A near-isogenic line for spikelet number in rice with a genetic background of IR64 under various fertilizer conditions

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ABSTRACT
The total spikelet number (TSN) per panicle is an essential factor involved in grain yield among yield components in rice. IR64-NIL12 carrying qTSN12.2-YP4, which was detected on chromosome 12 for TSN per panicle, has been developed in a previous study. It is important to characterize the environment of gene function to increase yields. Thus, we attempted to evaluate IR64-NIL12 grown under no, low, and high fertilizer conditions in 2019 and 2020. Grain yields of IR64-NIL12 were significantly higher than that of IR64 in all conditions. The average grain yield of each condition for IR64 and IR64-NIL12 ranged from 387 to 616 g m⁻². Therefore, qTSN12.2-YP4 would have an increasing effect on grain yield within this range. The ratio of fertility improved in IR64-NIL12. This rice line exhibited modified traits, such as tiller number and plant height (during the vegetative stage), as well as panicle number (at maturity), which may confer an advantage in terms of light-intercepting characteristics. We also focused on the number of unproductive tillers and dry weight per tiller at the heading stage to investigate the mechanism of the increasing effect of qTSN12.2-YP4 on TSN per panicle. There is a significant difference in dry weight per tiller at heading between IR64 and IR64-NIL12, but not in the percentage of unproductive tillers. Therefore, this result indicated that qTSN12.2-YP4 contributes to increasing dry weight per tiller until the heading stage and, consequently, TSN per panicle.

ARTICLE HISTORY
Received 11 May 2022
Revised 22 August 2022
Accepted 27 September 2022

KEYWORDS
Dry weight per tiller; near-isogenic line; productive tiller; rice; spikelet number

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Supplemental data for this article can be accessed online at https://doi.org/10.1080/1343943X.2022.2132966

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Introduction

Rice grain yield can be estimated using yield components, such as panicle number, single grain weight, the ratio of ripening, and total spikelet number (TSN) per panicle. TSN is a crucial factor because it is often positively associated with rice grain yield (Yoshida, 1981). Many quantitative trait loci (QTLs) have been identified in rice to improve yield potential by increasing TSN (Bai et al., 2012). Among the identified QTLs, four are associated with TSN, namely, SPIKE/LSCHL4/GPS (Fujita et al., 2013; Takai et al., 2013, 2019a; Zhang et al., 2014), SCM2/APO1 (Ookawa et al., 2010; Terao et al., 2010), DEP1 (Huang et al., 2009) and qTSN12.2-YP4 (Sasaki et al., 2017) were discovered to increase grain yield in near-isogenic lines (NILs) through changes in TSN under field conditions. Furthermore, interactions between genetic and environmental factors have been examined as the increasing effect of QTLs on grain yield through increasing TSN may be affected by growth conditions. Based on yield fields from 11 seasons, the increasing effect of SPIKE on grain yield depends on the paddy soil fertility and yield levels (Fujita et al., 2013; Takai et al., 2019a). NIL-SPIKE tended to be superior to IR64 for grain yield under low-N application, while the difference disappeared under high-N application (Takai et al., 2019a).

The increasing effect of APO1 on grain yield was observed under higher CO₂ concentrations, but not under ambient conditions (Nakano et al., 2017). Thus, it is critical to determine the growth conditions under which QTLs have an increasing effect on grain yield. However, the effect of qTSN12.2-YP4 on yield performance under various yield-level conditions has not been evaluated. Therefore, in this study, we attempted to assess IR64-NIL12 carrying qTSN12.2-YP4 grown under various fertilizer conditions.

