Effects of genes increasing the number of spikelets per panicle, TAW1 and APO1, on yield and yield-related traits in rice

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ABSTRACT
The genes TAWAWA1 (TAW1) and ABERRANT PANICLE ORGANIZATION1 (APO1) increase the number of spikelets per panicle (SN). In the present study, we examined the effects of these genes on morphological traits, yield, and yield-related traits including yield components using the near-isogenic lines (NILs) in the genetic background of a japonica rice variety, Koshihikari – NIL-taw1, NIL-apo1-D3, and NIL-apo1-D4 – in a field experiment. The SN and total number of spikelets per area of the three NILs were larger than those of Koshihikari. However, the yield of the three NILs did not exceed that of Koshihikari due to their low filling ability. Interestingly, our field experiments indicated that TAW1 did not affect the diameter of internodes and the PN, whereas APO1 decreased the PN and increased the diameter of internodes. These results suggest that TAW1 and APO1 differently affect yield-related traits.

One of the promising ways for breeding high-yield rice varieties is to introduce genes related to high yield into elite varieties with the assistance of DNA markers. Yield per area is determined by four yield components: the number of panicles per area (PN), the number of spikelets per panicle (SN), thousand grain weight (TGW), and the percentage of ripened grain (RP). Recently, several genes and quantitative trait loci (QTLs) that regulate the SN, such as \textit{Grain number 1a} \,(Gn1a) (Ashikari et al., 2005), DENSE AND ERECT PANICLE1 \,(DEP1) (Huang et al., 2009), ERECT PANICLE \,(EP) (Wang et al., 2009), ABERRANT PANICLE ORGANIZATION1 \,(APO1) (Ikeda-Kawakatsu et al., 2009), STRONG CULM2 \,(SCM2) (Ookawa et al., 2010), TAWAWA1 \,(TAW1) (Yoshida et al., 2013), SQUAMOSA PROMOTER BINDING PROTEIN-LIKE \,(OslPL14) (Jiao et al., 2010; Miura et al., 2010), SPIKELET NUMBER \,(SPIKE) (Fujita et al., 2013), and STRONG CULM3 \,(SCM3) (Yano et al., 2015), have been identified, and the near-isogenic lines (NILs) for high SN have been developed. However, since the four-yield components are often negatively correlated, the introduction of these genes may not always increase the yield in the field. One of the genes regulating the SN, TAW1, does not affect the PN (Yoshida et al., 2013). In contrast, SCM2, the APO1 allele of an indica rice variety, Habataki, increases the diameter of internodes (Ookawa et al., 2010) and its overexpression reduces the PN (Murai & Iizawa, 1994). In a previous study, we have revealed that tiller development is affected in apo1-D3 and apo1-D4 mutants, but not in a taw1-D2 mutant (Fukushima et al., 2015). In this study, we elucidated the effects of TAW1 and APO1, the genes that increase the SN, on morphological traits, yield, and yield-related traits including yield components using NILs in the genetic background of a japonica rice variety, Koshihikari, in field experiments conducted in the northern cold region of Japan.

Materials and methods
Development of NILs and their cultivation

Three NILs, NIL-taw1 \,(BC_5 F_5), NIL-apo1-D3 \,(BC_5 F_5), and NIL-apo1-D4 \,(BC_6 F_5), carrying taw1-D2 \,(a mutant of TAW1), apo1-D3, and apo1-D4 \,(mutants of APO1), respectively, were developed by backcrossing the japonica rice variety Koshihikari as the recurrent parent (Ikeda-Kawakatsu et al., 2009; Yoshida et al., 2013). Using the three NILs and the variety Koshihikari, field experiments were conducted in a paddy field \,(gray lowland soil) at NARO Tohoku Agricultural Research Center \,(Daisen City, northern Japan; 39°29′N, 140°29′E) in 2015 and 2016. The dates of seeding and transplanting were 17 April and 21 May in 2015 and

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21 April and 20 May in 2016, respectively. The seedlings at a leaf age of 4.0 to 5.0 were transplanted by hand, three seedlings per hill, at a hill spacing of 15 cm and row spacing of 30 cm. A compound fertilizer containing 14% N, 14% P₂O₅, and 14% K₂O was applied as basal dressing at a rate of 4 g m⁻² of N in 2015 and 3 g m⁻² of N in 2016; topdressing was not applied. A randomized complete block design with three replications was used. The area of each plot was approximately 6 m².

