Genetic studies for breeding of rice cultivars with superior grain appearance and lodging resistance from the rice cultivar ‘Emi-no-kizuna’

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ABSTRACT
We conducted a quantitative trait locus (QTL) analysis of grain appearance (GA) and agronomic traits of rice, using 128 recombinant inbred lines derived from ‘Emi-no-kizuna’ and ‘Tomohonami’. We detected two promising QTLs associated with GA: qGA4 on chromosome 4 and qGA8 on chromosome 8. qGA4 contributed highly to the greater percentage of perfect grains of the Emi-no-kizuna genotype. In the same region, we detected other QTLs associated with panicle number and spikelet number per panicle. In near-isogenic lines (NILs) in which Emi-no-kizuna alleles were introgressed in the genomic region of only the semi-dwarf 1 (sd1) locus (NIL_1) and both the sd1 locus and qGA8 (NIL_2), respectively, the percentage of perfect grains was significantly higher and the percentages of milky white, basal white, and white back grains were significantly lower than in Tomohonami; and the percentages of milky white and white back grains of NIL_2 were significantly lower than those of NIL_1. These results suggest that introgression in the sd1 region could improve GA, and that the addition of qGA8 could further improve GA. The culm lengths of the NILs were significantly shorter than that of Tomohonami, indicating improved lodging resistance. Grain weight of NIL_2 was significantly smaller than that of NIL_1, suggesting that the effect of qGA8 could be pleiotropic, or the gene that underlies qGA8 could be linked with genes associated with grain weight.

Abbreviations: ANOVA: analysis of variance; AT20: mean air temperature in the 20 days after heading; BW: basal white grain; CL: culm length; DAH: days after heading; GA: grain appearance; GW: 1000-grain weight; LOD: logarithm of odds; MW: milky white grain; NIL: near-isogenic line; PG: perfect grain; PL: panicle length; PN: panicle number; PTSN: putative total spikelet number; PVE: percentage of phenotypic variation explained; QTL: quantitative trait locus; RIL: recombinant inbred line; SN: spikelet number per panicle; SNP: single nucleotide polymorphism; WB: white back grain

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Introduction

Summer air temperatures in Japan are rising, spoiling the grain appearance (GA) of paddy rice. A typical problem is increased chalkiness due to insufficient starch accumulation (Morita, 2008). Lowered GA decreases the market value of the rice and thus the farmers’ incomes, and milling quality. As warming is predicted to continue and cause even more serious problems (Morita, 2008), it is necessary to breed cultivars that produce less chalkiness, particularly under high temperatures during the grain filling period.

The occurrence of chalkiness differs widely among cultivars (Iida, Yokota, Kihara & Suga, 2002; Nagato & Ebata, 1965) and is heritable (Tabata, Idaka & Ohsawa, 2005). It is thus likely that we can breed cultivars with improved GA. Quantitative trait loci (QTLs) associated with GA have been reported (Kobayashi et al., 2013; Tabata et al., 2007; Wada et al., 2015), and we identified QTLs for GA in the paddy rice cultivar Emi-no-kizuna, which has stable and superior GA (Nagaoa et al., 2017). These QTLs should prove useful for the improvement of GA, but the concrete effects of QTLs identified from Emi-no-kizuna have not yet been evaluated.

Relative to the popular cultivar Koshihikari, Emi-no-kizuna has a shorter culm length, lower spikelet number per panicle, lower 1000-grain weight (GW), and a higher panicle number (Sasahara et al., 2018). As GA is negatively correlated with the number of unhulled rice grains (Asano, Kanno & Endo, 2011) and GW (Takita, 1985), these traits should be investigated, because the superior GA of Emi-no-kizuna might be the result of the lower spikelet number or GW, i.e. yield.

On the other hand, the area of paddy fields under cultivation per farmer has been steadily increasing in Japan (Yagi, 2016). The resultant need to save costs has thus increased the demand for cultivars with not only superior GA but also lodging resistance. Hence, the semi-dwarf 1 (sd1) locus (Sasaki et al., 2002), which is very effective for improving lodging resistance, should be increased in breeding. However, as introgressing the genomic region of the sd1 locus might reduce GA (Terao & Hirose, 2015) the relationship between GA and sd1 of Emi-no-kizuna should be investigated.

