Cross-locational experiments to reveal yield potential and yield-determining factors of the rice cultivar ‘Hokuriku 193’ and climatic factors to achieve high brown rice yield over 1.2kg m\(^{-2}\) at Nagano in central inland of Japan

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\textbf{ABSTRACT}
Understanding the yield potential and yield-determining factors of recent high-yielding cultivars is essential for further increasing rice yield. In this study, a cross-locational field experiment was conducted across 3 years using ‘Hokuriku 193’ (H193), a high-yielding cultivar, at four sites including one in Nagano Prefecture, which is the highest-yielding region in Japan. The highest mean yields of 3 years, 1214 g m\(^{-2}\) for brown rice grains and 1586 g m\(^{-2}\) for rough grains, were recorded at the Nagano site. The yields from the 17 environments were strongly correlated with spikelet number per square meter while percentage of filled grain was relatively stable, suggesting that sink capacity is the primary determining factor for grain yield of H193. The climatic factors for high spikelet number at the Nagano site can be explained by the high cumulative radiation before heading associated with longer duration until heading by low night temperature. In addition, a large increase in shoot dry weight during grain filling (\(\Delta W\)) and high radiation use efficiency (\(\Delta W/\text{rad}\)) at the Nagano site could satisfy large source demand by the large sink size. The high \(\Delta W/\text{rad}\) at the Nagano site associated with low night temperature. This study demonstrated high yield potential of H193 and revealed an environment that achieves extra-high yields in H193, which provided insight to attain further increase in rice yield.

\textbf{Introduction}

Rice (\textit{Oryza sativa} L.) is one of the world’s most important crops (FAO, Godfray et al., 2010). Many high-yielding cultivars have been developed to feed the increasing global population. Understanding the yield potential and yield-determining factors of these cultivars is essential to reveal breeding targets and achieve further increases in yield. In China, a project for development of ‘super rice’ (rice with a super-high yield potential) was launched in 1996 (Wang & Peng, 2017). Many super rice cultivars were developed exploiting intersubspecific (\textit{indica} and \textit{japonica}) heterosis. Some of these cultivars

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have produced grain yields of more than 1500 g m⁻² (Amano et al., 1996; Katsura et al., 2008; Chang et al., 2016). In Japan, many high-yielding inbred cultivars have been developed from hybridization of indica and japonica cultivars, and the majority have a predominantly indica genetic background (Yonemaru et al., 2014). Among these indica-dominant cultivars, ‘Hokuriku 193’ (H193) and ‘Takanari’ are the highest-yielding cultivars grown in relatively warm regions in Japan, with more than 1100 g m⁻² recorded for brown rice grains (corresponding to more than 1400 g m⁻² if the ratio of rough grain yield to brown rice yield was 1.29) (Nagata et al., 2016).

The grain yield of rice is determined by the sink capacity (total number of spikelets per unit area x filled grain weight) and the filled grain ratio (percentage of grains that are filled). Yoshinaga et al. (2013) reported that the main factor for the high yield of recently developed high-yielding cultivars was large sink capacity, suggesting the importance of enlarging sink capacity as breeding target. On the other hand, the filled grain ratios of many cultivars often showed negative correlation with sink capacities (Yagioka et al., 2021; Yoshinaga et al., 2013) and some studies using near isogenic or mutant lines reported that increasing sink capacity did not substantially improve yield (Fukushima et al., 2017; Nakano et al., 2017; Ohsumi et al., 2011). In these cases, sink capacities did not determine grain yield. Therefore, understanding whether sink capacity or filled grain ratio (source abundance and/or translocation efficiency) limits yield of each cultivar under diverse conditions was important to achieve further increases in grain yield.

Nagano Prefecture, located in central inland of Japan, is the highest-yielding region for rice production in Japan. The mean farmers’ yield in the most recent 5 years (2016–2020) in Nagano Prefecture (619 g m⁻²) was the highest among Japanese prefectures, whereas the Japanese average brown rice yield was 533 g m⁻² (Ministry of Agriculture, Forestry and Fisheries, 2021). Murata (1964) analyzed the statistical nation-wide yield data published by Ministry of Agriculture, Forestry and Fisheries of Japan. He suggested that the high yield in cool regions, including Nagano Prefecture, partly depends on the high radiation use related to low temperature during grain filling stages. Horie et al. (1997) conducted cross-localational experiment at the sites including Nagano Prefecture using ‘Koshihikari’, a standard Japanese cultivar, and reported that high dry matter production at Nagano Prefecture compared with Kyoto Prefecture was attributed to higher cumulative radiation during the growing season. However, to the best of our knowledge, there are no precise studies investigating the potential yield and yield determining process associated with climatic variables in the sites including Nagano Prefecture at high-yielding level higher than 1000 g m⁻² for brown rice grains using recently developed high-yielding cultivars under high fertilized nitrogen levels.

In the present study, a cross-localational field experiment was conducted at four sites, including one in Nagano Prefecture, in 3 years under high nitrogen fertilizer application rate to reveal the yield potential of H193, selected as a representative high-yielding cultivar, under a favorable environment. Based on the relationships between yield traits related to sink and source capacity and climatic variables before and after heading stage, yield-determining factors of H193 in each environment and the climatic factors for achieving high yielding at Nagano Prefecture were discussed.

### Materials and methods

#### Plant materials and growth conditions

The rice cultivar ‘Hokuriku 193’ (H193) and ‘Nipponbare’ were planted in 17 and 11 environments, respectively, at four sites (listed in Table 1) across 3 years with some management differences (Supplemental Table S1). H193 is a Japanese high-yielding indica inbred cultivar. Nipponbare is a standard japonica inbred cultivar and was used as reference. The data for yield, yield components, shoot dry weight, and stem non-structural carbohydrate (NSC) content at the Ibaraki site in 2016 were identical to those reported by Okamura et al. (2018).