Increasing effects on grain yield in IR64-NIL12 by increasing TSN per panicle have been confirmed in a tropical area in multiple seasons (Sasaki et al., 2017). Because IR64-NIL12 also had significantly less tillers than IR64, the percentage of productive tillers may be associated with these increasing effects (Sasaki et al., 2017). The percentage of productive tillers is the ratio of productive and unproductive tillers. Unproductive tillers compete with productive tillers for assimilates, solar energy, and mineral nutrients, thus eliminating them could direct more nutrients to grain production (Nuruzzaman et al., 2000). It is crucial to minimize unproductive tillers and increase the sink size to improve the harvest index in rice (Peng et al., 2008). Moreover, no unproductive tiller is one of the target traits for developing new plant-type rice, including YP4, which was used as a donor during IR64-NIL12 development (Khush, 1995). In addition to the percentage of productive tillers, the dry matter produced by an individual tiller at the heading stage mostly determines TSN per panicle (Shiratsuchi et al., 2007; Yao et al., 2000). TSN per panicle was positively correlated with the dry weight of an individual tiller at the heading stage rather than with the leaf area or amount of nitrogen (Shiratsuchi et al., 2007). This correlation remained stable within each cultivar regardless of treatment, year, and tiller order (Shiratsuchi et al., 2007). However, it remains unknown how qTSN12.2-YP4 increases TSN per panicle. To elucidate the mechanism in this study, we hypothesized two possibilities: 1) qTSN12.2-YP4 contributes to improving grain yield through less unproductive tillers in IR64NIL-12; and 2) qTSN12.2-YP4 contributes to increasing shoot biomass per tiller, and consequently, TSN per panicle in IR64NIL-12. Thus, we evaluated the percentage of productive tillers in IR64-NIL12 grown under various fertilizer conditions. We also investigated the relationship between dry weight per tiller at the heading stage and TSN per panicle grown under various fertilizer conditions.

Materials and methods

Plant materials and cultivation

IR64-NIL12 carrying qTSN12.2-YP4 for TSN were developed from populations derived from crosses between IR64 and new plant-type rice, YP4 (Sasaki et al., 2017). IR64 and NIL were grown in paddy fields at an experimental station (36°3′15″N, 140°4′49″E) of Japan International Research Center for Agricultural Sciences (JIRCAS) in Tsukuba, Japan, in 2019 and 2020. Sterilized seeds were sown into a nursery box filled with nursing culture soil on 23 April 2019, and 21 April 2020. Four weeks after sowing, seedlings (one plant per hill) were transplanted at 18 cm between hills and 30 cm between rows. In each plot, 13 plants were transplanted in eight rows. There were four replications with randomized block design in the same fertilizer application. Fertilizer application methods in paddy fields have been fixed in the past multiple years due to efficient fertilizer management. In the low fertilizer application plot, nitrogen, P₂O₅, K₂O, and magnesia (Ajiyoshi ichi-gou, National Federation of Agricultural Cooperative Associations, Tokyo) were fertilized at 48, 64, 32, and 5 kg ha⁻¹, respectively, as basal fertilizer before puddling. No fertilizer was applied in the no fertilizer application plot. This management has been continued since 2017. Before puddling, the high fertilizer application plot received twice as much fertilizer as the low fertilizer application
plot. This management has been continued since 2018. Thus, the split-plot design was used, with fertilizer application as the main factor. Supplemental Material 1 shows the status of soils in paddy fields.

**Measurement of agronomic traits**

Tiller number and plant height were measured during the tillering stage. Five plants were measured in each plot. These same five plants were harvested at the maturity stage to evaluate the percentage of productive tillers, which was calculated by dividing the panicle number by the tiller number 76 days after sowing in 2019 and 80 days in 2020. At the heading stage, the shoots of the other five plants were harvested in a plot and divided into each tiller to weigh a single tiller weight at 2 days after drying at 70°C.

To measure grain yield and yield components, 24 plants (three rows × eight individuals) were harvested at the maturity stage. Harvested plants were dried in a well-ventilated greenhouse until measurement. Dried plants were threshed to determine shoot biomass. The grains were threshed after all panicles had been counted. Over 50 g subsample was made using a sample divider. According to Kobata et al. (2010), subsample grains were divided into fertile and sterile grains. Subsample grains were sawed into 70% ethanol and flowed grains were regarded as sterile grains, while the remaining grains at bottom of the beaker were regarded as fertile grains. A seed counter was used to count the number of fertile and sterile grains. TSN per square meter was calculated from the number of fertile and sterile grains of subsample grains. TSN per panicle was calculated by dividing TSN per square meter by the panicle number. Fertile grains were dried for 2 days at 70°C for measurement of 1000-gain weight, which revealed moisture content of 14%. Grain yield was estimated based on the results from yield components of subsamples. The harvest index was calculated by dividing the grain yield with shoot biomass. Sink capacity was calculated using the following equation:

Sink capacity = TSN per square meter × 1000-grain weight/1000

TSN per panicle per dry weight was calculated by dividing TSN per panicle at maturity by the dry weight per tiller at the heading stage.

