

Effects of yield-related QTLs *SPIKE* and *GPS* in two *indica* rice genetic backgrounds

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are continuing efforts to develop new rice cultivars with higher yielding potential than Takanari and IR 64 to ensure future food security.

Progress in rice genomics over the last two decades has enabled breeders to do molecular breeding using DNA markers and mapping information for quantitative trait loci (QTLs). With those tools, Fujita et al. (2013) recently identified *SPIKE*, as a QTL that controls spikelet number per panicle. They confirmed that a near-isogenic line (NIL) with the *SPIKE* allele from *tropical japonica* landrace, Daringan in the IR 64 genetic background produced more number of spikelets per panicle and higher grain yield than IR 64 in the field of the IRRI under tropical condition. The results highlight the value of this allele to increase yield potential in *indica* cultivars. Takai et al. (2013) similarly identified *GREEN FOR PHOTOSYNTHESIS* (*GPS*), which is a QTL responsible for high leaf photosynthesis in Takanari. NIL-*GPS*, with the *GPS* allele from *temperate japonica* cultivar, Koshihikari, in the Takanari genetic background, showed low photosynthesis rate and did not out-yield Takanari in the field of NARO Institute of Crop Science in Japan under temperate condition. Interestingly, the causal genes for both *GPS* and *SPIKE* were identical to *NALLOW LEAF1* (*NAL1*) (Qi et al., 2008). Why did the same gene result in different final yields between *SPIKE* and *GPS*; yield increase in *SPIKE* but not increase in *GPS*? Possible reasons include allelic differences between Koshihikari and Daringan, different genetic backgrounds between the two *indica* cultivars, or different environments between the trials.

In this study, we compared sequences of *NAL1* among the four rice cultivars used in the studies by Takai et al. (2013) and Fujita et al. (2013) to identify allelic differences. Then we conducted yield trials using Takanari, IR 64, and NILs for *GPS* and *SPIKE* to evaluate their yield potential in the same experimental paddy field in Japan. We discuss the effect of *SPIKE/GPS* on yield and its components toward the future use of *SPIKE/GPS*.

Materials and methods

Plant materials and sequence analysis

Takanari is a high-yielding *indica* cultivar released in Japan. Koshihikari is a leading *temperate japonica* cultivar in Japan with good eating quality and the largest area of cultivation during the last 30 years (Takeuchi et al., 2008). IR 64 is a high-yielding *indica* cultivar released by IRRI in the Philippines. Daringan is a *tropical japonica* landrace grown in Indonesia (Fujita et al., 2013). We amplified ~1.5-kb of the promoter region of *NAL1* of each cultivar by PCR with two primer sets: 5'-CAAGAATTGGCTCCATCTCC-3' and 5'-GCGCTGTTCTCTGTGTGTGT-3'; and 5'-CAGTGGAGATTCCCGTATCCATTCC-3' and 5'-GGCAACAGTTACAGCGACCTCAAGAA-3'. We amplified

the cDNA region with the primer sets used by Takai et al. (2013). We used 35 cycles of 1 min template DNA denaturing step at 94 °C, 1 min primer annealing step at 55 °C, and 2 min primer extension step at 72 °C in PCR program. The PCR products were sequenced with a BigDye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems) in an automated fluorescent laser sequencer (3130xl; Applied Biosystems).

Field experiments

Field experiments were conducted in the experimental paddy field at the NARO Institute of Crop Science, Tsukubamirai (36°02'N, 140°04'E), Ibaraki, Japan, in 2014 and 2015. The soil is a Gleyic Fluvisol. Takanari, NIL-*GPS*, IR 64, and NIL-*SPIKE* were grown under continuous flooded conditions. NIL-*GPS* has a Koshihikari-derived chromosome segment that includes *GPS* on the long arm of chromosome 4 in the Takanari genetic background (Figure 1). NIL-*SPIKE* has a Daringan-derived chromosome segment on the long arm of chromosome 4 in the IR 64 genetic background (Figure 1). In addition to the two NILs, a chromosome segment substitution line, SL2115, which carries a Koshihikari-derived chromosome segment on the long arm of chromosome 4 in the IR 64 background (Figure 1), was also grown in 2015 to compare the difference between *SPIKE* and *GPS* in the same IR 64 background.

Seeds were sown in a seedling nursery box on 23 April 2014 and 22 April 2015, and seedlings were transplanted at one per hill on 15 May 2014 and 14 May 2015, at 22.2 hills m⁻², with 15 cm between hills and 30 cm between rows. The experimental plots (18 m² each) were arranged in a randomized complete block design with three replicates. In 2014, basal fertilizer was applied 3 days before transplanting at 12 g N m⁻² as controlled-release fertilizer (4 g LP40 and 8 g LP100), 5.2 g P m⁻², and 7.5 g K m⁻². LP40 and LP100 release 80% of their total N content at a uniform rate by 40 and 100 days, respectively, after application at 20–30 °C. At the panicle initiation stage, 6 g N m⁻² was top-dressed as LP40. In 2015, we reduced the application to 4 g N m⁻² (1.3 g LP40 and 2.7 g LP100) as a basal dressing and 2 g N m⁻² (LP40) as a top-dressing to evaluate the influence of N fertility to the effect of *SPIKE/GPS*. The applications of P and K in 2015 were the same as those in 2014.