Seedlings (20–24 days old) were transplanted to paddy fields on the days listed in Supplemental Table S1. The plants were grown at a density of 22.2 hills m⁻² (spacing of 15 cm x 30 cm) except in the Aichi site where a density was 23.9–24.7 hills m⁻². The plot sizes were 5.3–15.0 m⁻². The plots were arranged in a randomized block design with three replicates except in the Aichi site where only single plot (>32.0 m²) was employed to accommodate three replicated samplings. Climatic

| Site name | City                  | Latitude (N) | Longitude (E) | Altitude above sea level (m) |
|-----------|-----------------------|--------------|---------------|------------------------------|
| Ibaraki   | Tsukubamirai, Ibaraki, Japan | 36°00’ | 140°02’ | 10                          |
| Nagano    | Suzaka, Nagano, Japan  | 36°39’ | 138°17’ | 334                         |
| Hiroshima | Fukuyama, Hiroshima, Japan | 34°50’ | 133°39’ | 1                           |
| Aichi     | Togo, Aichi, Japan     | 35°11’ | 137°09’ | 64                          |
variables at the four sites were measured by a weather station at each experimental station except for the Aichi site. The weather data for the Aichi site was obtained from the nearest weather station of Japan Meteorological Agency (2021), located in Nagoya city. Cumulative temperature and radiation for the period from the day X to day Y were calculated by sum of daily mean values from the day X to the 1 day before Y. The soil in the 10-cm-deep plow layer was sampled in each field of each site before fertilization in 2016. The soil chemical properties shown in Supplemental Table S2 were measured by a commercial service (Katakura and Co-op Agri Corporation, Tokyo, Japan).

Yield and yield components

At maturity, when approximately 85% of grains became yellow, 40–80 grains were air-dried for more than 2 weeks. The yield and yield components were measured in accordance with the procedure of Okamura et al. (2018). In brief, whole grains with the hull attached were weighed to determine the rough grain yield. Half of the rough grains were hulled and weighed to obtain the rough (whole) brown rice yield. The rough brown rice grains were sieved with a grain sorter with a sieve size of 1.6 mm, the retained grains were weighed to calculate the actual brown rice yield. Rough grain yield, rough brown rice yield, brown rice yield, and 1000-grain weight were adjusted to 15% (w/w) moisture content. Sink capacity was estimated as following formula based on Yoshinaga et al. (2013):

Sink capacity = (spikelets number × 1000-grain weight)/1000 (1)

Dry weight, non-structural carbohydrate content, and nitrogen content

At full heading and at maturity, 10–12 successive hills per plot were harvested. The heading date and full-heading date were defined as the dates when approximately 50% and 80%, respectively, of panicles had emerged. The sampling dates for each stage are shown in Supplemental Table S1. The samples were divided into panicles, leaf sheaths + culms (stems), and leaf blades, and then weighed in accordance with the method of Okamura et al. (2018). The divided samples were powdered in a mill for measurement of stem NSC content and shoot nitrogen content. The contents of starch, sucrose, glucose, and fructose in the powdered samples were measured in accordance with the methods of Okamura et al. (2016) using glucoamylase (Toyobo, Osaka, Japan), the F-kit #716,260 (J.K. International, Tokyo, Japan), and a microplate reader (Epoch 2, BioTek, Winooski, VT, USA). The NSC content was calculated as the sum of the contents of these carbohydrates.

The nitrogen content was measured using an elemental analyzer (Flash EA 1112, Thermo Fisher Scientific, Waltham, MA, USA or JM3000CN, J-Science Lab, Kyoto, Japan).

The increase in shoot dry weight during grain filling (ΔW) and potential source adjusted to 15% (w/w) moisture content, were calculated as following formula based on Morita and Nakano (2011):

\[ \Delta W = \text{shoot dry weight at maturity} - \text{shoot dry weight at full heading} \]

Potential source = (stem NSC content at full heading + ΔW) × 1.15 (3)

The spikelet number per shoot dry weight at full heading (Spik/Wₚₜₜ), the harvest index, the spikelet number per shoot nitrogen content at full heading (Spik/N), the brown rice yield per radiation until maturity (Y/rad), the shoot dry weight per radiation until maturity (Wₚₜₜ/rad), the spikelet number per radiation until heading (Spik/rad), the shoot dry weight per radiation until full heading (Wₚₜₜ/rad), ΔW per radiation during grain filling (ΔW/rad), and shoot nitrogen content per radiation until full heading (Nₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑ electorate

Dry weight, non-structural carbohydrate content, and nitrogen content

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site as fixed factors, respectively. The correlation coefficient and regression line were calculated using Microsoft Excel.

Results

Grain yield and yield components

Yield and yield components of Hokuriku 193 (H193) in the 17 environments at four sites for 3 years with some management differences were investigated (Table 2). Rough grain yield ranged from 1020 to 1708 g m$^{-2}$, and brown rice yield ranged from 757 to 1305 g m$^{-2}$, at the Ibaraki site in 2018 and the Nagano site in 2016, respectively. The spikelet number per square meter was strongly correlated with brown rice yield (Figure 1a). Conversely, the percentage of filled grains was not correlated with brown rice yield and fell within a relatively narrow range (Figure 1b and Table 2): from 78.9% at the Nagano site in 2018 to 94.1% at Aichi in 2017. Although only 11 of 17 environments, we investigated the yield and yield component of Nipponbare, a standard japonica cultivar, and the relationships of brown rice yield with spikelet number and percentage of filled grains were shown (Figure 1c,d). Both spikelet number and percentage of filled grains were significantly correlated with brown rice yield in Nipponbare unlike H193.