From this perspective, for effective breeding of rice cultivars with superior GA from Emi-no-kizuna without reduction of yield, we investigated the effects of QTLs for GA identified from Emi-no-kizuna and the relationships between GA and several agronomic traits via QTL analysis and evaluation of near-isogenic lines (NILs) by using hybrid and introgressed progeny of Emi-no-kizuna.

Materials and methods

Plant materials and examination of individual plants

We grew 180 recombinant inbred lines (RILs; F2) derived from Emi-no-kizuna and Tomohonami by the single-seed descent method and the two parents in 2016 in a paddy field at the Central Region Agricultural Research Center (Joetsu, Niigata, Japan: 37°12′29″N, 138°21′50″E). Tomohonami is derived from upland rice. It has the rice blast resistance gene pi21 and a genetic background similar to that of Koshihikari; it is prone to chalkiness and has poor resistance to heat stress (Saka et al., 2010). Seeds were sown on 26 April 2016, and 50 seedlings of each line were transplanted on 24 May in two ridges at intervals of 18 cm × 18 cm. The lines were spaced 36 cm apart. As a basal fertilizer, 5 g/m2 each of N, P2O5-equivalent, and K2O-equivalent (Nyouso-kasei toku No. 45; JA Higashinihon Kumiai Shiryou Co., Gunma, Japan) was applied. No additional fertilizer was used.

To ensure uniform grain-filling conditions, from among the 180 lines we used only the 128 lines in which panicles emerged within 3 days of the parents’ heading date. At 15 days after heading (DAH) we measured the culm length (CL), panicle length (PL), and panicle number (PN) in eight average plants per line or per parent as described by Sunohara and Horisue (1995). At 40 DAH we harvested the panicles of the same eight plants per line or per parent. We counted the spikelet number per panicle (SN) of the panicles used to measure the PL. All grains of each plant were hulled and examined. The heading date of each line was determined as the date when 50% of the panicles had headed. ‘Days to heading’ was determined as the number of days from the date of transplanting to the heading date. We defined PN × SN as the putative total spikelet number (PTSN) of a plant.

Air temperature measurement

The air temperature at the level of the panicles, about 80 cm above the ground surface, was recorded every 10 min on a data logger (model RTR-52A; T&D, Nagano, Japan).

Evaluation of GA and grain traits

We evaluated the GA and GW by using a grain quality inspector (model RQGI20A; Satake Co., Hiroshima, Japan). GW was based on 14.0% moisture content. All grains were examined and classified into perfect grains (PGs), milky white grains (MWs), basal white grains (BWs), or white back grains (WBs); the percentages of each were used as indexes of GA. Although the grain quality inspector cannot distinguish between white back and white belly, we regarded any blemish on a side face of a grain as white back, because we did not detect any white belly grains by
visual inspection in any of the lines or parents. We refer to MWS, BWs, and WBs collectively as chalkiness.

**QTL analysis**

DNA was extracted from each F$_6$ plant as described by Monna et al. (2002). We investigated the genotype by using 175 single nucleotide polymorphism (SNP) markers (Kim et al., 2016) spread throughout the genome. Linkage maps were prepared in MAPMAKER/EXP v. 3.0 software (Lander et al., 1987) using Kosambi’s mapping function (Kosambi, 1944). QTL analysis was conducted by using composite interval mapping in QTL Cartographer v. 2.5 software (Wang, Basten & Zeng, 2006). We determined a threshold value corresponding to a significance level of 5% by using 1000 permutations. When the logarithm of odds (LOD) score exceeded the threshold value, we concluded that a QTL was detected. In the analysis, the percentages of grains were arcsine-transformed to standardize dispersion.

**Breeding and evaluation of NILs**

Tomohonami and its progeny were backcrossed three times to Emi-no-kizuna. During backcrossing, we selected mother plants in which the genotypes of SNP markers at promising QTLs were heterozygous. Individuals were selected from the BC$_3$F$_2$ generation in 2016, and the BC$_3$F$_4$ lines were grown in 2018. We selected NIL_1 and NIL_2, in which Emi-no-kizuna alleles were introgressed only in the genomic regions of the sd1 locus and QTLs associated with GA. We compared several agronomic traits of these NILs with those of Tomohonami and Emi-no-kizuna. Seeds were sown on 18 April and seedlings were transplanted on 16 May. Plants were grown and evaluated as for the RILs. Following a significant analysis of variance (ANOVA) result at $P < 0.05$, the significance of differences was tested by Tukey’s test.