Evaluation of biomass production, yield, and yield components

Twelve hills were sampled from each plot at the panicle initiation stage (2014), heading stage (2014 and 2015), and maturity (2014 and 2015). Heading date was defined as the date when about half of the panicles in each plot had emerged. Days-to-heading was determined as the number

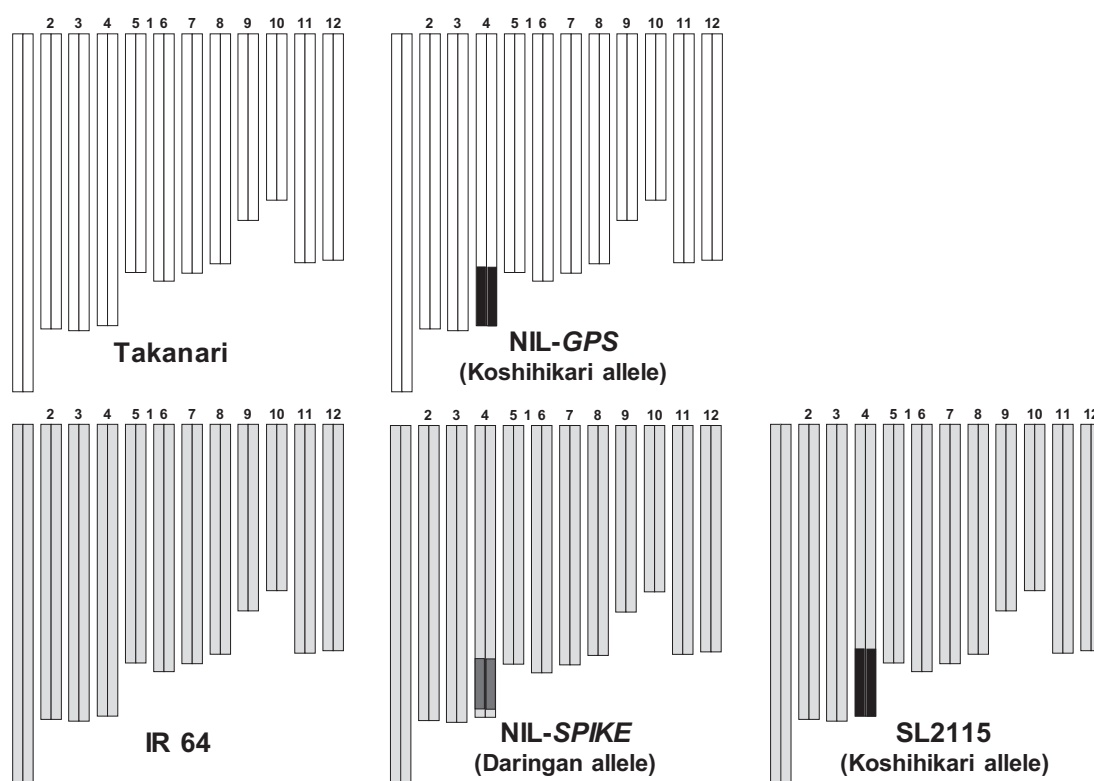


Figure 1. Graphical genotypes of Takanari, NIL-*GPS*, IR 64, NIL-*SPIKE*, and SL2115. White bars, Takanari; black bars, Koshihikari; light gray bars, IR 64; dark gray bars, Daringan.

of days from sowing to heading. In 2014, two representative hills with an average number of tillers were selected from the 12 hills, and the leaf blades were collected to measure leaf area with an area meter (AAM-9, Hayashi Denko, Tokyo, Japan). In both years, the entire shoots were dried for 72 h at 80 °C and weighed.

Leaf chlorophyll content was measured as the SPAD value with a chlorophyll meter (SPAD-502, Konica-Minolta, Japan) at the panicle initiation stage (2014), at heading (2014 and 2015), and at maturity (2014 and 2015). The top-most fully expanded leaves from the main stem were used for measurements. Six and 12 leaves in one experimental plot were used for the measurements in 2014 and in 2015, respectively.

At maturity, plants covering 2.7 m² (60 hills) were harvested from each plot for determination of yield and its components according to the method of Takai et al. (2014). Panicles were counted and then threshed to obtain unhulled grains, which were weighed and divided equally into subsamples A and B. Approximately 40 g of unhulled grains (subsampling C) was selected from subsample A and counted in an electronic seed counter (KC-10S, Fujiwara Scientific Co. Ltd., Tokyo, Japan). Spikelet number m⁻² was calculated by multiplying the grain number per unit weight in subsample C by the total weight of the unhulled grains m⁻². Spikelet number per panicle was calculated as the spikelet number m⁻² divided by panicle number m⁻².

The hulls from subsample B were subsequently removed with a rice huller (25 M, Ohya Tanzo G.K. Co., Aichi, Japan), and the hulled grains were weighed to determine brown rice yield. The grains were then screened with a grain sorter (TWS, Satake Co. Ltd., Tokyo, Japan; 1.6-mm sieve size), and the single-grain weight was calculated. The filled grain percentage was calculated as the number of screened hulled grains m⁻² divided by the spikelet number m⁻². Brown rice yield and single-grain weight were adjusted to 15% moisture content. Sink capacity was defined as single grain weight multiplied by the number of spikelets m⁻².