**Morphological traits of panicle, leaf, stem, spikelet, and brown rice grain**

Six shoots per plot (three longer shoots, excluding the longest shoot, from two hills) were sampled more than one week after heading. The number of primary rachis branches, primary spikelets, and secondary spikelets per panicle were counted. The number of degenerated spikelets per panicle was estimated from the vestige of spikelets and rachis branches. The average length and width of the first (flag), second, and third leaf blade, the average length of the first, second, and third leaf sheath, the culm length, and the average diameter of the second, third, and fourth internode were calculated.

The average size of spikelets and rice grain per plot was calculated from 10 spikelets and 10 mature brown rice grains randomly sampled from each plot and measured under a microscope.

**Yield, yield-related traits, and yield components**

Heading date was defined as the date when 50–60% of panicles emerged. The degree of lodging was estimated visually at maturity using a scale from 0.0 (no lodging) to 5.0 (perfect lodging). Forty hills per plot were harvested at maturity. The plants were dried outside and their total dry weight was measured. All panicles were counted to calculate the PN. After threshing, the unhulled rice grains were weighed. Approximately 7% of the unhulled rice grains was extracted as a sub-sample by a riffle sampler (Tsutsui Scientific Instruments Co., Ltd., Tokyo, Japan) and the number of spikelets of the sub-sample was counted by a multi-auto counter (Fujiwara Scientific Co., Ltd., Tokyo, Japan). The remaining unhulled rice grains were hulled and divided using a grain sorter into grains thicker than 1.8 mm (mature brown rice) and grains narrower than 1.8 mm (immature brown rice). The gross brown rice yield, mature brown rice yield (yield), immature brown rice weight, and TGW were measured and the values were adjusted to the standardized 15% moisture content. The total number of spikelets per area (TSN) was calculated as the number of spikelets of the sub-sample divided by the weight of the sub-sample and multiplied by the weight of total unhulled rice per area. The SN was calculated as the TSN divided by the PN. The RP was calculated as the number of grains thicker than 1.8 mm per area divided by the TSN.

**Results**

**Weather conditions**

Weather conditions during the study period in the northern cold region of Japan were compared with those in major Koshihikari cultivated regions (Table 1). Before heading the temperature at the study site was not largely different and the sunshine duration was slightly longer than those in major Koshihikari-cultivated regions. During ripening period, the temperature was lower in both years of the study period, and the sunshine duration was shorter in 2015 but longer in 2016, than those in major Koshihikari-cultivated regions.

**Morphological traits of the panicle, leaf, stem, spikelet, and brown rice grain**

The number of spikelets per panicle of the three NILs was significantly larger than that of Koshihikari due to a significantly larger number of either differentiated spikelets or secondary spikelets (Table 2). Among the three NILs, only NIL-aapo1-D3 had the number of primary rachis branches significantly larger than that in Koshihikari.

The culm, leaf blade, and leaf sheath were significantly longer in NIL-aapo1-D4 but not significantly different in NIL-aapo1-D3 from those in Koshihikari; the culm length of NIL-taw1 was significantly shorter than that of Koshihikari. In contrast, the leaf blade was significantly wider and the diameter of the internodes was significantly thicker in