To compare yield under different management practices in H193, the panicle number under 16 g m$^{-2}$ total nitrogen fertilizer content with one plant per hill (16 N) was significantly lower than that under 19 g m$^{-2}$ with three plants per hill (19 N) at the Ibaraki site in 2018–2019 (Table 3). However, no significant differences in spikelet number per square meter and yields were observed because the spikelet number per panicle was higher under 16 N. Similarly, although panicle number and spikelet number per square meter under 38 g m$^{-2}$ with three plants per hill (38 N) were significantly higher than those under 19 N at the Nagano site in 2018–2019, no significant differences in yields were observed because the percentage of filled grains tended to be lower under 38 N. Early transplanting increased yield at the Ibaraki site in 2018–2019 owing to the higher spikelet number per panicle, heavier 1000-grain weight, and higher percentage of filled grains.

To examine regional differences in yield, the yield and yield components at sites in Ibaraki, Nagano, and Hiroshima prefectures, where experiments were conducted for 3 years, were compared (Table 2). In this analysis, results under 19 N were used except for those of the Ibaraki site in 2016, for which results under 16 N were used. Given that the yield and yield components, except for panicle number and spikelet number per square meter, did not differ either under 16 N or 19 N.
as already mentioned (Table 3), the management practice had little or no effect on the results of analysis. Yield and all yield components except spikelet number per panicle showed significant differences among sites (Table 2). Mean rough grain and brown rice yield were highest at the Nagano site, followed by the Hiroshima site, and lowest at the Ibaraki site. Panicle number, spikelet number per square meter, and sink capacity were higher in the same order as that observed for brown rice yield. In contrast, 1000-grain weight was highest at the Hiroshima site, followed by the Nagano site, and lowest at the Ibaraki site.

**Yield-related traits**

Yield-related traits of H193 in the 17 environments are shown in Tables 4–6. The regional differences were compared in the same manner as the yield and yield components. The shoot dry weight at full heading and maturity were highest at the Nagano site (Table 4). The increase in shoot dry weight during grain filling (ΔW), potential source, sum of ΔW and stem NSC contents at full heading, were higher in the same order as those observed for brown rice yield: Nagano, Hiroshima, and Ibaraki. The spikelet number per shoot dry weight at full heading (Spik/WFH) and harvest index were not highest at the Nagano site. The shoot nitrogen content at full heading and maturity were higher at the Nagano site than other two sites and there was no regional difference in spike number per nitrogen content at full heading (Spik/N) (Table 5). The leaf dry weight and leaf nitrogen concentration at the Nagano site were not remarkably higher than those at the other two sites (Table 6).

The relationship between potential sources and sink capacities in H193 is shown in Figure 2. The potential sources were higher than the sink capacities at the Nagano site in all years and fertilized nitrogen levels (black and white square symbols).
Table 3. Comparison of yield under different management practices.

| Year | Site/ planting time | Total nitrogen fertilizer (g m⁻²) | Panicle number (m⁻²) | Spikelets per panicle | Spikelet number (×10⁴ m⁻²) | Thousand-grain weight (g) | Sink capacity (g m⁻²) | Percentage of filled grains (%) | Rough grain yield (g m⁻²) | Rough brown rice yield (g m⁻²) | Brown rice yield (g m⁻²) |
|------|-------------------|-----------------------------------|----------------------|----------------------|-----------------------------|--------------------------|----------------------|-------------------------------|-------------------------|-----------------------------|------------------------|
| 2018– Ibaraki 16 | 280 | 170 | 47.3 | 22.3 | 1056 | 83.4 | 1150 | 897 | 881 |
| 2019 Ibaraki 19 | 335 | 140 | 46.9 | 22.0 | 1034 | 81.8 | 1113 | 861 | 846 |
| 2018– Nagano 19 | 343 | 173 | 59.3 | 23.3 | 1382 | 84.6 | 1525 | 1191 | 1169 |
| 2019 Nagano 38 | 383 | 166 | 63.4 | 23.3 | 1480 | 81.7 | 1587 | 1234 | 1209 |
| 2018– Ibaraki 19 | 335 | 140 | 46.9 | 22.0 | 1033 | 81.8 | 1113 | 861 | 846 |
| 2019 Ibaraki Apr. 19 | 346 | 157 | 54.1 | 22.3 | 1207 | 86.9 | 1355 | 1059 | 1045 |

Sink capacity = (spikelet number × thousand-grain weight)/1000; Percentage of filled grains = filled spikelet number/total spikelet number. Values are means from 2 years. *P < 0.05, **P < 0.01, ***P < 0.001 (two-way ANOVA).

Table 4. Dry matter production-related traits.

| Year | Site/ planting time | Total nitrogen fertilizer (g m⁻²) | Full heading Maturity | ΔW (g m⁻²) | Stem NSC content at full heading (g m⁻²) | Potential source (g m⁻²) | Spik/WFH (g⁻¹) | Harvest index (%) |
|------|-------------------|-----------------------------------|----------------------|------------|------------------------------------------|--------------------------|----------------|-----------------|
| 2016 | Ibaraki 16    | 1511° | 2030° | 519 | 275° | 934 | 30.2 | 37.9 |
|      | Nagano 19     | 2257 | 3660 | 1393 | 270 | 1957 | 28.4 | 30.4 |
|      | Hiroshima 19  | 1814 | 2489 | 675 | 223 | 1517 | 30.2 | 38.2 |
|      | Aichi 19      | 1767 | 2539 | 772 | 202 | 1146 | 27.1 | 34.4 |
| 2017 | Ibaraki 16    | 1743 | 2512 | 769 | 134 | 1062 | 28.0 | 31.6 |
|      | Ibaraki 19    | 1786 | 2773 | 987 | 156 | 1345 | 28.3 | 28.6 |
|      | Nagano 19     | 1666 | 2817 | 1151 | 163 | 1546 | 34.3 | 34.3 |
|      | Nagano 38     | 1819 | 3230 | 1412 | 160 | 1849 | 34.2 | 31.9 |
|      | Hiroshima 19  | 1545 | 2493 | 948 | 162 | 1306 | 36.9 | 36.1 |
|      | Aichi 19      | 2070 | 2561 | 490 | 231 | 849 | 19.7 | 28.9 |
|      | Ibaraki – Apr. 19 | – | 2752 | – | – | – | – | 35.1 |

| Mean of three years | Ibaraki 19 | 1784 | 2355 b | 572 c | 240 a | 955 c | 26.4 | 32.7 b |
|                     | Nagano 19  | 1988 a | 3364 a | 1376 a | 235 a | 1895 a | 30.9 b | 31.0 b |
|                     | Hiroshima 19 | 1643 c | 2458 b | 815 b | 188 b | 1181 b | 34.2 a | 37.9 a |