**Results**

**Distribution of heading dates and air temperature**

The number of days to heading of the 128 RILs ranged from 71 to 77, and that of both Emi-no-kizuna and Tomohonami was 74 days (Figure 1). The mean air temperature during the 20 DAH (AT20) ranged from 26.0 to 26.3°C, with no clear differences.

**Distributions of traits in the RILs, and correlations among traits**

All traits showed continuous distributions (Figure 2). Emi-no-kizuna had a higher percentage of PGs, lower percentages of MWS, BWs, and WBs, shorter CLs and PLs, a lower SN, and a larger PN than Tomohonami (Figure 2). The

![Figure 1](image1.png)

**Figure 1.** Distribution of days to heading in an F$_7$ hybrid population between Emi-no-kizuna and Tomohonami. Values within parentheses denote the mean air temperature in the 20 days after heading. Days to heading of both parents were 74.

![Figure 2](image2.png)

**Figure 2.** Frequency distributions of each trait in an F$_7$ hybrid population between Emi-no-kizuna and Tomohonami. Arrows, values of the parents: E, Emi-no-kizuna; T, Tomohonami; PG, perfect grain; MW, milky white grain; BW, basal white grain; WB, white back grain; CL, culm length; PL, panicle length; PN, panicle number; SN, spikelet number per panicle; PTSN, putative total spikelet number; GW, 1000-grain weight.
percentages of PGs, MWs, BWs, and WBs showed mostly significant correlations with PN, SN, and GW (Table 1).

**QTL analysis**

In the analysis of traits related to GA, two QTLs associated with the percentage of PGs were detected, one on chromosome (Chr.) 3 and one on Chr. 4 (Figure 3, Table 2). Two QTLs associated with the percentage of MWs were detected, both on Chr. 3. Three QTLs associated with the percentage of BWs were detected, one on each of Chrs. 3, 6, and 10. Two QTLs associated with the percentage of WBs were detected, one on Chr. 7 and one on Chr. 8. The QTLs associated with the percentage of WBs on Chrs. 7 and 8 corresponded with qGA7 and qGA8, respectively (Nagaoka et al., 2017).

Of the loci associated with GA, the QTL associated with the percentage of PGs on Chr. 4 explained 34.5% of phenotypic variation (percentage of phenotypic variation explained: PVE). At the end of the short arm of Chr. 3, we detected QTLs associated with the percentages of MWs and BWs at almost the same location. On the long arm of Chr. 3, QTLs associated with the percentages of PGs and MWs and PTSN were detected at almost the same location. On the long arm of Chr. 4, QTLs associated with the percentage of PGs, PN, and SN were detected at almost the same location. On the short arm of Chr. 8, QTLs associated with the percentage of WBs, SN, and GW were detected at almost the same location. We designated these newly detected QTLs associated with GA as qGA3, qGA4, qGA6, and qGA10, respectively (Figure 3).

The QTLs associated with CL and PL were detected at almost the same location on Chr. 1 with high PVEs (56.4% and 25.4%, respectively, Table 2).

**Evaluation of NILs**

In the NILs, Emi-no-kizuna alleles were introgressed in the genomic region of the sd1 locus in NIL_1 and of the sd1 locus and of qGA8 in NIL_2, and Tomohonami alleles were found in all other regions (Figure 4).

The percentage of PGs of NIL_1 was significantly higher and those of MWs, BWs, and WBs were significantly lower than those of Tomohonami (Table 3). The percentages of MWs and WBs of NIL_2 were significantly lower than those of NIL_1. The GW of NIL_2 was significantly lower than those of Tomohonami and NIL_1. The CLs of the NILs were significantly shorter than that of Tomohonami.

**Discussion**

We tested RILs derived from Emi-no-kizuna and Tomohonami. The parents headed on the same day, and all selected RILs headed within 3 days of that date. The AT20 was 26.0 to 26.3°C and was similar among heading dates (Figure 1). When AT20 exceeds around 26–27°C, the occurrence of chalkiness increases strongly (Terashima et al., 2001; Wakamatsu, Sasaki, Uezono & Tanaka, 2007). It is therefore likely that the grain filling conditions in this study were nearly uniform among the parents and RILs, and that the environmental conditions were conducive to a high incidence of chalkiness.

All traits investigated had continuous distributions (Figure 2), suggesting that several genes were associated with each trait. The percentages of PGs, MWs, BWs, and WBs showed mostly significant correlations with PN, SN, and GW (Table 1), suggesting that the traits were genetically correlated with the GA of Emi-no-kizuna.

