Nursery Management for Improving Seedling Length and Early Growth after Transplanting in a Semi-Dwarf Rice Cultivar Hokuriku 193

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Abstract: Nursery management methods for elongating seedlings are needed for stabilization of early growth after transplanting in the semi-dwarf rice cultivar Hokuriku 193, unlike japonica commercial cultivars. This study aimed to investigate how keeping the plants at 28ºC for 5 d after sowing (H treatment) and nitrogen top-dressing (N treatment) affects seedling quality and early growth after transplanting. Control plants had seedling lengths of 6.5 cm to 10.9 cm at transplanting. The H and N treatments significantly increased the seedling length, and the increase was greater in the H-treated plants. The H × N treatment elongated seedlings by 2.5 – 3.7 cm compared to the control plants. The N treatments, but not the H treatments, improved biomass production and tillering early after transplanting owing to the high nitrogen concentration in the seedlings. Combining H and N treatments can contribute for improving seedling length and early plant growth after transplanting in Hokuriku 193.

Key words: Early growth after transplanting, Low temperature, Nitrogen top-dressing, Rice, Seedling length, Tiller number.
weight to length by increasing the length. In commercial japonica cultivars, decreasing seedling length and increasing the ratio of shoot dry weight to the length improve seedling quality and early biomass production after transplanting (Kusutani, 1986). However, these results suggest that enforced elongation of seedlings might impair seedling quality and decrease early biomass production in Hokuriku 193. In addition, Horai et al. (2013) reported that poor plant growth early after transplanting reduces the final panicle number and grain yield in cool regions. Thus, evaluating biomass production and tillering ability early after transplanting is necessary to assess the seedling elongation. This study aimed to investigate the effects of heating and top-dressing of nitrogen during the nursery period on seedling traits in Hokuriku 193, and to evaluate plant growth early after transplanting for the seedlings subjected to different treatments.

Materials and Methods

1. Nursery and field management

In 2011, 2012 and 2013, four nursery treatments combining heat (H) or control (C) treatments with top-dressing nitrogen (N) or no top-dressing (0N) treatments were conducted; each treatment had three replicates. Six packs of well-ripened 100 g seeds of Hokuriku 193 were germinated at 28°C in a water pool after abundant water absorption. A pack of fully imbibed seeds was sown on a nursery box (584 mm × 281 mm) filled with soil containing 1.6 g N, 1.6 g P$_2$O$_5$, and 1.6 g K$_2$O, and covered with soil containing no nitrogen. The nursery boxes were incubated at 28°C for 2 d in the dark to ensure seedling emergence as a control; this is the usual cultivation practice in the study area. For H-treatment, nursery boxes were kept in an incubator at 28°C for about three more days, 5 d in total, to elongate seedlings over 5 cm, which is similar to the method used for growing nurseling seedlings for machine transplanting in Japan (e.g. Hoshikawa et al., 1995). After emergence, seedlings were grown in a plastic greenhouse at Hokuriku Research Center, Jo-etsu, Japan (37°6’N, 138°16’E). All the nursery boxes were partitioned equally by using a plastic plate, and the seedlings in a compartment of each nursery box were top-dressed twice with ammonium sulfates (N treatment): 0.75 g N per compartment when the leaf number was two, and 1.25 g N per compartment when the leaf number was three. The total amount of nitrogen top-dressing was double of the recommended amounts for japonica commercial cultivars, because seedling length of indica cultivars become further shorter than that of japonica cultivars at lower temperature (Hiraoka et al., 1987; Redoña and Mackill, 1996). Four treatments were arranged in a split-block design with heat treatment as the main plot and top-dressing as the subplot.

Seedlings were hand-transplanted at a depth of 2 cm into paddy fields at the rate of two plants per hill with a total of three replicates. The plots were arranged in a randomized complete block design. Spacing between plants was 15 cm, and that between rows was 30 cm. Plot size was larger than 5 m$^2$, with five-plant rows in all the years. All fertilizers were applied as basal for each plots; the fertilizer composition was 4 g m$^{-2}$ P$_2$O$_5$, 4 g m$^{-2}$ K$_2$O, 4 g N m$^{-2}$ ammonium sulfate, and 7 g N m$^{-2}$ slow-release coated urea (LP140 that releases 80% of the nitrogen at a uniform rate until 140 d after application). Plants were grown under irrigated conditions and protected from diseases and insects by using chemicals.