Statistical analysis

Statistical analyses were performed using a general linear model in the SPSS 22.0 software (IBM, Chicago, IL, USA) in each genetic background. Cultivar was considered as a fixed effect, and replication was considered as a random effect. Analysis of variance (ANOVA) was conducted to examine the effects of *GPS* or *SPIKE* on each trait in each year, because SL2115 was grown only in 2015. Significant *GPS* or *SPIKE* effects ($p < .05$) were tested using Student's *t*-test except for IR 64 genetic background in 2015. The effects in IR 64 genetic background in 2015 was tested using Tukey's test. Difference in each trait in each cultivar across the two years was also tested by ANOVA.

Results

Sequence comparisons among the four rice cultivars

Comparison of *NAL1* cDNA sequences between Takanari and Koshihikari represented six single nucleotide polymorphisms (SNPs), three of which caused amino acid substitutions, between the cultivars as well as one retrotransposon insertion in Koshihikari (Figure 2). The sequences of IR 64 and Daringan were identical to those of Takanari and Koshihikari, respectively (Figure 2). In the promoter region, there were nine polymorphisms among the four cultivars. The sequences of Takanari and IR 64 were identical (Figure 2). Koshihikari and Daringan had seven and nine polymorphisms, respectively, against the two *indica* cultivars, which indicate that two SNPs were existed between Koshihikari and Daringan in the promoter region (Figure 2). The base sequences of the two SNPs in Koshihikari were the same as those in the two *indica* cultivars.

Climate conditions and growth durations

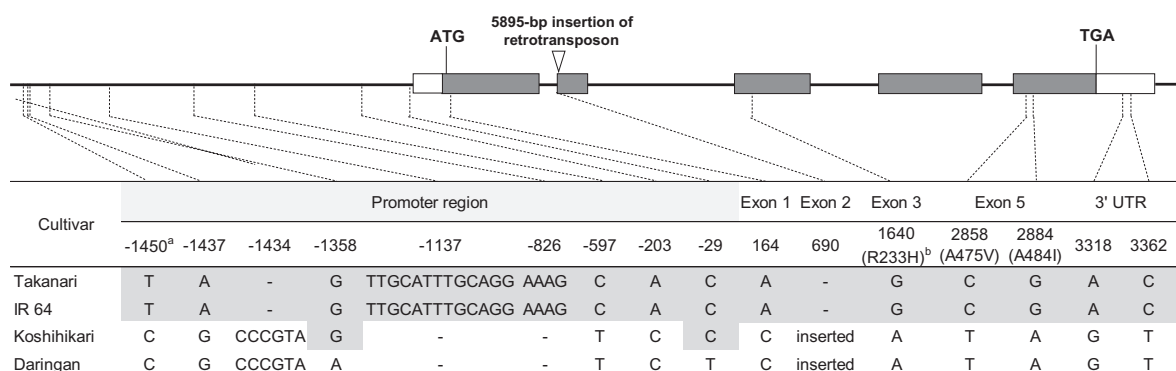
Mean temperatures during the growth period were similar in 2014 and 2015 (Figure 3(A)). They showed a gradual increase until heading in mid-August, and then a gradual decrease during grain filling until maturity. Solar radiation also showed similar patterns in 2014 and 2015, except for a remarkable decline in early July 2015 (Figure 3(B)). Radiation levels were high after transplanting in May and during the reproductive period between mid-July and mid-August. Takanari and NIL-*GPS* headed at ~105 days after sowing, and IR 64, NIL-*SPIKE*, and SL2115 headed at 108–114 days after sowing (Table 1). Accordingly, total growth durations were longer in IR 64, NIL-*SPIKE*, and SL2115 than in Takanari and NIL-*GPS* (Table 1).

Comparisons of yield and its components

In 2014, Takanari yielded 1241 g m⁻² of unhulled grain and 919 g m⁻² of brown rice (Table 2). The yield of IR 64 was lower than that in Takanari, namely 781 g m⁻² of unhulled grain and 562 g m⁻² of brown rice. In 2015, yields of unhulled grain and brown rice in Takanari were 13 and 10% lower than those in 2014 due to low N application, respectively, while the yield in IR 64 did not decrease compared with that in 2014. The yields in NIL-*GPS* and NIL-*SPIKE* also showed the similar pattern to those in their parental cultivars in both years. Consequently, NIL-*GPS*, NIL-*SPIKE*, and SL2115 did not out yield their parental cultivars in both years (Table 2).

For yield components, our field experiments confirmed that *SPIKE* controls the number of spikelets per panicle (Fujita et al., 2013): the number was significantly larger in NIL-*SPIKE* than in IR 64 in 2014 (Table 2). NIL-*GPS* and SL2115 also produced a significantly greater number of spikelets per panicle than their parental cultivars in 2015. The extent of the increase was smaller in Takanari background than that in IR 64 background. On the other hand, there was no difference in the number of spikelets m⁻² among them except for between Takanari and NIL-*GPS* in 2014, because NILs and SL2115 had fewer panicles than their parental cultivars. Takanari and NIL-*GPS*, with the Takanari genetic background, produced more spikelets m⁻² than IR 64, NIL-*SPIKE*, and SL2115, with the IR 64 genetic background, because of their large panicles (~200 vs. ~110 spikelets per panicle). However, decreased rate of spikelet number m⁻² in Takanari and NIL-*GPS* in 2015 relative to in 2014 due to low N application was >20%, while the decreased rate in IR 64 and NIL-*SPIKE* was <20%.

