1982; Yamamoto et al., 1995). Leaf number and plant length were determined for the harvested plants. The plants harvested at 18 DAT were separated into leaf blade (hereafter referred to as “leaf”) and other parts (hereafter referred to as “stem”). The dry weights of the shoots and all organs were determined after drying at 70ºC for 48 hr. The ratio of leaf dw (dry weight)/stem dw at 18 DAT was calculated and compared among the different genotypes. The seedling character index (SCI) was calculated as following equation:

\[
SCI \ (g \ m^{-1}) = \frac{\text{Plant age in leaf number} \times \text{shoot dry weight} (mg)}{\text{shoot length} (mm)}.
\]

Effects of genotype, year and their interactions on the plant characteristics were analyzed by analysis of variance (ANOVA). As a result, no interaction effect of genotype and year was found, and the data were represented as the average for each genotype and year on Tables 3, 4 and 5. Significant differences among them were analyzed using the least significant differences (LSD) test (p<0.05).

Results

1. Temperature regimes

The daily mean, minimal, and maximal air temperatures during the seedling period and during the 18 days after transplanting were recorded in two years. The minimal and maximal temperatures and solar radiation in the fields are shown in Table 2. The air temperatures inside the greenhouse and the water temperature at the surface of the soil in the fields were recorded in the second year of the study (2010). All varieties were grown in three separate rows, in a random block design, with one seedling per hill and more than 12 hills per row. Spacing was kept at 15 cm between plants and 30 cm between rows. ET and LT plants were grown in separate plots. The fields were irrigated to a depth of 5 cm when the water level decreased to about 1 cm in depth. Environmental interferences by the different neighboring varieties were considered to be negligible because the rice plants were small until the time of harvest. A slow-release coated urea, LP140, and ammonium sulfate were each applied at 4 g N m⁻² to make the total amount 8 g N m⁻² and applied to all the plots before transplanting.
transplanting in the fields were lower in ET than in LT for both years (Table 2). However, the differences in the cumulative air temperatures during the seedling period between ET and LT was slight, less than 10.1%, due to the longer seedling period in greenhouse in ET in both years. The mean air temperature after transplanting was about 4°C lower in ET than in LT in both years. Although the maximal temperature inside the greenhouse was 3.2°C higher than that outside in both ET and LT, the minimal temperature inside the greenhouse was 1.0°C lower, probably because of radiational cooling. As a result, the difference between daily mean air temperatures inside and outside the greenhouse was less than 0.1°C during the growth of the seedlings. The daily mean, minimal, and maximal water temperatures were 2.5, 5.2, and 1.9°C higher than the respective air temperatures in ET, and 3.1, 5.1, and 2.6°C higher than the respective air temperatures in LT. The daily solar radiation was higher before transplanting in 2009 than in 2010, but was lower after transplanting. Cumulative values of air temperature and solar radiation were lower during the seedling period in 2010 compared to those in 2009.

### Table 3. Seedling characteristics at the time of transplanting in 2009 and 2010.

| Genotype | Plant age in leaf number | Shoot length (mm) | Shoot dry weight (mg) | Seedling character index (SCI) (g m⁻¹) |
|----------|--------------------------|-------------------|-----------------------|----------------------------------------|
|          | ET                       | LT                | ET                    | LT                                    |
| japonica | 4.01 4.29                | 118 a 156 a       | 27.2 26.6             | 0.92 0.74 b                           |
| mixed    | 4.02 4.50                | 116 a 152 ab      | 28.3 28.1             | 1.01 0.85 ab                          |
| indica   | 3.89 4.23                | 94 b 139 b        | 24.2 27.9             | 1.04 0.86 a                          |
| commercial | 4.06 4.18              | 106 ab 169 a      | 23.9 23.8             | 0.86 0.60 c                          |
| Year     |                          |                   |                       |                                        |
| 2009     | 4.63 a 4.54 a            | 132 a 160 a       | 38.8 a 35.5 a         | 1.38 a 1.02 a                        |
| 2010     | 3.38 b 4.01 b            | 91 b 148 b        | 14.0 b 17.8 b         | 0.52 b 0.48 b                        |

ANOVA

| Genotype (G) | ** | ns | ns | ns | ns | ns | ns | ns |
| Year (T)     | ** | ** | ** | ** | ** | ** | ** | ** |
| G × Y        | ns | ns | ns | ns | ns | ns | ns | ns |

Seedling character index (SCI) is the product of plant age (leaf number) and shoot dry weight per unit shoot length. Values are the averages for each genotype or year. Values followed by different letters differ significantly (P<0.05, LSD). ns, not significant. *,**, significant at the 0.05 and 0.01 probability level, respectively.
2. Seedling characters

The seedlings in ET had a shorter shoot length than those in LT in all the genotypes in both years (Table 3). Plant age in leaf number was younger and shoot dry weight of the seedlings lighter in ET than in LT in 2010. All the traits studied were significantly higher in 2009 than in 2010 (p<0.05). The seedling weight and SCI in 2010 were less than the halves in 2009 in all the genotypes. The indica genotypes had a shorter seedling length than the japonica varieties, although this difference was not significant in 2010 (not shown). High-yielding indica genotypes, except for Mohretsu, tended to have higher SCIs in both ET and LT in 2010 (Fig. 1). Similar varietal differences of SCIs were observed in 2009.