| City, prefecture | Year  | Heading date | Temperature (°C) 60-31 DBH | 30-1 DBH | 1-30 DAH | Sunshine duration (hour) 60-31 DBH | 30-1 DBH | 1-30 DAH |
|------------------|-------|--------------|-----------------------------|---------|---------|-------------------------------|---------|---------|
| Daisen, Akita    | Average | 10-Aug | 20.3 | 23.6 | 23.3 | 5.0 | 5.6 | 5.7 |
|                  | 2015 | 09-Aug | 20.7 | 25.3 | 22.6 | 6.9 | 6.0 | 3.6 |
|                  | 2016 | 11-Aug | 21.0 | 24.8 | 24.9 | 4.3 | 7.1 | 7.0 |
| Tukuba, Ibaraki  | Average | 03-Aug | 20.5 | 24.2 | 25.4 | 3.9 | 4.7 | 5.7 |
| Takada, Niigata  | Average | 07-Aug | 21.4 | 25.4 | 25.9 | 4.4 | 5.5 | 6.0 |

Notes: Data publicized by Japan Meteorological Agency were used. Average: Average from 1981 to 2010, DBH: Days before heading, DAH: Days after heading.
These characteristics were not significantly different between NIL-taw1 and Koshihikari. The length of the spikelets of the three NILs was significantly shorter compared to that of Koshihikari (Table 3). Accordingly, the length of mature brown rice of NIL-taw1 and NIL-apo1-D4 was significantly shorter than that of Koshihikari.

Table 3. Morphological differences in the spikelets and brown rice grain between the rice variety Koshihikari and three NILs.

| Variety and lines | Spikelet | Brown rice grain |
|-------------------|----------|------------------|
|                   | Length (mm) | Width (mm) | Length (mm) | Width (mm) | Thickness (mm) |
| Koshihikari       | 6.40      | 3.47          | 5.09        | 3.00        | 2.17          |
| NIL-taw1          | 6.25**    | 3.39NS        | 4.94*       | 2.93NS      | 2.05**        |
| NIL-apo1-D3       | 6.32**    | 3.48NS        | 5.07NS      | 2.99NS      | 2.15NS        |
| NIL-apo1-D4       | 6.25**    | 3.32*         | 4.99*       | 2.89*       | 2.09*         |

Notes: Significance: *p < .05; **p < .01; NS, not significant (Paired t-test compared with Koshihikari, N = 6 (2 years x 3 replications)).

NIL-apo1-D3 and NIL-apo1-D4 than in Koshihikari, whereas these characteristics were not significantly different between NIL-taw1 and Koshihikari.

The length of the spikelets of the three NILs was significantly shorter compared to that of Koshihikari (Table 3). Accordingly, the length of mature brown rice of NIL-taw1 and NIL-apo1-D4 was significantly shorter than that of Koshihikari.

Yield and yield-related traits including yield components

The heading date did not differ significantly between Koshihikari and the three NILs; it occurred on 10 Aug. in Koshihikari and NIL-taw1 and 11 Aug. in NIL-apo1-D3 and NIL-apo1-D4 (Table 4). The degree of lodging was greater in 2015 than in 2016; the variety and all the lines were severely lodged in 2015, whereas in 2016, the most severe lodging was observed in NIL-apo1-D4, followed by NIL-apo1-D3.

The gross brown rice yields of NIL-taw1 and NIL-apo1-D3 were not significantly different from that of Koshihikari, whereas the yield of NIL-apo1-D4 was significantly smaller. The immature brown rice weight of the three NILs was significantly larger than that of Koshihikari. As a result, the yield of the three NILs, NIL-taw1 (p < .05), NIL-apo1-D3 (not significant), and NIL-apo1-D4 (p < .01), was slightly smaller than that of Koshihikari. The yield in 2015 due to severe lodging was smaller than that in 2016, particularly, the yield of NIL-apo1-D3 and NIL-apo1-D4, the two NILs that experienced severe lodging.