We found significant differences in single-grain weight and percentage of filled spikelets between the parental cultivars and the NILs or SL2115 (Table 2). The single-grain weight of brown rice in both NILs and SL2115



^a Position from start of the 5' UTR based on Takanari genome.

^b Amino acid substitution from *indica* to *japonica* cultivars

Figure 2. Gene structure and mutation sites of *NAL1* in Takanari, IR 64, Koshihikari, and Daringan. Gray bars represent exons; white bars represent 5'- and 3'-untranslated regions.

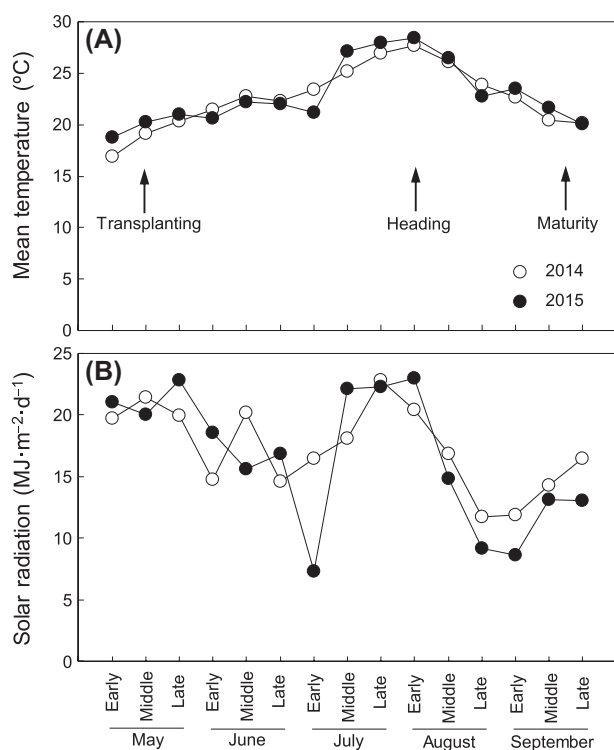


Figure 3. (A) Mean temperature and (B) solar radiation during the rice growth period measured in the experimental field.

Table 1. Days-to-heading and growth duration in five cultivars in 2014 and 2015.

Cultivar	2014		2015	
	Days-to-heading (d)	Growth duration (d)	Days-to-heading (d)	Growth duration (d)
Takanari	104	148	106	145
NIL-GPS	105	148	106	145
IR 64	108	159	111	149
NIL-SPIKE	112	159	114	149
SL2115	–	–	112	149

Note: Days-to-heading indicates the number of days from sowing to heading. Growth duration indicates the number of days from sowing to the final harvest.

was significantly lower than that in their parental cultivars except for between Takanari and NIL-GPS in 2015. NIL-SPIKE showed relatively higher percentage of filled spikelets than IR 64, while NIL-GPS did not show higher percentage of filled spikelets than Takanari.

The comparison between NIL-SPIKE and SL2115 is useful to examine the difference of the effects between SPIKE and GPS in the same IR 64 genetic background. No significant difference was observed for yield and its components except for single-grain weight between NIL-SPIKE and SL2115 in 2015, indicating that the effects on yield and its component are similar between SPIKE and GPS.

Eventually, NIL-GPS, NIL-SPIKE, and SL2115 did not have a significantly larger sink capacity than their parental

cultivars (Table 2). The number of spikelets m^{-2} was significantly correlated with brown rice yield ($r = .77, p < .05$; Table 3).

Comparisons of leaf area index (LAI), chlorophyll content, and biomass production

GPS controls leaf size and chlorophyll content (Takai et al., 2013). LAI peaked at heading in all four cultivars in 2014 (Figure 4(A) and (B)). At heading, IR 64 had 25% higher LAI than Takanari, and NIL-SPIKE and NIL-GPS had 11 and 14% higher LAI than IR 64 and Takanari, respectively, although the differences were not significant. The SPAD value, an index of leaf chlorophyll content, differed significantly between the parental cultivars and the NILs or SL after heading: Takanari had a higher SPAD value than IR 64, and the NILs and SL2115 had a significantly lower SPAD value than the parental cultivars at heading (Figure 4(C)–(E)). The differences remained significant during grain filling (Figure 4(C) and (D)).

There was no difference in biomass production between Takanari and IR 64 or between parental cultivars and the NILs or SL2115 in either year (Figure 5(A)–(C)). Despite this, Takanari and NIL-GPS produced 39–45 spikelets per unit biomass, but IR 64, NIL-SPIKE, and SL2115 produced 29–34 (Figure 6).