Seed-sowing and transplanting were done at different times in 2011, 2012 and 2013, to verify if the two nursery treatments have stable effects on plant growth under different climatic conditions (Table 1). The seedlings were grown in the nursery for 30 d in 2011 and 2013, and for 21 d in 2012. Since the sowing and transplanting times were later in 2012, the daily mean, maximum, and minimum air temperatures were higher in 2012 in the nursery period, as well as during 3 wk after transplanting. The transplanting times in 2011 and 2013 were the recommended times for Hokuriku 193 to achieve its yield potential in the study area (Ohsumi et al., 2014).

2. Measurements of plant traits

Plants were harvested at the transplanting time ($n = 30$), as well as 3 wk after transplanting ($n = 16$), in all the plots over the three years. Plant traits at 3 wk after transplanting represent early growth, according to the previous studies (Murakami et al., 1982; Yamamoto et al., 1995). Plant age in leaf number, plant length, and shoot dry weight were determined for the harvested plants. The dry weights were determined after drying the plant parts at 80°C for 72 hr.
The oven-dried shoots were used to determine the nitrogen concentration by using the combustion method (JM3000CN; J Science Labo, Kyoto, Japan). Tiller number was counted for six plants in each plot at the initial tillering stage, when the first tiller appeared in most of the plants (> 88.8 tiller m\(^{-2}\)) and at plant maturity over the three years. Three-way analysis of variance was used to compare the effects of H and N treatments, year, and their interaction, followed by Student’s t-test. Statistical analyses were performed using the statistical software, JMP 9.0 (SAS Inst. NC, USA). Plant age in leaf number (leaf number on the main tiller) and tiller number were measured at 3 – 4-d intervals for 3 wk after transplanting in 2012.

### Results

The seedlings of the C × 0N treatment were the shortest in all the years (\(P < 0.05\)); and the shortest length of 6.5 cm was recorded in 2011 (Table 2). The H treatments significantly increased seedling length (\(P < 0.05\)) and decreased the leaf number, although the leaf number in H × 0N treatment was similar to that in C × 0N plants in 2012. The N treatments significantly increased both plant age (leaf number on the main tiller) and seedling length (\(P < 0.05\)). The H × N plants showed the greatest seedling length among the treatments, although the increase of seedling length in H × N plants, compared to that of C × 0N plants, was less than the sum of the respective increases in H × 0N and C × N plants. The H and N treatments respectively increased the shoot dry weight at the transplanting time, and H × N plants showed the greatest shoot dry weight (\(P < 0.05\)). The H × 0N plants showed the lowest ratios of shoot dry weight to length in all the years, but the effects of H and N treatments on the ratios were not consistently observed through the years; the H × N plants showed the highest ratios in 2011 and 2012, and showed the lowest ratio in 2013. The H treatments did not markedly increase nitrogen content, and the nitrogen concentrations of seedlings subjected to H treatments were significantly lower than those in the control, excepting H × 0N plants in 2012 and 2013 (\(P < 0.05\)). The N treatments increased nitrogen content of plants, which was approximately double of that in 0N treatments on average of the years, and increased nitrogen concentration in seedlings. The plants grown in 2012 showed lower seedling

### Table 2. Seedling traits of Hokuriku 193 in heat \times N treatments.

| Yr   | Treatment | Leaf number | Seedling length (cm) | Seedling weight (mg plant\(^{-1}\)) | shoot dry weight / length (mg cm\(^{-1}\)) | Nitrogen concentration (%) | Nitrogen content (mg plant\(^{-1}\)) |
|------|-----------|-------------|-----------------------|-------------------------------------|------------------------------------------|-----------------------------|-------------------------------------|
| 2011 | C × 0N    | 4.0 b       | 6.5 c                 | 10.3 b                              | 1.60                                     | 4.3 c                       | 0.45 b                              |
|      | H × 0N    | 3.5 c       | 8.7 a                 | 13.8 a                              | 1.59                                     | 3.1 d                       | 0.43 b                              |
|      | C × N     | 4.2 a       | 7.5 b                 | 12.6 ab                             | 1.68                                     | 5.5 a                       | 0.69 a                              |
|      | H × N     | 3.9 b       | 9.0 a                 | 15.1 a                              | 1.67                                     | 4.9 b                       | 0.73 a                              |
| 2012 | C × 0N    | 3.4 c       | 10.9 d                | 9.1 b                               | 0.84 a                                   | 2.0 c                       | 0.18 c                              |
|      | H × 0N    | 3.3 c       | 13.8 b                | 9.4 b                               | 0.68 b                                   | 2.0 c                       | 0.19 c                              |
|      | C × N     | 4.1 a       | 12.3 c                | 10.5 b                              | 0.86 a                                   | 4.8 a                       | 0.51 b                              |
|      | H × N     | 3.6 b       | 14.6 a                | 13.8 a                              | 0.95 a                                   | 4.6 b                       | 0.63 a                              |
| 2013 | C × 0N    | 4.0 b       | 10.9 c                | 18.2 b                              | 1.67 a                                   | 3.0 c                       | 0.54 b                              |
|      | H × 0N    | 3.5 d       | 13.9 c                | 21.3 a                              | 1.33 ab                                  | 2.7 c                       | 0.58 b                              |
|      | C × N     | 4.3 a       | 13.0 b                | 19.9 ab                             | 1.53 ab                                  | 5.0 a                       | 1.00 a                              |
|      | H × N     | 3.7 c       | 14.3 a                | 21.6 a                              | 1.51 b                                   | 4.6 b                       | 0.99 a                              |