Analysis of variance (ANOVA) revealed a significant difference between line, fertilizer application, year, and these interactions. A significant difference between IR64 and IR64-NIL12 was calculated using the t-test. Heritability was calculated with the results from ANOVA, which was performed in each condition, according to Asante et al. (2019).

**Results**

Tiller numbers and plant height of IR64 and IR64-NIL12 were counted and measured during the tillering stage. The maximum tiller number stage was around 76 and 80 days after sowing in 2019 and 2020 (supplemental material 2). At this stage, IR64-NIL12 carrying qTSN12.2-YP4 had a significantly lower tiller number than that of IR64 in all fertilizer applications. The plant height of IR64-NIL12 was significantly higher than that of IR64 during the tillering stage in all fertilizer applications, except for that in low fertilizer condition at 49 days after sowing in 2020.

Panicle number and dry weight of IR64 and IR64-NIL12 were measured at the full heading stage. All measured traits differed significantly based on year, fertilizer application rate, and line (Table 1). The numbers of days to heading in IR64-NIL12 were slightly higher than that in IR64 under all fertilizer conditions in both years (Table 1). The dry weights per plant and per tiller of IR64-NIL12 were significantly higher than that of IR64, although the panicle number of IR64-NIL12 was lower. The panicle number of IR64-NIL12 was significantly lower than that of IR64 grown under all conditions except for no fertilizer application in 2020. The dry weight of IR64-NIL12 was significantly higher than that of IR64 grown without fertilization in 2019 and under all conditions in 2020. The dry weight per tiller of IR64-NIL12 was significantly higher than that of IR64 grown under all conditions.

The percentage of productive tillers was calculated using the maximum tiller number and panicle number counted from the same individuals. The maximum tiller and panicle numbers varied according to years and line, although the percentage of productive tillers remained stable. Meanwhile, all the traits varied according to the fertilizer application rate (Table 2). The maximum tiller and panicle numbers of IR64-NIL12 were significantly lower than those of IR64 under all conditions. No significant difference was observed for the percentage of productive tillers between IR64 and IR64-NIL12 under all conditions except for high fertilizer application in 2020.

At maturity, grain yield, biomass, yield components, sink capacity, and TSN per panicle per dry weight of IR64 and IR64-NIL12 were evaluated. There were significant differences among years in the grain yield, harvest index, ratio of fertility, and 1000-grain weight, whereas there were significant differences among fertilizer application rates under all conditions (Table 3). There were significant differences between lines under all conditions except for TSN per panicle per dry weight. The grain yield, harvest index, and 1000-grain weight of IR64-NIL12 were significantly higher than that of IR64 in all
conditions, whereas the panicle number of IR64-NIL12 was significantly lower than that of IR64. Shoot biomass and TSNs per square meter of IR64-NIL12 were significantly higher than those of IR64 in all fertilizer conditions in 2019, although these traits did not differ between lines under all fertilizer conditions in 2020. The TSNs per panicle and sink capacity of IR64-NIL12 were significantly higher than that of IR64 in all conditions except for high fertilizer application in 2020. TSN per panicle per dry weight of IR64-NIL12 was significantly higher than that of IR64 grown without fertilization in 2019, whereas in 2020, the reverse occurred.