Discussion

The effect of sequence differences within alleles of NAL1

In this study, we found no DNA sequence difference in the promoter and cDNA regions between Takanari and IR 64 but found some differences between Takanari and Koshihikari as well as IR 64 and Daringan (Figure 2). Within these differences, one of the most remarkable differences is a retrotransposon insertion in the second exon of NAL1 both in Koshihikari and Daringan (Figure 2). Although an insertion of retrotransposon generally destroys the inserted gene and leads to loss-of-function of the gene, NAL1 was expressed in both Koshihikari and Daringan in previous studies and seems to be functional alleles (Fujita et al., 2013; Takai et al., 2013). Expression of many splice variants in NAL1 transcripts observed in Koshihikari and Takanari is another conspicuous point of this gene (Takai et al., 2013). However, it is still not clear how the retrotransposon insertion and splice variants affected the gene expression level and further expression analysis might be necessary.

Another candidate of sequence difference that may influence the effect of NAL1 is the SNPs in the cDNA region. Recently, Taguchi-Shiobara et al. (2015)

Table 2. Yield and yield components in five cultivars in 2014 and 2015.

Cultivar	Unhulled grain yield (g m ⁻²)	Brown rice yield (g m ⁻²)	No. of panicles (m ⁻²)	No. of spikelets per panicle	No. of spikelets (m ⁻²)	Filled spikelets (%)	Single-grain wt. of brown rice (mg)	Sink capacity (g m ⁻²)
2014								
Takanari	1241n.s.	919n.s.	265*	216n.s.	57,145*	69.8*	22.2*	1268n.s.
NIL-GPS	1240n.s.	871n.s.	284*	211n.s.	60,035*	64.7*	21.2*	1275n.s.
IR 64	781n.s.	562n.s.	404**	105*	42,433n.s.	54.4n.s.	23.3*	987n.s.
NIL-SPIKE	891n.s.	625n.s.	342**	123*	41,939n.s.	65.7n.s.	22.2*	931n.s.
2015								
Takanari	1083*	830*	229*	195*	44,609n.s.	87.2n.s.	21.3n.s.	952n.s.
NIL-GPS	1020*	778*	210*	206*	43,207n.s.	87.1n.s.	20.7n.s.	893n.s.
IR 64	899n.s.	678n.s.	326n.s.	111a	36,273n.s.	80.8	23.1a	838n.s.
NIL-SPIKE	849n.s.	642n.s.	280n.s.	123ab	34,281n.s.	90.3n.s.	20.8c	712n.s.
SL2115	935n.s.	699n.s.	288n.s.	130a	37,383n.s.	86.8	21.6b	809n.s.
2014 vs 2015								
Takanari	(87)*	(90)*	(87)n.s.	(90)*	(78)**	(125)**	(96)**	(75)**
NIL-GPS	(82)**	(89)*	(74)**	(97)n.s.	(72)***	(135)***	(97)*	(70)***
IR 64	(115)*	(121)*	(81)**	(106)n.s.	(85)*	(149)***	(99)n.s.	(85)*
NIL-SPIKE	(95)n.s.	(103)n.s.	(82)***	(100)n.s.	(82)***	(137)**	(94)**	(76)***

Notes: Sink capacity is defined as single-grain weight of brown rice multiplied by the number of spikelets per area. ** and * show $p < .01$ and $p < .05$, respectively, by Student's t test between the parent and its NIL within each trait and year except for IR 64 genetic background in 2015. Different letter shows $p < .05$ by Tukey's test among IR 64, NIL-SPIKE, and SL2115 within each trait in 2015.

***, **, and * show $p < .001$, $p < .01$, and $p < .05$, respectively, by student's t test for each trait within each cultivar between 2014 and 2015. n.s. shows not significant. Numerals in parentheses show percentage of each trait in 2015 relative to that in 2014 within each cultivar.

Table 3. Correlation coefficient (Pearson's r) between brown rice yield and the three yield components.

	No. of spikelets m ⁻²	Percentage of filled spikelets	Single grain wt. of brown rice
Brown rice yield	.77*	.14	-.37

* $p < .05$.

suggested that an R233H amino acid substitution in the third exon may explain the phenotypic difference for leaf morphology between *indica* and *japonica* cultivars. The *indica* cultivars Takanari and IR 64 carry the R-233 allele, and the *japonica* cultivars Koshihikari and Daringan carry the H-233 allele. This suggests that the amino acid substitution may cause the phenotypic difference both in Daringan SPIKE substitution in the IR 64 background and Koshihikari GPS substitution in the Takanari background.

The factor that may cause the difference between Koshihikari and Daringan is the two SNPs in the promoter region in *NAL1* (Figure 2). It is suggested that either or both SNPs may be related to the higher gene expression level of the Daringan allele than the IR 64 allele in young panicles (Fujita et al., 2013) although no increase of *NAL1* expression was observed in young flag leaves between Koshihikari and Takanari (Takai et al., 2013). However, these two experiments were conducted at the different organs and stages. Comparisons of gene expression level among the parental cultivars or NILs in the same stage organ such as young

panicles are necessary to conclude whether the two SNPs are the key factors to regulate the gene expression or not.