The PN of NIL-taw1 was not significantly different from that of Koshihikari, whereas the PN of NIL-apo1-D3 and NIL-apo1-D4 was significantly smaller. The SN of the three NILs was significantly larger than that of Koshihikari, resulting in larger TSN in the three NILs as compared to that of Koshihikari. The TGW of the three NILs was significantly smaller and their RP was significantly lower than that of Koshihikari.
Compared to Koshihikari, the unhulled rice weight was larger in NIL-taw1 but smaller in NIL-apo1-D4, whereas it did not differ between NIL-apo1-D3 and the variety. The straw weight of NIL-taw1 and NIL-apo1-D3 was significantly smaller and that of NIL-apo1-D4 was not different from the straw weight of Koshihikari. As a result, the total dry weight of the three NILs was slightly smaller than that of Koshihikari, with a significant decrease being observed only in NIL-taw1 (p < .05).

Discussion

In this study, we showed that TAW1 and APO1 differently affect the yield-related traits, although both genes increase the SN and TSN. Most of the previous studies cited in the introduction show that the genes that increase the SN also increase the diameter of internodes, while the PN is decreased. Interestingly, our field experiments indicated that TAW1 did not affect the diameter of internodes and PN, whereas APO1 decreased the PN and increased the diameter of internodes (Tables 2 and 4). In addition, APO1, but not TAW1, decreased the number of tillers (Fukushima et al., 2015) and increased the width of leaf blade (Table 2). These results suggest that APO1, but not TAW1, affects traits that are determined before heading, such as the number of tillers, PN, the diameter of internodes and the width of leaf blade.

Among the APO1 alleles, SCM2, an APO1 allele of the indica rice variety Habataki, increases the SN, diameter of internodes, and yield but does not affect the PN (Ookawa et al., 2010). Another study reports similar effects of the same APO1 allele in Habataki on the SN and yield without affecting the PN (Terao et al., 2010). In the present study, however, we showed that dominant alleles of APO1, namely apo1-D3 and apo1-D4 identified from the mutant analysis, reduced the PN while increased the SN and diameter of the internodes (Tables 2 and 4). This discrepancy in the effects of APO1 on morphological traits suggests that the spatial and temporal patterns of gene expression vary between alleles, leading to different contributions to yield-related traits.

The effects of genes that control the SN on TWG differ slightly among the genes and their alleles. Gn1a does not affect the TGW (Ashikari et al., 2005) and OsSpL14 increases the TGW (Jiao et al., 2010). In contrast, EP (Huang et al., 2009; Wang et al., 2009), APO1 (this study), and TAW1 (this study) decrease the TGW. We revealed that TAW1 and APO1 shortened the spikelets, increased the weight of immature brown rice, and decreased the RP (Tables 3 and 4). In other words, both small spikelet size and low filling ability might restrict the TGW.

In this study, TAW1 and APO1 increased the SN and TSN but not the yield, mainly due to low RP that was attributed
to the high proportion of immature brown rice grains (Table 4). This might be because the NILs of Koshihikari did not provide a suitable genetic background for high yield due to late heading in the northern cold region of Japan (Table 1) and weak lodging resistance, which might be promoted by the longer culm in APO1-NILs. Therefore, introduction of TAW1 and APO1 into varieties with early heading and short culm might be effective to increase RP and yield in the northern cold region of Japan. Ohsumi et al. (2011) also suggested that SBN1 and PBN6, two QTLs that increase the SN, increase the TSN but not the yield due to insufficient filling ability.

In conclusion, we revealed the similarities and differences between TAW1 and APO1 in their effects on yield. The genes that increase the SN including TAW1 and APO1 have not been used actively in high-yield breeding programs in Japan probably because these genes increase the TSN but do not improve other yield-related traits, particularly filling ability. Genes and their expression pattern that are suitable for improving the yield remain to be identified. The optimal combination of genes regulating the SN and genes controlling other yield-related traits needs to be elucidated in field experiments.

Author contribution
A. F. and J. K. designed the study; M.M., A. Y., and J. K. generated the NILs; A. F., H.O., N.Y., and N. T. performed the field experiments; A. F. and J. K. wrote the paper.

Disclosure statement
No potential conflict of interest was reported by the authors.

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