**Discussion**

Grain yield of IR64-NIL12 had to be evaluated in multiple fertilizer applications to identify the grain yield level at which the increasing effect of qTSN12.2-YP4 on grain yield was observed. To determine environments in which gene function and the increasing effect of qTSN12.2-YP4 on grain yield, we evaluated yield performance of IR64 and IR64NIL-12 carrying qTSN12.2-YP4 grown under no, low, and high fertilizer conditions in paddy fields for two years. IR64-NIL12 showed a higher grain yield than the background variety, IR64, in all six conditions in temperate and tropical environments (Table 3, Sasaki et al., 2017). In addition to qTSN12.2-YP4, SPIKE was detected as a QTL for TSN and increased grain yield by enhancing TSN in field conditions (Fujita et al., 2013; Fujita et al., 2012). NIL for SPIKE tended to be superior to IR64 for grain yield under the low-N application, in which the mean grain weight of NIL and IR64 was 431 g m⁻², while the difference disappeared under the high-N application, in which the mean grain yield of NIL and IR64 was 670 g m⁻² (Takai et al., 2019a). In this study, the average grain yield of each fertilizer application for IR64 and IR64-NIL12 ranged from 388 g m⁻² to 615 g m⁻² (Figure 1A). Within these ranges, qTSN12.2-YP4 had an increasing effect on grain yield. We were successful in proposing a wider range of grain yield that could be exploited by IR64-NIL12 than the previous study (Sasaki et al., 2017). Among the yield components, the ratio of fertility and 1000-grain weight improved under all conditions in IR64-NIL12. Consequently, grain yield in IR64-NIL12 increased through increasing the harvest index. Meanwhile, the TSN per square meter of IR64-NIL12 was higher than that of IR64 under all fertilizer application rates in 2019 but not in 2020 (Table 3). Furthermore, TSN per square meter and shoot biomass values of IR64-NIL12 were higher than those of IR64 under all fertilizer application rates in 2019 (Table 3). A similar trend was also observed in yield performance trials in Philippines (Sasaki et al., 2017). The TSN and shoot biomass values of IR64-NIL12 per square meter were higher than those of IR64 in the dry season of 2012 but not in the wet season in 2011.

**Table 1.** Panicle number and dry weight of IR 64 and IR64-NIL12 at heading stage grown under various fertilizer conditions.

| Year | Fertilizer application | Line | Days to heading (day) | Panicle number (plant⁻¹) | Dry weight (g plant⁻¹) | Dry weight (g tiller⁻¹) |
|------|------------------------|------|-----------------------|--------------------------|------------------------|------------------------|
| 2019 | No                     | IR64 | 112.0                 | 13.2                     | 38.5                   | 2.9                    |
|      | IR64-NIL12             |      |                       | 115.3**                 | 11.6**                 | 45.9**                 | 3.9**                  |
|      | Low                    | IR64 | 108.5                 | 17.4                     | 53.9                   | 3.0                    |
|      | IR64-NIL12             |      |                       | 110.3*                  | 14.6**                 | 59.6ns                 | 4.0**                  |
|      | High                   | IR64 | 110.5                 | 9.5                      | 60.9                   | 3.0                    |
|      | IR64-NIL12             |      |                       | 113.5**                 | 16.5**                 | 67.3ns                 | 4.0**                  |
| 2020 | No                     | IR64 | 113.0                 | 14.5                     | 39.0                   | 2.7                    |
|      | IR64-NIL12             |      |                       | 116.0**                 | 11.6ns                 | 48.1*                  | 4.2**                  |
|      | Low                    | IR64 | 111.0                 | 17.0                     | 58.8                   | 3.5                    |
|      | IR64-NIL12             |      |                       | 113.3**                 | 13.7**                 | 67.0*                  | 4.9**                  |
|      | High                   | IR64 | 111.5                 | 18.7                     | 67.3                   | 3.6                    |
|      | IR64-NIL12             |      |                       | 113.3**                 | 14.0*                  | 73.0*                  | 5.2**                  |

ANOVA

| Year | **c** | *   | **   | **   |
|------|-------|-----|------|------|
| Fertilizer application | **      | **  | **   | **   |
| Line | **    | **  | **   | **   |
| Year×Fertilizer application | **    | **  | ns   | **   |
| Fertilizer application×Line | *      | ns  | ns   | ns   |
| Year×Line | ns  | *   | ns   | **   |
| Year×Fertilizer application×Line | ns  | ns  | ns   | ns   |

** and * indicate significant difference between IR64 and IR64-NIL12 by t-test at 1% and 5% significant level, respectively (n = 4).

ns indicates that this combination was not tested due to the lack of variance.