Effect of SPIKE/GPS on yield and its components

In yield trials, Takanari produced larger panicles (a larger number of spikelets per panicle) than IR 64, which resulted in a larger number of spikelets m⁻² and thus higher yield than IR 64 across the two years (Table 2). However, Takanari remarkably declined yield in 2015 compared to 2014, while yield level in IR 64 was consistently stable across the two years. Main difference between the two years is the N application level; 18 g N m⁻² in 2014 and 6 g N m⁻² in 2015. Takanari seems to require more N, but IR 64 seems to be stable under various environmental conditions including poorly fertilized one.

Our results suggest that the effects of SPIKE/GPS may be influenced by the difference in genetic backgrounds. Both Koshihikari GPS and Daringan SPIKE alleles affected the increase of the number of spikelets per panicle and decrease of chlorophyll content of leaves irrespective of the genetic backgrounds as well as regardless of the two SNPs. However, the effect of increasing the number of spikelets per panicle was smaller in the Takanari genetic background than in the IR 64 genetic background. The alleles of SPIKE/GPS might not be effective in the genetic background of high yielding *indica* cultivars with low panicle number and big panicles such as Takanari because Takanari already attains large panicles at least by the alleles of *GN1a* and *APO1* for the direction of increasing

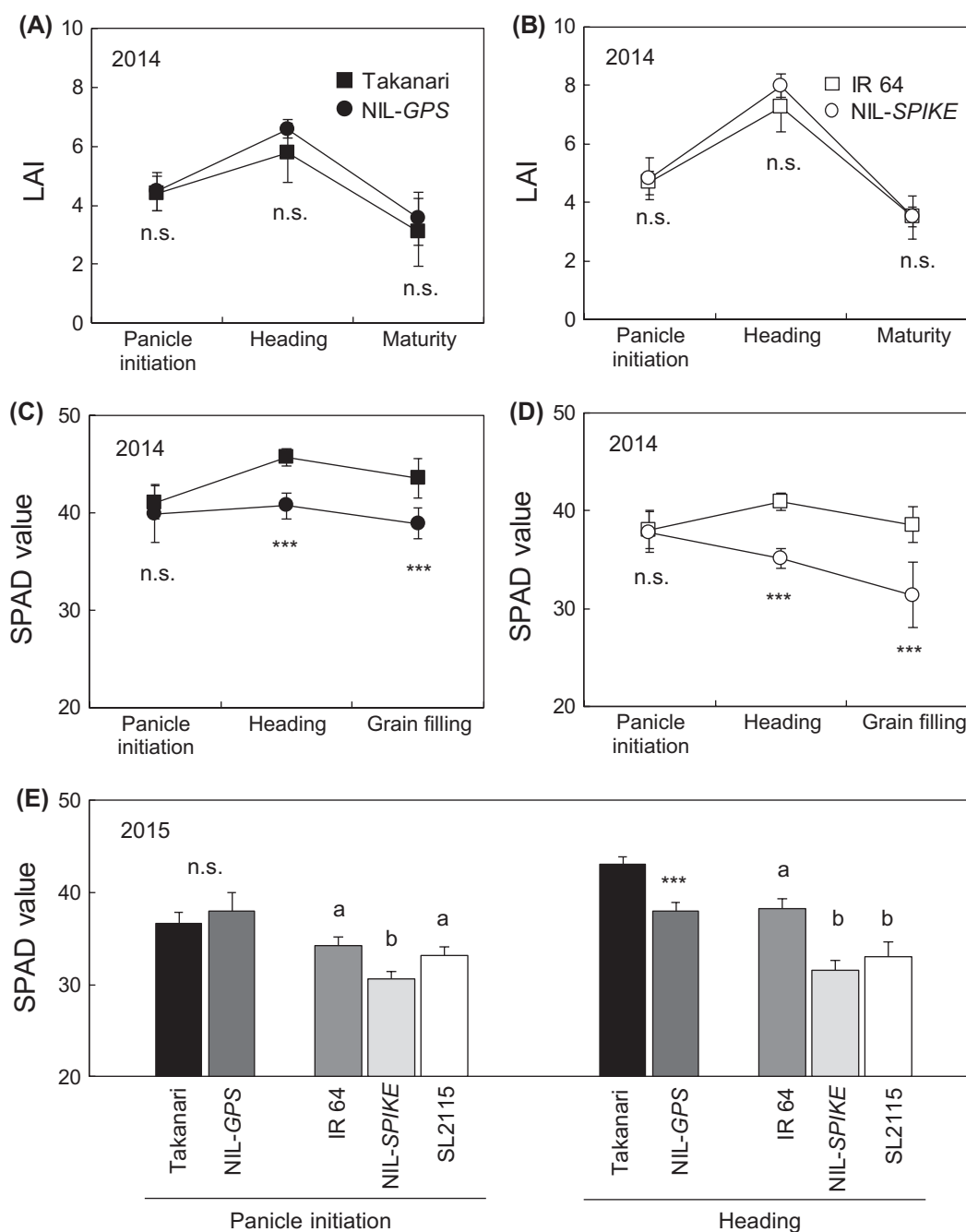


Figure 4. Change of (A, B) leaf area index (LAI) in 2014 and (C–E) chlorophyll content (as the SPAD value) in the topmost fully expanded leaves in (C, D) 2014 and (E) 2015 in five cultivars. *** shows $p < .001$ by student's t test between the parent and its NIL in 2014 and in the Takanari background in 2015. Different letter shows $p < .05$ by Tukey's test among IR 64, NIL-SPIKE, and SL2115 in 2015. n.s. shows not significant.

spikelet number (Takai et al., 2014) and hence already has higher sink capacity than IR 64. Therefore, there may not be an enough room to raise the number of spikelets in the Takanari background by *SPIKE/GPS*.