\(F\) values:

|          | Heat    | N        | Yr       | Heat × N | Heat × Yr | N × Yr | Heat × N × Yr |
|----------|---------|----------|----------|----------|----------|--------|--------------|
|          | 436**   | 859**    | 64**     | 2        | 138**    | 4      |              |
|          | 323**   | 175**    | 30**     | 3        | 2887**   | 802**  |              |
|          | 114**   | 1995**   | 371**    | 380**    | 280**    | 320**  |              |
|          | 10**    | 41**     | 0        | 5*       | 0        | 1      |              |
|          | 14**    | 8**      | 1        | 1        | 40**     | 3      |              |
|          | 10**    | 5*       | 3*       | 6**      | 98**     | 12**   |              |
|          | 26**    | 5*       | 5*       | 2        | 14**     | 5**    |              |

Different letters within each column denote significant difference at the 5% probability level within each column (Tukey’s test). * and ** indicate at 5% and 1% level of significance, respectively.
ages, shoot dry weights, and nitrogen content; this could be attributed to the shorter nursery period (Table 1). Significant \( H \times N \) interactions were observed in the leaf number, seedling length \( (P < 0.01) \) and the ratio of shoot dry weight to length \( (P < 0.05, \text{Table 2}) \). Significant interactions of \( H \times N \times Yr \) were also observed in most of the seedling traits. However, the effects of the \( H \) and \( N \) treatments on the seedling traits, excepting the ratio of shoot dry weight to the length, were consistent across the years.

The \( H \) treatment did not significantly affect plant traits at 3 wk after transplanting (Table 3). On the other hand, the \( N \) treatments increased plant length, shoot dry weight, and nitrogen content at 3 wk after transplanting \( (P < 0.05) \). The plants grown in 2012 showed greater plant length, shoot dry weight, and nitrogen content, probably because of the higher temperature (Table 1).

The days at which the first tiller appeared in most of the plants (initial tillering stage) were 43 d, 17 d, and 30 d after transplanting in 2011, 2012, and 2013, respectively. Although \( H \) treatments did not significantly increase the tiller number at the initial tillering stage, \( N \) treatments increased it \( (P < 0.05; \text{Table 4}) \). However, no significant difference was observed in panicle number at plant maturity among the nursery treatments. Tiller number and plant age (leaf number on the main tiller) were measured periodically for 3 wk after transplanting in 2012 (Fig. 1). Although there were differences among the nursery treatments in the initiation time of tillering and tiller number with respect to days after transplanting, there was no difference in the increases in tiller number with respect to plant age in leaf number.

### Discussion

The \( C \times 0N \) plants showed the shortest seedling length of 6.5 cm in 2011 when the air temperature in nursery

| Yr       | Treatment | Plant length (mm) | Shoot dry weight (mg plant\(^{-1}\)) | Nitrogen concentration (%) | Nitrogen content (mg plant\(^{-1}\)) |
|----------|-----------|-------------------|---------------------------------------|----------------------------|------------------------------------|
| 2011     | \( C \times 0N \) | 171               | 52.9                                  | 3.02                       | 1.60                               |
|          | \( H \times 0N \) | 173               | 50.3                                  | 2.94                       | 1.47                               |
|          | \( C \times N \) | 177               | 58.4                                  | 3.03                       | 1.77                               |
|          | \( H \times N \) | 168               | 58.1                                  | 2.85                       | 1.65                               |
| 2012     | \( C \times 0N \) | 316               | 406.1                                 | 3.73                       | 15.1                               |
|          | \( H \times 0N \) | 328               | 420.4                                 | 3.68                       | 15.4                               |
|          | \( C \times N \) | 335               | 407.8                                 | 3.74                       | 18.7                               |
|          | \( H \times N \) | 345               | 541.7                                 | 3.79                       | 20.6                               |
| 2013     | \( C \times 0N \) | 196               | 59.5                                  | 3.19                       | 1.90                               |
|          | \( H \times 0N \) | 193               | 60.1                                  | 3.14                       | 1.89                               |
|          | \( C \times N \) | 198               | 65.6                                  | 3.20                       | 2.11                               |
|          | \( H \times N \) | 204               | 75.9                                  | 3.28                       | 2.49                               |
| 2011−2013| \( C \times 0N \) | 228 b             | 173 b                                 | 3.31                       | 6.21 b                             |
|          | \( H \times 0N \) | 231 ab            | 177 b                                 | 3.25                       | 6.27 b                             |
|          | \( C \times N \) | 236 a             | 207 ab                                | 3.33                       | 7.51 ab                            |
|          | \( H \times N \) | 239 a             | 225 a                                 | 3.31                       | 8.23 a                             |