** and * indicate significant difference by ANOVA at 1 and 5% significant levels, respectively (n = 4).

ns indicate there is no significance.
Table 2. The percentage of productive tillers of IR 64 and IR64-NIL12 at the maturity stage grown under various fertilizer conditions.

| Year | Fertilizer application | Line       | Maximum tiller numbera (plant−1) | Panicle number (plant−1) | Percentage of productive tiller (%) |
|------|------------------------|------------|----------------------------------|--------------------------|------------------------------------|
| 2019 | No                     | IR64       | 22.0                             | 15.1                     | 69.9                               |
|      |                        | IR64-NIL12 | 16.9**b                          | 11.8**                   | 70.3ns                             |
|      | Low                    | IR64       | 36.5                             | 16.7                     | 47.6                               |
|      |                        | IR64-NIL12 | 24.7*                           | 13.5**                   | 56.6ns                             |
|      | High                   | IR64       | 30.8                             | 18.5                     | 50.8                               |
|      |                        | IR64-NIL12 | 22.6**                          | 13.4*                    | 60.6ns                             |
| 2020 | No                     | IR64       | 23.6                             | 14.2                     | 60.6                               |
|      |                        | IR64-NIL12 | 19.0*                           | 11.2**                   | 59.6ns                             |
|      | Low                    | IR64       | 25.6                             | 14.9                     | 59.1                               |
|      |                        | IR64-NIL12 | 19.6*                           | 11.7**                   | 59.9ns                             |
|      | High                   | IR64       | 32.6                             | 16.3                     | 50.2                               |
|      |                        | IR64-NIL12 | 24.2**                          | 13.0**                   | 54.3*                              |

ANOVA

| Factor                      | df    | F     | P    |
|-----------------------------|-------|-------|------|
| Year                        | 1     | 1.88  | ns   |
| Fertilizer application      | 3     | 1.39  | ns   |
| Line                        | 1     | 1.39  | ns   |
| Year×Fertilizer application | 3     | 1.39  | ns   |
| Fertilizer application×Line | 3     | 1.39  | ns   |
| Year×Fertilizer application×Line | 3     | 1.39  | ns   |

aMaximum tiller number is tiller number at 76 and 80 days after sowing in 2019 and 2020, as shown in Supplemental Material 2.
b** and * indicate significant difference between IR64 and IR64-NIL12 by t-test at 1% and 5% significance level, respectively (n = 4).

** and * indicate significant difference by ANOVA at 1% and 5% significance levels, respectively (n = 4).
ns indicate there is no significance.

Thus, increasing shoot biomass increases TSN per square meter in IR64-NIL-12. This conclusion is supported by the small difference in the efficiency of spikelet production per dry weight between IR64-NIL12 and IR64, as well as the similar levels of TSN per panicle per dry weight between IR64-NIL12 and IR64 (Table 3).

Although qTSN12.2-YP4 was detected as a QTL for spikelet number per panicle, other traits, such as panicle number, also changed in IR64NIL-12, as well as TSN. It is important to minimize unproductive tillers and increase the sink size to improve the harvest index in rice (Peng et al., 2008). Moreover, no unproductive tiller is one of the target traits for developing new plant-type rice, including YP4 (Khush, 1995). Thus, we hypothesized that qTSN12.2-YP4 contributes to improving grain yield through less unproductive tiller in IR64NIL-12. In fact, during the vegetative stage, the tiller number of IR64NIL-12 was significantly lower than that of IR64.
Table 3. Grain yield, biomass, and yield components of IR64 and IR64-NIL12 at maturity under various fertilizer conditions.