ANOVA

| Year (Y) | Site (S) | Y × S |
|----------|----------|-------|
| *** n.s. | *** n.s. | *** n.s. |

ΔW = shoot dry weight at maturity – shoot dry weight at full heading; NSC = non-structural carbohydrate; Potential source = (ΔW + Stem NSC content at full heading) × 1.15; Spik/WFH = spikelet number/shoot dry weight at full heading; Harvest index = (brown rice yield × 0.85/shoot dry weight at maturity) × 100; °The data from Okamura et al. (2016). °The results under the 16 g m⁻² application rate were used in 2016. Values are means (n = 3). Different lower-case letters within a column indicate a significant difference among sites (P < 0.05, Tukey’s test after two-way ANOVA); *P < 0.05, **P < 0.01, ***P < 0.001.

Growth duration and climatic variables

The mean growth duration at the three sites in 3 years are shown in Table 7. The total growth duration was longer at the Nagano site than the other two sites by the longer days to heading and grain filling duration.

The climatic variables at the three sites in 3 years are shown in Table 8. The daily mean temperature was the lowest at the Nagano site through growing period mainly due to lower minimum temperature. The mean temperature after heading was remarkably higher at the Hiroshima site than the other two sites. The mean...
radiation until heading was higher at the Nagano site, while that after heading had no significant difference from the other two sites. The cumulative radiations until and after heading were both higher at the Nagano site than the other two sites mainly due to extended duration of the grain filling.

**Relationships between climatic variables and yield-related traits**

The relationships between climatic variables and spikelet number in the 17 environments are shown in **Figure 3**. The daily mean and minimum temperature until heading showed significantly negative correlation with spikelet number. The daily mean and cumulative radiation until heading showed significantly positive correlation with spikelet number. The days to heading also negatively correlated with minimum temperature until heading (**Figure 4**). During grain filling, increase of shoot dry weight (ΔW) showed negative correlation with mean and minimum temperature and positive correlation with cumulative radiation (**Figure 5**). There was no significant correlation between ΔW and daily mean radiation.

As indices for radiation use efficiency, the ratios of yield and yield-related traits to cumulative incident radiation and shoot nitrogen content were calculated and regional difference at the tree site was shown in **Table 9**. Among the three sites, the W_{M}/rad and ΔW/rad were highest at the Nagano site. Y/rad and Spik/rad were higher at the Hiroshima site than at the other two sites. The W_{FH}/rad was highest at the Ibaraki site, followed by the Hiroshima site, and lowest at the Nagano site, which was the opposite order observed for yield. No significant differences in N_{FH}/rad was observed. The relationships between minimum temperature during grain filling and ΔW/rad in the 17 environments are shown in **Figure 6**. They showed significantly negative correlation.

**Discussion**

**Highest yield of ‘Hokuriku 193’: 1708 g m⁻² for rough grain yield**

The highest yields of 1305 g m⁻² for brown rice yield and 1708 g m⁻² for rough grain yield were recorded at the Nagano site in 2016 (**Table 2**). In previous studies, the highest yields in Japan recorded in a field experiment were 1173 g m⁻² for ‘Takanari’ and 1131 g m⁻² for H193

| Year | Site/ planting time | Total nitrogen fertilizer (g m⁻²) | Shoot nitrogen concentration (%) | Shoot nitrogen content (g m⁻²) | Spik/N (g⁻¹) |
|------|---------------------|-----------------------------------|---------------------------------|-----------------------------|-------------|
|      |                     |                                   | Full heading | Maturity | Full heading | Maturity |             |
| 2016 | Ibaraki             | 16                                | 0.89         | 1.02     | 13.4        | 20.7      | 3399        |
|      | Nagano              | 19                                | 1.10         | 1.02     | 24.8        | 37.1      | 2578        |
|      | Hiroshima           | 19                                | 1.13         | 0.86     | 20.5        | 21.5      | 2670        |
|      | Aichi               | 19                                | 0.94         | 1.01     | 16.7        | 25.7      | 2847        |
| 2017 | Ibaraki             | 16                                | 1.32         | 1.03     | 23.0        | 26.0      | 2113        |
|      | Nagano              | 19                                | 1.21         | 0.93     | 21.6        | 25.9      | 2340        |
|      | Hiroshima           | 19                                | 1.27         | 0.78     | 21.2        | 21.9      | 2695        |
|      | Nagano              | 38                                | 1.49         | 0.96     | 27.2        | 31.0      | 2289        |
|      | Hiroshima           | 19                                | 1.22         | 0.93     | 18.8        | 23.2      | 3023        |
|      | Aichi               | 19                                | 1.16         | 0.90     | 24.0        | 23.0      | 1688        |
| 2018 | Ibaraki             | 16                                | 0.88         | 0.67     | 17.2        | 14.1      | 2671        |
|      | Nagano              | 19                                | 1.03         | 0.75     | 21.2        | 17.1      | 2038        |
|      | Hiroshima           | 19                                | 1.16         | 0.78     | 23.7        | 28.3      | 2596        |
|      | Aichi               | 19                                | 1.17         | 0.94     | 26.2        | 35.3      | 2466        |
|      | Ibaraki – April     | 19                                | 1.03         | 0.58     | 19.9        | 15.3      | 2455        |
|      | Nagano              | 19                                | 1.18 ab       | 0.86 a   | 23.2 a       | 29.1 a    | 2638 n.s.   |
|      | Hiroshima           | 19                                | 1.25 a        | 0.90 a   | 20.4 ab      | 22.2 b    | 2747 n.s.   |