\( F \) values

|          | Heat       | N          | Yr          | Heat \(\times N\) | Heat \(\times Yr\) | N \(\times Yr\) | Heat \(\times N \times Yr\) |
|----------|------------|------------|-------------|-------------------|-------------------|----------------|-----------------------------|
|          | 1.3        | 0.5        | 0.6         | 0.5               | 8.5**             | 7.3*          | 0.4                         |
|          | 1294.9**   | 313.9**    | 76.7**      | 335.8**           | 8.3**             | 3.3           | 4.5*                        |

Different letters within each column denote significant difference at the 5% probability level within each column (Tukey’s test). * and ** indicate at 5% and 1% level of significance, respectively.
period was the lowest (Table 2). The length was shorter than 7.4 cm ensuring accurate machine transplanting (Kiriyama, 1991). Seedling length and weight varied with years. While the lighter seedling weights in 2012 were due to the short nursery period, the short seedling length and lighter seedling weight in 2011 were due to the lower temperature in the nursery period. Plant length and shoot dry weight at 3 wk after transplanting were markedly smaller in 2011 and 2013, compared to those in 2012. These results confirmed that air temperature significantly affects plant growth, as supported by the previous studies (Hiraoka et al., 1987; Redoña and Mackill, 1996; Ohsumi et al. 2012). In the present study, both H and N treatments contributed to elongation of seedlings under any climatic conditions, while the increase in seedling length was greater in the H-treated plants. Because H and N treatments additively influenced seedling length, the plants with combined treatment of H × N had the largest seedling length, which was 2.5 – 3.7 cm longer than in the C × 0N plants under different climatic conditions. Thus the combined treatment was an effective management method to stably elongate seedlings in the semi-dwarf cultivar Hokuriku 193.

Saito and Goto (1998) showed that the seedling length of japonica commercial cultivar Sasanishiki was over 7 cm after 5 d incubation at 28°C in the dark, while that of Hokuriku 193 was about 5 cm after the same treatment in the present study. This suggests that seedling elongation of Hokuriku 193 is inferior to that of japonica cultivars. H treatment was imposed until the plants reached an age of about 1.5 leaf number, which increased seedling length equaling the length sum of the third leaf and leaf sheath or fourth leaf and leaf sheath (Table 2). This is because temperature influenced rapidly growing organs (Sasaki,}

Table 4. Tiller number at initial tillering stage and panicle number at plant maturity.

| Treatment | Tiller number | Panicle number |
|-----------|---------------|---------------|
|           | m⁻²           | m⁻²           |
| 2011      |               |               |
| C × 0N    | 86            | 256           |
| H × 0N    | 101           | 246           |
| C × N     | 95            | 239           |
| H × N     | 102           | 246           |
| 2012      |               |               |
| C × 0N    | 80 b          | 256           |
| H × 0N    | 101 a         | 252           |
| C × N     | 111 a         | 242           |
| H × N     | 100 a         | 249           |
| 2013      |               |               |
| C × 0N    | 90 b          | 267           |
| H × 0N    | 90 b          | 278           |
| C × N     | 119 a         | 264           |
| H × N     | 110 ab        | 258           |
| 2011−2013 |               |               |
| C × 0N    | 86 b          | 259           |
| H × 0N    | 98 ab         | 258           |
| C × N     | 108 a         | 248           |
| H × N     | 104 a         | 251           |

Different letters within each column denote significant difference the at 5% probability level within each column (Tukey’s test). * indicates at 5% level of significance, respectively.

Fig. 1. Increases in tiller number with increasing days after transplanting and with increasing leaf number on the main tiller (plant age in leaf number). The treatments in the nursery period consist of heat treatments (C: control, H: heat) × nitrogen treatments (0N: no top dressing, N: top-dressing).
as well as the increased seedling age in leaf number (Table 2).