We also observed that *SPIKE/GPS* in the IR 64 genetic background improved the percentage of filled spikelets in addition to the number of spikelets per panicle. On the contrary, no increase of the percentage of filled spikelets was observed in the Takanari background. Lack of

surplus supply of carbohydrate caused by the high sink capacity in Takanari might be one of the reasons of the low improvement of filled spikelet percentages. These results also confirm that the effects of *SPIKE/GPS* on the yield components are affected by the difference in genetic backgrounds. *SPIKE/GPS* may be more useful to improve the yield components in *indica* cultivars with small panicles such as IR 64 rather than those with large panicles such as Takanari.

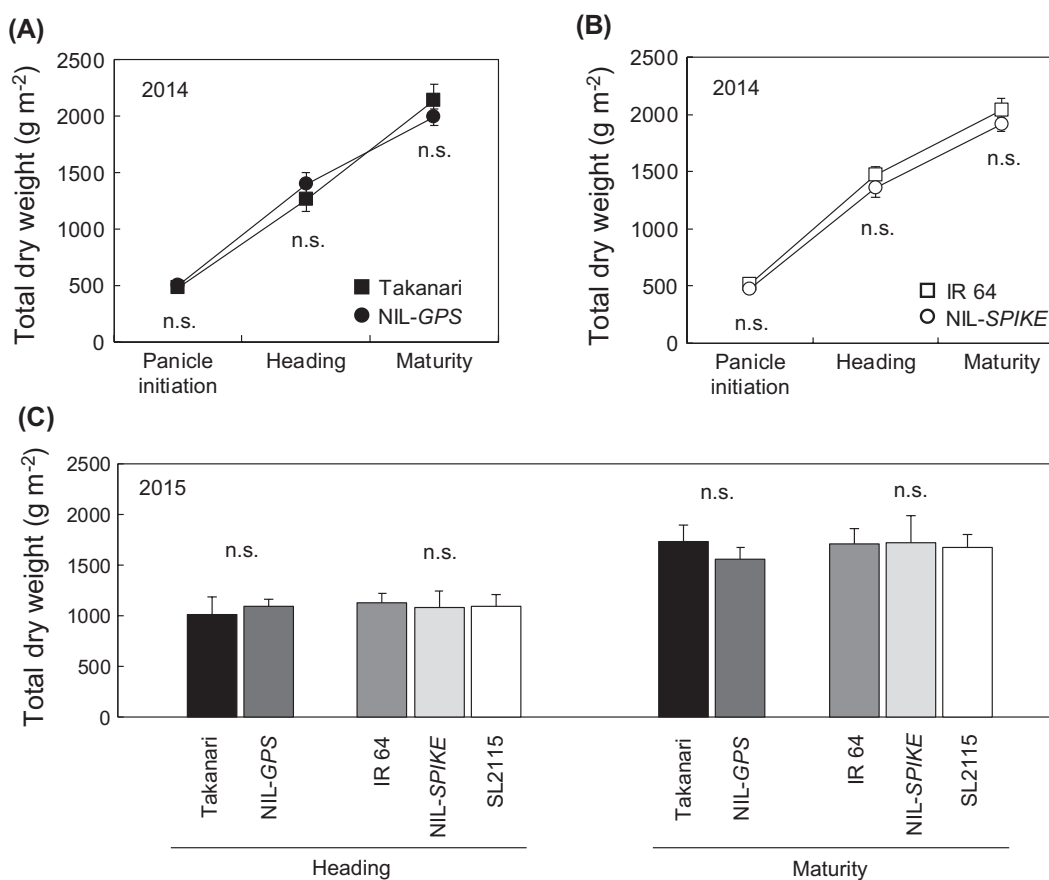


Figure 5. Change of total dry weight in (A, B) 2014 and (C) 2015 in five cultivars. n.s. shows not significant by student's *t* test between the parent and its NIL in 2014 and in the Takanari background in 2015, and by Tukey's test among IR 64, NIL-SPIKE, and SL2115 in 2015.

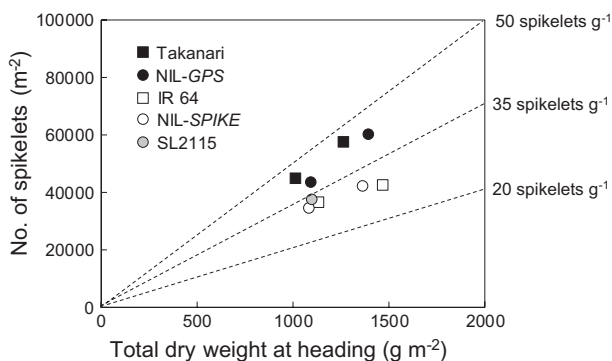


Figure 6. Relationship between total dry weight at heading and the number of spikelets m^{-2} among five cultivars. Dashed lines indicate production of stated spikelets per unit dry weight. Data from 2014 to 2015 are combined.