ANOVA

| Year (Y) | Site (S) | Y x S |
|----------|----------|-------|
| *        | n.s.     | *     |
| **       | n.s.     | ** n.s. |
| Y x S   | n.s.     | *** n.s. |

Spik/N = spikelet number/shoot nitrogen content at full heading. *The results under the 16 g m⁻² application rate were used in 2016. Values are means (n = 3). Different lower-case letters within a column indicate a significant difference among sites (P < 0.05, Tukey’s test after two-way ANOVA). *P < 0.05, **P < 0.01, ***P < 0.001.
Table 6. Leaf nitrogen concentration and content at full heading.

| Year | Site/planting time | Total nitrogen fertilizer content (g m⁻²) | Leaf dry weight (g m⁻²) | Leaf nitrogen concentration (%) | Leaf nitrogen content (g m⁻²) |
|------|--------------------|------------------------------------------|------------------------|--------------------------------|-------------------------------|
| 2016 | Hiroshima          | 19                                       | 437                    | 2.58                            | 11.3                          |
|      | Nagano             | 19                                       | 466                    | 2.27                            | 10.6                          |
|      | Aichi              | 19                                       | 387                    | 2.02                            | 8.0                           |
|      | Ibaraki            | 19                                       | 437                    | 2.58                            | 11.3                          |
|      | Ibaraki-3 Apr.     | 19                                       | 405                    | 2.07                            | 8.4                           |
| 2017 | Nagano             | 19                                       | 437                    | 2.58                            | 11.3                          |
|      | Ibaraki            | 19                                       | 474                    | 2.36                            | 11.3                          |
|      | Ibaraki-3 Apr.     | 19                                       | 426                    | 2.28                            | 9.8                           |
|      | Nagano             | 19                                       | 474                    | 2.44                            | 11.6                          |
|      | Ibaraki            | 19                                       | 414                    | 2.84                            | 11.8                          |
|      | Ibaraki-3 Apr.     | 19                                       | 426                    | 2.28                            | 9.8                           |
| Means of three years | Nagano          | 19                                       | 430 n.s.               | 2.49 a                          | 10.6 n.s.                     |
|      | Hiroshima          | 19                                       | 422 n.s.               | 2.55 a                          | 10.8 n.s.                     |
| ANOVA | Site (S)          | **                                       | *                      | n.s.                            |                              |
|       | Y X S             | ***                                      | *                      | n.s.                            |                              |

*The results in 16 g m⁻² were used in 2016. Values are means (n = 3). The difference letters indicate the significant differences among site (P < .05, Tukey’s test after two-way ANOVA). *P < 0.05, **P < 0.01, ***P < 0.001.

Table 7. Mean growth duration at the three sites as mean values of 3 years.

| Site     | Total nitrogen fertilizer (g m⁻²) | Total growth duration (d) | Days to heading (d) | Grain filling duration (d) |
|----------|-----------------------------------|--------------------------|---------------------|----------------------------|
| Ibaraki  | 19*                               | 141 b                    | 87.8 b              | 53.6 b                     |
| Nagano   | 19                                | 161 a                    | 97.1 a              | 63.9 a                     |
| Hiroshima| 19                                | 135 b                    | 83.8 b              | 51.6 b                     |

Total growth duration is days from transplanting to maturity. Days to heading is days from transplanting to heading. Grain filling duration is days from heading to maturity. *The results in 16 g m⁻² were used in 2016. Values are means of three years. Different lower-case letters within a column indicate a significant difference among sites (P < 0.05, Tukey’s test after one-way ANOVA).

for rough brown rice yield at Fukuyama city, Hiroshima Prefecture (Nagata et al., 2016), to the best of our knowledge. The present record of H193 in Nagano Prefecture was higher than all the yield records obtained in selected previous studies (Table 10). These studies used the different cultivars from the present study or applied lower nitrogen fertilizer. Therefore, it is considered that the present highest yield at Nagano in Japan in this study was achieved by using H193 partly due to high fertilizer input.

Many field experiments have been conducted in various regions regarded as a high-yielding region for rice production worldwide using inbred and hybrid cultivars and the results are summarized in Table 10. The present result at the Nagano site in 2016 with H193 is the second-highest record among these studies, suggesting high yield potential of H193, an inbred cultivar, under favorable conditions.

**Yield determining factor for H193**

While it was reported that the main factor for the high yield of recently developed high-yielding cultivars was large sink capacity (Yoshinaga et al., 2013), some recently developed cultivars with a large sink capacity show poor grain-filling even if the source abundance is adequate as a result of low translocation efficiency of carbohydrate (Okamura et al., 2018; 2021). It is therefore important to understand the determinant mechanism of the percentage of filled grains in each cultivar. In this study with H193, the spikelet number per square meter was strongly correlated with brown rice yield but not with the percentage of filled grains (Figure 1a,b). Meanwhile, both spikelet number and the percentage of filled grains showed moderate correlation with brown rice yield in Nipponbare, a standard japonica cultivar.
suggesting the grain filling of H193 was more stable than that of Nipponbare and the sink capacity is the primary limiting factor for grain yield of H193.