3. Plant traits at 18 days after transplanting

Plant age in leaf number, shoot length, and dry weight of the shoots at 18 DAT were higher in LT than in ET in all the genotypes (Table 4). Increase in plant age in leaf number during the 18 days after transplanting was greater in the indica genotypes than in the japonica high-yielding varieties, and the differences were significant (P<0.05, Table 5). The indica genotypes showed a lower increase in shoot length in ET than the japonica high-yielding and commercial varieties (p<0.05), but showed a greater increase in shoot length in LT (p<0.05). Varietal differences were observed in shoot dry weights at 18 DAT both in ET and in LT (Fig. 2). The variations in shoot dry weights at 18 DAT were larger in ET than in LT, with the coefficients of variance being 31 and 16%, respectively in 2009, and 22 and 16%, respectively, in 2010. Two japonica varieties, Kusayutaka and Beko-aoba, consistently showed the greatest shoot dry weights among the varieties in ET in both years. On the other hand, most of the indica genotypes and two japonica varieties, Akenohoshi and Kusahonami, had lower biomass productivity in ET. The dry weight ratios of leaves to stems in ET were similar to those in LT in each year (Table 4). The dry weight ratios of the indica genotypes were significantly greater than those of the high-yielding japonica varieties.

Variatel differences in SCIs were not related with the varietal differences in shoot dry weights at 18 DAT in ET in

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**Table 4. Plant characteristics at 18 days after transplanting in 2009 and 2010.**

| Plant age in leaf number | Shoot length (mm) | Shoot dry weight (mg) | Dry weight ratio of leaves/stems |
|-------------------------|-------------------|-----------------------|----------------------------------|
| Genotype                | ET                | LT                    | ET                              | LT                              |
| japonica                | 6.26              | 7.33                  | 250 a                           | 321                             | 92 a                             | 300 | 0.83 b | 0.80 b |
| mixed                   | 6.40              | 7.60                  | 230 ab                          | 325                             | 88 a                             | 289 | 0.80 b | 0.73 b |
| indica                  | 6.45              | 7.51                  | 211 b                           | 321                             | 59 b                             | 260 | 0.99 a | 0.96 a |
| commercial              | 6.46              | 7.38                  | 252 a                           | 310                             | 92 a                             | 271 | 0.83 b | 0.77 b |
| Year                    | 2009              | 6.69 a                | 7.58 a                          | 260 a                           | 349 a                            | 101 a | 360 a | 0.81 b | 0.79 b |
|                        | 2010              | 6.00 b                | 7.22 b                          | 219 b                           | 289 b                            | 67 b  | 206 b | 0.92 a | 0.87 a |

ANOVA

Genotype (G): ns ns ** ns ** ns ** **
Year (T): ** ** ** ** ** ** ** **
G×Y: ns ns ns ns ns ns ns ns

Values are the averages for each genotype or year.
Values followed by different letters differ significantly (P<0.05, LSD).
ns, not significant.
**, significant at the 0.01 probability level.

**Table 5. Increases in plant age in leaf number and shoot length for 18 days after transplanting in 2009 and 2010.**

| Plant age in leaf number | Shoot length (mm) |
|-------------------------|-------------------|
| Genotype                | ET                | LT |
| japonica                | 2.25 b            | 3.04 b |
| mixed                   | 2.38 ab           | 3.10 ab |
| indica                  | 2.52 a            | 3.27 a |
| commercial              | 2.40 a            | 3.19 ab |
| Year                    | 2009              | 2.07 b | 3.03 b |
|                        | 2010              | 2.62 a | 3.21 a |

ANOVA

Genotype (G): ** ** ns **
Year (T): ** ns ns ns
G×Y: ns ns ns ns

Values are the averages for each genotype or year.
Values followed by different letters differ significantly (P<0.05, LSD).
ns, not significant.
*,**, significant at the 0.05 and 0.01 probability level, respectively.
either 2009 or 2010 (Fig. 3). A significant correlation between SCI and shoot dry weight was observed only in LT in 2009 ($P<0.05$). Varietal differences in shoot dry weights in ET were tightly linked to those in shoot length at 18 DAT ($P<0.001$, Fig. 4), whereas their correlation coefficients were low in LT in both years ($P<0.05$). The shoot lengths of most of the indica genotypes at 18 DAT were shorter than those of the japonica varieties with the higher shoot dry weights in both years. Varietal differences in shoot length at 18 DAT in ET were negatively correlated to those in the dry weight ratio of leaves/stems ($P<0.01$, Fig. 5), but the relationships differed with the year.