| Year | Fertilizer application | Line | Grain yield (g m⁻²) | Shoot biomass (g m⁻²) | Harvest index | Panicle number (m⁻²) | TSN (m⁻²) | Ratio of fertility (%) | 1000-grain weight (g) | Sink capacity | TSN (panicle⁻¹) dry weight⁻¹ |
|------|------------------------|------|---------------------|-----------------------|---------------|----------------------|-----------|------------------------|----------------------|---------------|-----------------------------|
| 2019 | No                     | IR64 | 321.8               | 1026.8                | 0.31          | 270.4                | 20906.0   | 77.5                   | 61.7                 | 24.9          | 520.8                       |
|      | NIL12                  |      | 569.1**             | 1116.5**              | 0.51**        | 201.2**              | 25743.0**| 128.6**                | 83.6**               | 26.4**        | 680.7**                     |
|      | IR64-NIL12             |      | 569.1**             | 1116.5**              | 0.51**        | 201.2**              | 25743.0**| 128.6**                | 83.6**               | 26.4**        | 680.7**                     |
|      | Low                    | IR64 | 358.9               | 1253.2                | 0.29          | 287.3                | 26007.0   | 90.5                   | 53.1                 | 26.0          | 676.2                       |
|      | NIL12                  |      | 675.1**             | 1403.5**              | 0.48**        | 241.3**              | 29528.7**| 122.4**                | 81.2**               | 28.2**        | 831.6**                     |
|      | IR64-NIL12             |      | 675.1**             | 1403.5**              | 0.48**        | 241.3**              | 29528.7**| 122.4**                | 81.2**               | 28.2**        | 831.6**                     |
|      | High                   | IR64 | 446.9               | 1487.8                | 0.30          | 316.4                | 31789.9   | 100.4                  | 54.4                 | 25.8          | 820.3                       |
|      | NIL12                  |      | 784.9**             | 1634.0*               | 0.48**        | 276.2**              | 36268.9*  | 131.7**                | 76.1**               | 28.4**        | 1031.7**                    |
|      | IR64-NIL12             |      | 784.9**             | 1634.0*               | 0.48**        | 276.2**              | 36268.9*  | 131.7**                | 76.1**               | 28.4**        | 1031.7**                    |
| 2020 | No                     | IR64 | 283.7               | 1034.4                | 0.27          | 271.3                | 20975.5   | 77.3                   | 52.0                 | 26.1          | 548.0                       |
|      | NIL12                  |      | 491.3**             | 1114.4ns              | 0.44**        | 209.5**              | 24256.2ns | 115.9**                | 71.9**               | 28.1**        | 682.6*                      |
|      | IR64-NIL12             |      | 491.3**             | 1114.4ns              | 0.44**        | 209.5**              | 24256.2ns | 115.9**                | 71.9**               | 28.1**        | 682.6*                      |
|      | Low                    | IR64 | 322.0               | 1326.8                | 0.24          | 296.6                | 24806.4   | 83.7                   | 47.3                 | 27.4          | 680.7                       |
|      | NIL12                  |      | 484.2**             | 1380.7ns              | 0.35**        | 234.9**              | 25094.8ns | 106.5**                | 64.5**               | 30.0**        | 751.4**                     |
|      | IR64-NIL12             |      | 484.2**             | 1380.7ns              | 0.35**        | 234.9**              | 25094.8ns | 106.5**                | 64.5**               | 30.0**        | 751.4**                     |
|      | High                   | IR64 | 462.2               | 1596.1                | 0.29          | 330.3                | 36741.9   | 111.0                  | 44.6                 | 28.4          | 1045.2                      |
|      | NIL12                  |      | 688.4*              | 1632.0ns              | 0.42**        | 247.6**              | 34138.8ns | 137.4ns                | 66.5**               | 30.4**        | 1034.2ns                    |
|      | IR64-NIL12             |      | 688.4*              | 1632.0ns              | 0.42**        | 247.6**              | 34138.8ns | 137.4ns                | 66.5**               | 30.4**        | 1034.2ns                    |

ANOVA

| Source                      | df | Sum of squares | Mean square | F value | p value |
|-----------------------------|----|---------------|-------------|---------|---------|
| Year                        | 1  | 153.2         | 153.2       | 3.4     | 0.07    |
| Fertilizer application      | 1  | 54.3          | 54.3        | 1.2     | 0.27    |
| Line                        | 1  | 54.3          | 54.3        | 1.2     | 0.27    |
| Year×Fertilizer application | 1  | 153.2         | 153.2       | 3.4     | 0.07    |
| Year×Line                   | 1  | 54.3          | 54.3        | 1.2     | 0.27    |
| Year×Fertilizer application×Line | 1  | 153.2       | 153.2       | 3.4     | 0.07    |

*This parameter was calculated by dividing TSN per panicle at maturity by the dry weight per tiller at the heading stage.