To investigate the reason for the stable grain filling in H193, the relationships between sink capacity (product of spikelet number and single grain weight) and
potential source for grain filling (sum of ΔW and stem NSC content at heading) are shown in Figure 2. The potential sources were adequate to sink capacity in most of the environments, suggesting the stable grain filling was supported by a high source ability of H193. High biomass production of H193 obtained in this study with the highest value 3650 g m⁻² recorded in 2016 in the Nagano site reflected a high potential of source ability of H193. High source ability with high photosynthetic activity and translocation of NSC is generally observed in indica-dominant high-yielding cultivars (Hirasawa et al., 2010; Ohsumi et al., 2007; Yoshinaga et al., 2013). Grain filling percentage was low <85% in some years and locations for unclarified reasons (Table 2). A further investigation is required to clarify the possibility of involvement of sterility caused by low assimilation around flowering (Kobata et al. 2017).

**The reason for high spikelet number of H193 at Nagano prefecture**

Although the previous study with Koshihikari showed higher dry matter accumulation in Nagano Prefecture was responsible for higher cumulative radiation during growing season (Horie et al., 1997), the effect of radiation and other climatic factors during growing periods on yield determining process remain ununderstood, especially in recently developed high-yielding cultivars. In the present study with H193, not only cumulative radiation until maturity, but also the shoot dry weight at maturity per radiation (W₆/μ) were the highest at the Nagano site (Tables 8 and 9). This result suggests that the reason for high yielding of H193 at the Nagano site cannot be explained only by cumulative radiation during the whole growing season. Since we showed a primary yield-determining factor for H193 is the sink capacity in the above section, we analyzed the relationships between sink production and the climatic variables until heading separately from grain-filling period.

Until heading, mean daily temperature was lowest at the Nagano site mainly due to the lowest minimum temperature (Table 8). Mean and cumulative radiation were highest at the Nagano site. These climatic variables were closely correlated with spikelet number in the 17 environments (Figure 3). The correlation coefficient was higher in cumulative radiation than mean radiation (Figure 3c,d), and the spikelet number per cumulative radiation until heading (Spik/μ) at the Nagano site

![Figure 4. Relationship between mean minimum temperature until heading and days to heading. Values are means (n = 3). ***p < 0.001.](image-url)
Figure 5. Relationship between climatic variables and ΔW. ΔW is the increase of shoot dry weight during grain-filling. Values are means (n = 3). **P < 0.01, ***P < 0.001.

Table 9. Indices for radiation use efficiency at the three sites as mean values of 3 years.

| Site/planting time | Total nitrogen fertilizer (g m⁻²) | Y/rad (g MJ⁻¹) | Wₘ/rad (g MJ⁻¹) | Spik/rad (MJ⁻¹) | Wₜₜ/rad (g MJ⁻¹) | ΔW/rad (g MJ⁻¹) | Nₜₜ/rad (mg MJ⁻¹) |
|--------------------|-------------------------------------|----------------|-----------------|----------------|-----------------|----------------|-----------------|
| Ibaraki            | 19                                  | 0.380 c         | 1.03 b          | 29.3 b         | 1.08 a          | 0.98 b         | 11.3 n.s.       |
| Nagano             | 19                                  | 0.404 b         | 1.12 a          | 28.9 b         | 0.92 c          | 1.79 a         | 10.8 n.s.       |
| Hiroshima          | 19                                  | 0.465 a         | 1.04 ab         | 35.4 a         | 1.02 b          | 1.22 b         | 12.7 n.s.       |
| ANOVA              |                                     | *** n.s.        | ***             | ***            | ***             | *** n.s.       |
| Year (Y)           | ***                                 | ***             | ***             | ***            | ***             | *** n.s.       |
| Site (S)           | ***                                 | *               | ***             | ***            | ***             | *** n.s.       |
| Y × S              | ***                                 | ***             | *               | ***            | ***             | *** n.s.       |

Y/rad = brown rice yield/cumulative radiation until maturity; Wₘ/rad = shoot dry weight at maturity/cumulative radiation until maturity; Spik/rad = spikelet number/cumulative radiation until heading; Wₜₜ/rad = shoot dry weight at full heading/cumulative radiation until full heading; ΔW/rad = ΔW/cumulative radiation during grain filling; Nₜₜ/rad = shoot nitrogen content at full heading/cumulative radiation until full heading. *The results under the 16 g m⁻² application rate were used in 2016. Different lower-case letters within a column indicate a significant difference among sites (P < 0.05, Tukey’s test after two-way ANOVA). **P < 0.01, ***P < 0.001, n.s. not significant.
Table 10. Summary of previous studies at high-yielding regions including Nagano Prefecture.

| Year | Site            | Grain yield (g m⁻²) | Cultivar name | Cultivar type | Total nitrogen fertilizer content (g m⁻²) | Mean temperature (°C) | Mean Radiation (MJ m⁻² day⁻¹) | Method for Filled-grain selection | Reference  |
|------|-----------------|---------------------|---------------|---------------|------------------------------------------|-----------------------|-------------------------------|-----------------------------------|------------|
| 2016 | Nagano, Japan   | 1645                | H193          | indica        | 19                                       | 22                    | 19                            | calculated from rough grain specific gravity | Present study |
| 1992 | Nagano, Japan² | 968                 | Koshihikari   | japonica      | 20                                       | 20⁴                    | 16                            | calculated from rough grain specific gravity | (>1.06 g m⁻³) |
| 2002 | Nagano, Japan² | 919                 | Takanari      | indica        | no data                                  | 22⁴                   | no data                       | calculated from rough grain specific gravity | Yoshi & Horie, 2009 |
| 2008–2010¹ | Nagano, Japan | 1333               | H193          | indica        | 10                                       | 21⁹                   | 19⁹                          | calculated from brown rice specific gravity | Sakai et al., 2011 |
| 2017 | Nagano, Japan   | 1306                | Oonari        | indica        | 19                                       | 21⁹                   | 19⁹                          | calculated from brown rice specific gravity | Arai-Sanoh et al., 2020, Arai-Sanoh, Okamura, Hosoi et al., 2020 (>1.06 g m⁻³) |
| 1985 | Kafl El Shak, Egypt | 1571             | Giza 172      | Egyptian      | local                                    | 15                    | 25                            | 26                                |                                      |
| 1991 | Yanco, Austria  | 1341                | Koshihikari   | japonica      | 13                                       | 22⁴                   | 24                            | specific gravity                   | (>1.06 g m⁻³) |
| 1994 | Yunnan, China   | 2005                | Yu-Za 29      | japonica      | 14                                       | 23                    | 20                            | wind selection                     | Amano et al., 1996 |
| 2003 | Yunnan, China   | 1589                | Liangyoupeijiu| indica        | 28                                       | 25                    | 17                            | calculated from rough grain specific gravity | Katsura et al., 2008 |
| 2003 | Yunnan, China   | 1454                | Takanari      | indica        | 28                                       | 25                    | 17                            | calculated from rough grain specific gravity | Katsura et al., 2008 |
| 2013 | Ghuizhou, China | 1409                | Y-liangyou 1  | indica        | 23                                       | 23⁴                   | 21                            | specific gravity                   | (>1.00 g m⁻³) |