**Discussion**

Our study showed that the varietal differences in biomass production early after transplanting in rice were larger at the low temperatures in ET than in LT in the field (Fig. 2). The japonica varieties Kusayutaka and Beko-aoba produced the greatest biomass early after transplanting in
ET and LT in both years. On the other hand, the indica genotypes showed the lowest shoot dry weights in ET at 18 DAT (Table 4). The shoot dry weights of the indica genotypes were 35% smaller in ET than those of the japonica high-yielding varieties, and 13% lower in LT. This indicates that the biomass productivity of the indica genotypes is more sensitive to low temperatures than other genotypes under field conditions.

There are some reports that plant growth of indica genotypes is more strongly restricted under low temperature conditions than that of other genotypes, and that there is a greater incidence of chilling injuries (Kabaki and Tajima, 1981; Mackill and Lei, 1997; Andaya and Mackill, 2003), irreversible root damage and poor water absorption (Kabaki and Tajima, 1981), decreased leaf development (Hiraoka et al., 1987), and suppression of shoot elongation (Hiraoka et al., 1987; Redona and Mackill, 1996). In the present study, there was a strong positive correlation between the varietal differences in shoot dry weights at 18 DAT and those in shoot lengths in ET in both years (Fig. 4). The high-yielding indica genotypes which showed the lowest level of biomass production in ET also exhibited the lowest shoot length (Table 4). The short shoot length in the indica genotypes was associated with greater biomass allocation to the leaves (Fig. 5), which would be an inherent characteristic of semi-dwarf high-yielding indica genotypes. Carbohydrate accumulation in sink organs increases at low temperatures (Paul et al., 1991; Kanno et al., 2009). However, the decreased shoot elongation at the low temperatures in ET would mean a smaller volume of sink tissues (i.e. in stem) in which to store photoassimilates. Taken together, the low biomass production in indica genotypes at low temperatures could be partly due to the feedback inhibition of photosynthesis (Bagnall et al., 1988). This warrants an examination of the relative importance of sink capacity for carbohydrate accumulation, leaf photosynthesis, and water absorption (Kabaki and Tajima, 1981) as factors influencing genotypic variation in biomass productivity at low temperatures.

The semi-dwarf indica genotype seedlings grown under low temperature conditions may have been to some extent more negatively affected by the water management in our experiment; we maintained the water depth below 5 cm after transplanting, which could have limited the leaf area above the surface of the water, especially for the small shoots of the semi-dwarf indica genotype seedlings. This may be similar to the observation of disadvantages of the rice plants with less vigorous shoot elongation under prolonged submergence (Sakagami et al., 2009).

Two high-yielding japonica varieties, Kusayutaka and Beko-aoba, showed the greatest biomass production and longest shoot lengths (Fig. 4) early after transplanting at low temperatures. The superior growth after transplanting might be attributed to their higher seedling dry weight (not shown) due to the large amount of accumulated carbohydrates at the time of transplanting (Honda and Usuda, 1959). The seedling vigor in these varieties might be associated with their grain size, which was much larger than the commercial cultivars (Kato, 2008), because the endosperm nutrients serve as a source for growth during the early seedling period (Sasaki, 2004).

Murakami et al. (1982) reported that seedlings with greater leaf number or greater ratio of shoot dry weight to length showed vigorous growth early after transplanting at low temperatures. As reported previously by Gui et al. (2000), SCI, which is the product of leaf number and the ratio of shoot dry weight to length, was the highest in the indica genotypes among all the rice varieties studied (Fig. 1).

Fig. 4. Relationships between lengths and dry weights of shoots at 18 days after transplanting for 26 rice genotypes in 2009 and 2010. ET and LT represent early and late transplanting, respectively. * and *** indicate significant correlation at 5% level and 0.1% level, respectively.

Fig. 5. Relationships between dry weight ratios of leaves/stems and shoot lengths at 18 days after transplanting for 26 rice genotypes in the early transplanting treatment in 2009 and 2010. ** indicates significant correlation at 1% level.
Gui et al. (2000) reported that high-yielding varieties with high SCIs produced a large biomass early after transplanting due to superior leaf development. In our study, the indica genotypes that tended to have higher SCIs also showed faster leaf emergence after transplanting both in ET and LT (Table 5). However, the varietal differences in SCI alone did not account for the differences in biomass productivity early after transplanting at low temperatures (Fig. 5), due to the disadvantages of the shorter seedlings in the indica genotypes as discussed above. Also, the similar levels of biomass production observed in the indica genotypes that were supposed to be high-yielding and in the commercial varieties in LT in our study were not consistent with the findings of Gui et al. (2000). This could be attributed to the higher temperature conditions in the study by Gui et al. (2000), which would indicate that the indica genotypes with high SCIs may demonstrate greater biomass productivity at higher temperatures.

In this study, we showed that the high-yielding japonica varieties with greater shoot lengths produced greater biomass early after transplanting at low temperatures. This characteristic of superior shoot elongation with larger biomass allocation to the stems would be beneficial to achieve stable yields of rice in cool regions.

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