** and * indicate a significant difference between IR64 and IR64-NIL12 by t-test at 1% and 5% significant levels, respectively (n = 4).

ns indicate there is no significance.

TNS, total spikelet number.
However, contrary to our hypothesis, the percentage of productive tillers of IR64NIL-12 was significantly the same level as IR64 under all conditions (Table 2). These results indicated that the increasing effect of IR64NIL-12 on grain yield was not the reason for the change in the percentage of productive tillers. Our second hypothesis is that qTSN12.2-YP4 contributes to shoot biomass and, consequently, TSN in IR64NIL-12 because TSN per panicle is mostly determined by dry matter production of an individual tiller at the heading stage (Shiratsuchi et al., 2007). Additionally, Takai et al. (2019b) reported that varieties having high dry weight per tiller, such as Takanari and Hokuriku193, at heading stage had higher TSN per panicle at maturity stage than that of other varieties or lines. In addition to these studies, there is a positive relationship between dry weight per tiller at the heading stage and TSN per panicle at the maturity stage (Figure 2). IR64NIL-12 showed significantly higher dry weight per tiller at the heading stage than that of IR64 and, consequently, higher TSN per panicle at the maturity stage in all fertilizer conditions (Table 1 and Figure 2). Because the levels of TSN per panicle per

Figure 2. Correlation between dry weight per tiller at heading stage and total spikelet number at maturity stage in IR64 and IR64-NIL12. Boxes and circles indicate IR64 and IR64-NIL12 values. White, light gray, and dark gray indicated values grown under no, low, and high fertilizer conditions, respectively. Mean values are shown with standard deviation (n = 4).
dry weight of IR64NIL-12 and IR64 were similar, efficient spikelet production per dry weight could be small. Therefore, qTSN12.2-YP4 would increase dry weight per tiller until the heading stage and contribute to improving TSN per panicle in IR64NIL-12 at the maturity stage. Moreover, the heritability of dry weight per tiller until the heading stage was consistently higher in a range of 0.86–0.97 than that of panicle number and dry weight per plant (Supplemental Material 3). These results imply that the dry weight per tiller is effective for the genetic analysis of biomass productivity at the heading stage to improve TSN per panicle at maturity. The increasing dry weight per tiller in IR64NIL-12 may also affect the ratio of fertility, which was improved in IR64-NIL12 under all conditions, although TSN per panicle showed increase (Table 3).

Kobata et al. (2013) discussed that the spikelet sterility of high-yielding varieties was caused by inadequate assimilate supply during flowering. In addition, IR64NIL-12 has fewer tillers and tends to be taller than IR64 during the vegetative stage and has less panicles than IR64 at maturity. These traits confer advantages to IR64-NIL12 in terms of light-intercepting characteristics. Kuroda et al. (1989) showed that tall cultivars tended to have a higher photosynthetic capacity than short cultivars. Laza et al. (2004) reported that rice crops with less tillers have a better canopy structure for light interception than ones with more tillers. Having fewer tillers is an important characteristic for maintaining a high canopy photosynthetic rate when light is a limiting factor. Therefore, it is likely that the light-intercepting characteristics of IR64NIL-12 were improved. Consequently, the adequate supply of assimilates contributed to the improved ratio of fertility. Further study is needed to assess the amount of carbohydrates, such as nonstructural carbohydrates in each developmental stage.

Acknowledgments

We thank Dr. T. Takai, Dr. S. Yanagihara, Dr. Y. Fukuta, Dr. H. Saito, and Dr. N. Kobayashi for their suggestions. We also thank the staff of the research planning and partnership division in JIRCAS for their technical support in paddy fields.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by the JIRCAS under the research projects ‘Food Security in Africa’ and ‘Africa rice farming system.’

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