H treatments increased the shoot dry weight of seedlings (Table 2), although carbon assimilation did not occur during the treatments in the dark. Hoshikawa et al. (1995) reported that increase in shoot dry weight of growing nursling seedlings was closely associated with consumption of endosperm nutrients in japonica commercial cultivars. In our study, a rapid decrease in seed dry weight till 10 d after seed sowing was also observed in the H-treated plants (not shown). In addition, increased carbon assimilation due to longer leaf length after H treatments would be partly associated with the increased seedling weight. Similarly, increased seedling weight in N treatment would be resulted from enhanced carbon assimilation due to increased leaf length and number (Table 2), as well as the increased photosynthetic rate due to the high nitrogen concentration (Cook and Evans, 1983).

The H-treatment decreased nitrogen concentration in seedling but did not decrease nitrogen content compared to control plants (Table 2), suggesting that increase in biomass does not increase nitrogen content of plant. No increase in nitrogen content in H × 0N plants was not attributed to the limited amounts of nitrogen applied to the nursery boxes because variation in nitrogen content of seedlings was observed across the years. The N treatments increased the nitrogen content and concentration in seedlings irrespective of the climatic conditions (Table 2). The result is in agreement with that of a previous study showing that the amount of nitrogen fertilizer affects the nitrogen concentration in rice seedlings more strongly than temperature (Hoshino et al., 1971). Interestingly, the N-treated plants had a higher nitrogen content as well as biomass production early after transplanting with the same amounts of basal fertilizer applied for each year (Table 3). This suggests that the high nitrogen concentration in seedlings, and not high biomass productivity, increases nitrogen absorption after transplanting. The increase in nitrogen content after transplanting in N-treated plants might be attributed to the increased root number, because increased shoot dry weight and high nitrogen concentration in seedlings are known to enhance the rooting ability (Honda and Usuda, 1959).

H treatment did not reduce early biomass production (dry weight at 3 wk after transplanting) (Table 3) and tillering after transplanting (tiller number at initial tillering stage) (Table 4), although larger seedlings lead to poor plant growth early after transplanting in japonica commercial cultivars (Kusutani, 1986). This might be attributed to the lack of marked change in the ratio of seedling weight to the length in the present study, because the increase in shoot dry weight were accompanied by increases in seedling length (Table 2). However, the H × 0N plants showed the lowest ratios of shoot dry weight to length in all the years, and the ratios decreased at high temperature in 2012. These results warn that excessive heating in the nursery period might inhibit early growth after transplanting, through the further decreased ratio of shoot dry weight to length, under low temperature conditions with early transplanting time, while the effects of the decreased ratios on early growth after transplanting were not evident in 2012 because of the higher temperature (Table 1). The high nitrogen concentration in the seedlings subjected to N treatments led to vigorous biomass production (Table 3) and tillering early after transplanting (Table 4), as has been reported for japonica (Kitano, 1989) and semi-dwarf indica cultivars (Ehara et al., 1992). Thus, combining H and N treatments is advantageous not only to elongate seedlings but also to improve early plant growth after transplanting.

N treatment improved early tillering, but did not increase the panicle number (Table 4). Ehara et al. (1992) reported that seedlings with a high nitrogen concentration generate first tiller at a lower node than control plants, and that increased tillers from lower nodes increases panicle number and yield when plants are grown with small amounts of basal fertilizer. In the present study, increase in tiller number with respect to plant age in leaf number was not improved with N treatments (Fig. 1), which suggests that the first tiller did not emerge at the lower nodes. In addition, larger amounts of basal fertilizer were applied in our study. However, since Horai et al. (2013) showed that panicle number is reduced by poor early biomass production with slow leaf expansion at low temperature, improving early tillering with N treatment would be beneficial for ensuring sufficient panicle number for stable and high grain yield under unfavorable conditions.

In conclusion, we showed that H and N treatments significantly increased seedling length under any climatic conditions in indica-dominant semi-dwarf cultivar Hokuriku 193, and that the increase in seedling length with H treatment was greater than that with N treatment. Enforced elongation of seedlings with H treatment did not reduce early biomass production and tillering after transplanting in Hokuriku 193. On the other hand, N treatment improved both early biomass production and tillering after transplanting. Because H and N treatments additively influenced the plant growth, combined treatment of H × N can be useful for improving seedling length and early growth after transplanting in semi-dwarf cultivars.
Acknowledgements

We would like to thank Makoto Hirokawa, Hiroyuki Nakagawa, Chinatsu Saito, and the staffs of the Hokuriku Research Center, NARO, for their technical assistance.

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