*14% Moisture. *Mean during the growing season. *Rough grain yield was multiplied by 0.963, which is the mean ratio of wind selected filled-grain yield to rough grain obtained from the present data (n = 24). *The different site from the present study, located at Minamininowa village. *Mean from May to September in the nearest weather station of Japan Meteorological Agency (2021), located in Iida city. *Only the mean for three years was obtained. *Mean from May to September in the nearest weather station of Japan Meteorological Agency (2021), located in Nagano city. *Brown rice yield was multiplied by 1.289, which is the mean ratio of wind selected filled-grain yield to brown rice obtained from the present data (n = 24). *Calculated by Namba (2003). *Median between maximum and minimum temperature.

were not higher than those at the other two sites (Table 9). These results indicated that a primarily important environmental determining factor for spikelet number of H193 is probably cumulative radiation until heading. Given that the latitude of the experimental sites and the transplanting days were similar among all environments, the day length during the vegetative period differed negligibly. In these environments, the days to heading was determined largely by temperature because the phenological development of a cultivar is well explained by two environmental factors: temperature and day length (Horie & Nakagawa, 1990). In support of the present results, no significant difference in cumulative temperature until heading was observed among experimental sites (Table 8) and mean minimum temperature until heading was strongly correlated with days to heading (Figure 4). Therefore, not only higher mean radiation but also the long duration to heading caused by lower minimum (night) temperature were responsible for the higher spikelet number at the Nagano site as compared with other sites. Somewhat similar trends were reported by Ying et al. (1998) who compared yield between subtropical condition at Yunnan, China and tropical conditions at IRRI, the Philippines, using high-yielding
cultivars with high N rate (20 g m\(^{-2}\) in total) and showed that the much higher yield in Yunnan than IRRI was mainly related to longer duration in Yunnan associated with lower minimum temperature, but not maximum temperature, which led to larger panicle number and sink capacity (spikelet number per area).

Cool night temperature alone may increase spikelet number because night-time warming is considered to cause declines in yield and biomass owing to complex reasons, such as increase in respiration loss, early leaf senescence, and other factors (Sadok & Jagadish, 2020). Estimations of yield and/or biomass loss in response to night-time warming in previous studies have varied widely: 0–10% decrease in yield for each 1°C increase in night temperature (Lyman et al., 2013; Peng et al., 2004; Peraudeau et al., 2015). Shoot dry weight at full heading was increased by 2.3% for each 1°C decrease in daily minimum temperature on average in a comparison of the Nagano and Ibaraki sites in the present study (Tables 4 and 8). While the high biomass production at heading was one of factors for high spikelet number under high cumulative radiation at Nagano, biomass production may not be only determinant factor for spikelet number since spikelet number per shoot dry weight at full heading (Spik/WFH) varied by locations (Table 2) and correlation between biomass and spikelet number was not high (\(r = 0.205, p = 0.430\), data not shown). High nitrogen uptake at heading is also considered to be related to the high spikelet number at Nagano (Table 5). However, nitrogen uptake could not totally explain variation in spikelet number since its correlation with spikelet number was not very high including all environments (\(r = 0.571, p = 0.021\), data not shown). It would be interesting to further examine the direct effect of a cool night temperature on spikelet number.

### The reason for high dry matter production during grain filling of H193 at Nagano prefecture

The high yields at the Nagano site were achieved by not only high spikelet number but also adequate source abundance to fill them (Figure 2). There were regional differences in potential source (sum of \(\Delta W\) and stem NSC content at heading) mainly due to the differences of dry matter production after heading (\(\Delta W\) (Table 4)). During grain filling, the Nagano site showed the lowest daily mean temperature due to the lowest minimum temperature and the highest cumulative radiation owing to the long grain-filling duration despite that the mean radiation was not so high at the Nagano site (Tables 7 and 8). The mean and minimum temperature and cumulative radiation were correlated with \(\Delta W\) (Figure 5). It is well known that the grain-filling duration was determinant by cumulative temperature after heading (Arai-Sanoh, Okamura, Mukouyama et al., 2020; Takahashi et al., 2007) and cumulative temperature after heading had no regional differences in this study (Table 8), when the

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**Figure 6.** The relationship between mean minimum temperature during grain filling and \(\Delta W/\text{rad}\). \(\Delta W\) = The increase of shoot dry weight during grain-filling; \(\Delta W/\text{rad} = \Delta W/\text{cumulative radiation during grain filling. **P < 0.01.}**
maturity was judged by grain color. Therefore, one possible reason for high $\Delta W$ at the Nagano site was as follows. The low minimum temperature prolonged the grain-filling duration. Then, the high cumulative radiation caused by prolonged grain-filling duration was one reason for high $\Delta W$.