Of the detected QTLs associated with GA, the Emi-no-kizuna allele of qGA4, on the long arm of Chr. 4, increased the percentage of PGs, with a high PVE of 34.5%. In the same region, QTLs associated with PN and SN were detected (Figure 3, Table 2). It is thus possible that the effect of qGA4 is due to the combination of PN and SN alleles. Although increases in PN and decreases in SN in the Emi-no-kizuna genotype could have been caused by a single factor because PN and SN are in a trade-off relationship (Kuroda, Abe, Ishibashi, Hirano & Murata, 1999), it is also possible that decreases in SN reduced the sink capacity per stem and resulted in

**Table 1. Coefficients of correlation between traits.**

|        | PG  | MW  | BW  | WB  | CL  | PL  | PN  | SN  | PTSN |
|--------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| MW     | -0.87*** | 0.30*** | 0.15ns | 0.19ns | 0.16ns |     |     |     |     |
| BW     | -0.62*** | 0.47*** | 0.15ns | 0.25** | 0.22* | 0.55*** |     |     |     |
| WB     | -0.57*** | 0.47*** | 0.15ns | 0.25** | 0.22* | 0.55*** |     |     |     |
| CL     | -0.12ns | 0.06ns | 0.19*  | 0.16ns |     |     |     |     |     |
| PL     | -0.25** | 0.25** | 0.15ns | 0.22* | 0.55*** |     |     |     |     |
| PN     | 0.26**  | -0.14ns | -0.25** | -0.29** | -0.09ns | -0.23** |     |     |     |
| SN     | -0.35*** | 0.26**  | 0.24** | 0.35*** | 0.24** | 0.44*** | -0.68*** |     |     |
| PTSN   | -0.16ns | 0.18*  | 0.03ns | 0.12ns | 0.21* | 0.31*** | 0.23** | 0.55*** |     |
| GW     | -0.21*  | 0.26** | 0.12ns | 0.15ns | -0.19* | 0.01ns | -0.07ns | -0.14ns | -0.28** |

***, **, and *: Significant at levels of 0.1%, 1%, and 5%, respectively. ns: Not significant at the 5% level according to Pearson product-moment correlation analysis.

PG, perfect grain; MW, milky white grain; BW, basal white grain; WB, white back grain; CL, culm length; PL, panicle length; PN, panicle number; SN, spikelet number per panicle; PTSN, putative total spikelet number; GW, 1000-grain weight.
Figure 3. Locations of quantitative trait loci (QTLs) associated with each trait. Major markers are shown on the right of each chromosome. Bars on the left of each chromosome denote intervals equal to, or higher than, the logarithm of odds (LOD) value threshold. Triangles: location of LOD peak; ▲Emi-no-kizuna increased; ◄Tomohonami increased. Shaded arrows: Loci where QTLs associated with GA were estimated to exist. The positions of the sd1 locus are shown on the left side. QTLs were not detected on chromosomes 2, 5, 9 and 11. Locations of qGA7 and qGA8 are from Nagaoka et al. (2017).

PG, perfect grain; MW, milky white grain; BW, basal white grain; WB, white back grain; CL, culm length; PL, panicle length; PN, panicle number; SN, spikelet number per panicle; PTSN, putative total spikelet number; GW, 1000-grain weight.

Table 2. Quantitative trait loci associated with traits detected by composite interval mapping analysis.