Despite the effects of *SPIKE/GPS* to increase the number of spikelets per panicle, none of NIL-SPIKE, SL2115, or NIL-GPS significantly out-yielded their parental cultivars under the present field conditions regardless of the amount of N applications (Table 2). No increase of final yield is considered to be caused by a trade-off between the number of spikelets per panicle and the number of panicles, which resulted in the similar number of spikelets

m^{-2} and the similar sink capacity, which significantly correlated to the final yield (Table 3). This indicates why the NILs and SL2115 did not out-yield their parental cultivars in this study.

The reason why the effect of SPIKE differs in tropics and temperate Japan

So why did NIL-SPIKE out-yield IR 64 in the study by Fujita et al. (2013)? A possible reason is the difference in vegetative growth period between their study and ours. Days-to-heading in IR 64 and NIL-SPIKE was approximately 80–90 days in the Philippines (see Figure S2 of Fujita et al., 2013) and 108–114 days in this study (Table 1). Under the tropical climate conditions such as short day and high temperature, acceleration of flowering restricts the period of vegetative growth. In such condition, IR 64 did not produce a greater number of panicles per plant compared with NIL-SPIKE (see Figure S2 of Fujita et al., 2013). On the other hand, the difference of producing tillers and hence panicles between IR 64 and NIL-SPIKE will become bigger under prolonged vegetative growth condition such as in this study, and therefore the effects of *SPIKE* to increase

the number of spikelets per panicle was canceled by the lower number of panicles (Table 2). These results suggest that *SPIKE* may have an advantage in increasing the number of spikelets m^{-2} under the conditions that tillering is restricted due to such as short vegetative growth period as observed in Fujita et al. (2013). If this principle is true, *SPIKE* may be also useful to the paddy field where vegetative growth period is restricted due to late transplanting after the harvesting of winter crops such as wheat or barley. Also, under the conditions that plants cannot attain enough tillering, growing NIL-*SPIKE* with high plant density may compensate for the fewer panicles m^{-2} and may have high sink capacity. Further trials to apply *SPIKE/GPS* to increase grain yield are necessary under conditions where plants can have adequate vegetative growth period such as in Japan.

Another possible reason is that *SPIKE/GPS* is effective under low yield level conditions as observed in Fujita et al. (2013). Yield level of IR 64 in Fujita et al. (2013) was lower than that in our low fertility experiment in 2015. In such low soil fertility condition in Fujita et al. (2013), tillering and thus the number of panicle formation are also restricted. Consequently, the effect of increasing spikelet number per panicle by *SPIKE/GPS* may have advantage under such low yield level conditions by low soil fertility.

Effect of *SPIKE/GPS* on biomass production

Large biomass production is also an important trait for achieving high yield potential (Dingkuhn et al., 2015; Peng et al., 2008; Takai et al., 2009). Although the NILs had a lower leaf chlorophyll content and a relatively greater LAI than the parental cultivars at heading (Figure 4), biomass production was not affected by *GPS* or *SPIKE* (Figure 5). This absence of an effect of *GPS* or *SPIKE* on biomass production may be another reason why the NILs and SL2115 did not out-yield the parental cultivars. Although there is no information on biomass production in the experiments by Fujita et al. (2013), NIL-*SPIKE* is possible to increase biomass production under tropical conditions. As described above, both IR 64 and NIL-*SPIKE* produced the similar panicle number in the Philippines. Under such conditions, the effect of *SPIKE* to enlarge each leaf in each tiller may raise LAI more greatly in the IR 64 background and therefore NIL-*SPIKE* may produce higher biomass than IR 64. Taken together, our results indicate that the Daringan *SPIKE* allele and the Koshihikari *GPS* allele may not play a role in increasing yield potential at least under the present field conditions, and that other genetic factors that increase sink capacity or biomass production are necessary to improve yield potential in Takanari and IR 64. Interestingly, Takanari yielded significantly greater than IR 64 despite the similar biomass production. Takanari produced more spikelets per

unit biomass than IR 64 (Figure 6). The higher efficiency of spikelet production per unit biomass in Takanari may be due to the alleles of *GN1a* and *APO1* that increase spikelet number per panicle (Takai et al., 2014). We are currently conducting genetic analysis using materials derived from a cross between Takanari and IR 64 to understand the genetic mechanisms underlying the higher efficiency of spikelet production per unit biomass and to clarify whether the genetic factors in Takanari can improve yield potential in IR 64.

Conclusion

Although two SNPs were revealed in the promoter region between Koshihikari and Daringan in the *NAL1* gene (the causal gene of both *SPIKE* and *GPS*), the effects of both alleles were similar but were affected by the difference of the genetic backgrounds. The environment factors such as different vegetative growth period and soil fertility conditions may also influence the effect of *SPIKE/GPS* on yield potential. The interaction between QTLs and environment should be important to utilize them to breeding programs and to farmers. Further yield trials across multiple environments (as well as cultivation method improvement that can compensate for the negative environmental effects) will confirm the utility of *SPIKE/GPS*.

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Disclosure statement

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