However, $\Delta W$ per radiation during grain filling ($\Delta W$/rad) was also much higher at the Nagano site than at the other sites (Table 9), indicating that efficient utilization of radiation during grain filling supported high source abundance at the Nagano site as well as the high cumulative radiation. One possible reason for the high $\Delta W$/rad at the Nagano site is the direct effect of cooler night temperature to the biomass production similar to that before heading (Table 7) because $\Delta W$/rad was correlated with minimum temperature (Figure 6). The further study to estimate the effect of cool night temperatures to mitigate biomass loss due to respiration quantitatively is needed.

The additional factors for the high biomass production at the Nagano site should not be ignored. For example, radiation interception ratio by canopy should be considered. Although leaf dry weight and leaf nitrogen content at full heading were not higher at the Nagano site (Table 6), it is possible that the difference of plant architecture affects radiation use efficiency (San et al., 2018; Yang & Hwa, 2008) and further analysis for canopy structure was needed. The shoot nitrogen content was highest at the Nagano site, especially at maturity (Table 5), resulting highest nitrogen absorption during grain filling at the Nagano site (2.5 g m$^{-2}$, 5.9 g m$^{-2}$, 1.8 g m$^{-2}$, at the Ibaraki, Nagano and Hiroshima sites, respectively). This might lead to delayed leaf senescence followed by high $\Delta W$/rad at late grain filling, although no significant differences in leaf nitrogen content at full heading and maturity were observed (data not shown), and a further time-course analysis of leaf nitrogen content is needed. This delayed leaf senescence might be associated with the differences in soil characteristics (Supplemental Table S2) to affect root activity such as N uptake. In addition, photosynthesis rates in diverse plants, including rice, are affected by the sink–source balance owing to feedback regulation from carbohydrate (Fabre et al., 2019; Kasai, 2008; Sugiuura et al., 2019). Therefore, the possibility that the high sink capacity at the Nagano site enhanced photosynthesis, which was responsible for the high $\Delta W$/rad, cannot be denied. If so, the increase in sink capacity may be directly linked to improvement in yield at the other sites. Further studies are needed to test whether genetic increase in sink capacity enhances dry matter production at these sites. As discussed here, high yield at the Nagano site in H193 were probably achieved by several interactions of plant characters of H193, such as phenological development, tiller and spikelet formation, dry matter productivity, N uptake and others, and climatic factors inferred from the results in this study. To quantitatively estimate the effect of these factors on yield determination quantitatively, sensitivity analysis using crop growth modeling (such as Yoshida & Horie, 2009, 2010) would be a valuable option in future study.

**Yield determining factors in other sites**

The relationship between spikelet number and cumulative radiation until heading was plotted (Figure 3d). Although the greater spikelet number at the Nagano site (square symbols) and the early planting at the Ibaraki site (grey-colored circle symbols) could be explained by cumulative radiation, it was not the case at the Hiroshima site (triangle symbols). At the Hiroshima site, which was the second-highest-yielding site in the present study (Table 2), the Spik/rad was highest whereas $W_{FH}$/rad was lower than that at the Ibaraki site (Table 9). These results indicated that additional environmental and/or cultural factors limit spikelet number of H193 as well as cumulative radiation. Given that the difference of shoot nitrogen content per radiation until full heading ($N_{FH}$/rad) between the Ibaraki site and the Hiroshima site tended to be larger than that of spikelet number per shoot nitrogen content at full heading (Spik/N) (Tables 5 and 9, 12.3% and 3.7% larger in the Hiroshima site, respectively), this unknown factor responsible for higher Spik/rad at the Hiroshima site (Table 9) may be associated with nitrogen absorption. Spik/$W_{FH}$ was also highest in Hiroshima indicating efficient sink production in this site, which possibly resulted in high harvest index (Table 4). It is possible that the differences in timing of application or type of fertilizer (Supplemental Table S1) were related to the higher Spik/rad and Spik/$W_{FH}$. The difference in the nitrogen mineralization characteristics of soil to release available nitrogen among sites might be also involved as indicated in Table S2. There may be a research opportunity to optimize nitrogen supplying patterns and amount for attaining maximum Spik/rad in H193. In any case, the present results suggested that increasing Spik/rad was an effective strategy to increase yield of H193 where radiation is limited. On the other hand, there were limited regional difference in Spik/N among sites (Table 9), which is consistent with previous finding that the number of differentiated spikelets of a cultivar is well explained.
by nitrogen content of the plant at 2 weeks before heading and this efficiency in spikelet differentiation per nitrogen is largely affected by genotypic factor (Yoshida et al., 2006). Therefore, it is also useful to seek to increase the_spike/N by breeding for further increasing yield potentials. Since the 1000-grain weight showed less variation among environments than spikelet number in the current study (Table 2), breeding to increase sink capacity by increasing grain size might be also effective.

Conclusion
The results of cross-locational experiments achieved extra high yield, over 1.2 kg m⁻² on three-year average, in brown rice yield, and suggest that sink capacity is the primary determining factor for grain yield of H193 rather than grain filling rate. The stable grain filling was supported by the high source ability of H193. Therefore, a breeding target for further increase in grain yield of H193 is improvement in grain number or grain size under favorable conditions such as Nagano. The high sink capacity at the Nagano site can be explained by the high cumulative radiation before heading. In addition, high dry matter production per incident radiation after heading stage at the Nagano site could satisfy large source demand by the large sink size. Although this high radiation use efficiency at the Nagano site associated with low night temperature, further studies are needed to clarify the factors attributed to regional differences in dry matter production. This study has revealed attainable yield of H193 and a climatic factor that enables extra-high yield production in rice, and provides valuable information to attain further increases in rice yield.

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