| Trait | Chr. | Nearest marker | Peak position (cM) | LOD | Additive effect | PVE (%) | LOD threshold value |
|-------|------|----------------|-------------------|-----|----------------|---------|---------------------|
| CL    | 1    | FA0749         | 162.8             | 29.35 | −6.31          | 56.4     | 3.35                |
| PL    | 1    | FA0749         | 163.1             | 10.17 | −0.54          | 25.4     | 2.88                |
| MW    | 3    | FA0199         | 1.0               | 6.78  | 2.25           | 18.1     | 2.88                |
| BW    | 3    | FA0191         | 4.4               | 4.72  | 1.26           | 11.9     | 2.97                |
| PL    | 3    | FA0209         | 60.9              | 6.43  | −2.41          | 8.3      | 3.35                |
| MW    | 3    | FA2511         | 69.7              | 2.97  | −0.28          | 6.5      | 2.88                |
| PN    | 3    | FA0935         | 143.5             | 3.11  | −71.54         | 14.1     | 2.90                |
| PG    | 3    | FA0234         | 158.1             | 2.96  | 1.59           | 8.2      | 2.77                |
| MW    | 3    | FA0234         | 158.1             | 4.27  | −1.57          | 9.9      | 2.88                |
| PG    | 4    | FA2867         | 239.9             | 3.58  | 3.24           | 34.5     | 2.77                |
| PN    | 4    | FA2867         | 256.9             | 18.96 | 1.27           | 40.3     | 3.44                |
| SN    | 4    | FA2867         | 256.9             | 26.85 | −12.37         | 52.3     | 3.57                |
| BW    | 6    | FA0369         | 21.3              | 3.08  | −1.10          | 8.9      | 2.97                |
| WB    | 7    | FA1847         | 98.8              | 4.54  | −0.91          | 10.8     | 2.87                |
| CL    | 7    | FA0465         | 109.3             | 5.30  | −2.77          | 6.9      | 3.35                |
| GW    | 8    | FA0472         | 0.0               | 3.86  | −0.25          | 10.2     | 3.08                |
| SN    | 8    | FA0472         | 2.9               | 4.08  | −3.96          | 5.4      | 3.57                |
| WB    | 8    | FA0475         | 6.9               | 5.85  | −1.07          | 16.0     | 3.08                |
| BW    | 10   | FA0573         | 8.7               | 3.19  | −1.09          | 9.2      | 2.97                |
| GW    | 10   | FA0596         | 51.3              | 4.35  | −0.27          | 11.2     | 3.08                |
| GW    | 12   | FA1767         | 69.8              | 3.59  | −0.28          | 12.1     | 3.08                |

Additive effect refers to the Emi-no-kizuna allele.
PG, perfect grain; MW, milky white grain; BW, basal white grain; WB, white back grain; CL, culm length; LOD, the logarithm of odds; PL, panicle length; PN, panicle number; SN, spikelet number per panicle; PTSN, putative total spikelet number; PVE, percentage of phenotypic variation explained by each quantitative trait locus; GW, 1000-grain weight.
improved grain filling. This explanation agrees with the findings of Shiotsu, Liu, Bian, Toyota and Kusutani (2008), who noted that the grain filling performance was improved in plants with a large PN and a small SN. Of course, Emi-no-kizuna might have a gene associated with GA in the region that might be linked with genes associated with PN and SN, because a QTL associated with the percentage of WBs has been reported in this region (Kobayashi, Genliang, Shenghai & Tomita, 2007). The evaluation of these traits with NILs will offer an effective approach to reveal the details. In addition, several genes have been reported in this region, including d17, which controls tillering (Umehara et al., 2008), and SPIKE (Fujita et al., 2013), GPS (Takai et al., 2013), and LSCHL4 (Zhang et al., 2014), which are involved in the number of spikelets, and should be taken into consideration in future research.

On the short arm of Chr. 3, we detected QTLs associated with increased percentages of MWs and BWs in the presence of the Emi-no-kizuna genotype (Figure 3, Table 2). Although we do not have enough information, GA of Emi-no-kizuna might be improved by introgressing the Tomohonami allele in this region.

We expect that qGA8, detected in our previous study (Nagaoka et al., 2017), will be useful for the improvement of GA. On the other hand, we detected a QTL associated with CL on Chr. 1 with a high PVE (56.4%) (Figure 3, Table 2), which we suspect resulted from an effect of the sd1 locus. The Emi-no-kizuna allele should be introgressed in this region, because the lodging resistance of Tomohonami is not strong (Saka et al., 2010). However, as it is unclear whether the sd1 locus of Emi-no-kizuna affects GA or not we bred NILs from Tomohonami by introgressing Emi-no-kizuna alleles in the genomic regions of the sd1 locus and qGA8 (Figure 4). The percentage of PGs of NIL_1 was significantly higher and the percentages of MWs, BWs, and WBs were significantly lower than those of Tomohonami (Table 3). Although no QTL associated with GA was detected near the sd1 locus (Figure 3), the percentages of PGs and chalkiness showed associations with the genotype of the sd1 locus in F2 lines (Figure 5). These results suggest that there is some genetic factor which improves GA

Figure 4. Graphical genotypes of near-isogenic lines NIL_1 and NIL_2. Important markers are shown on the right side of each chromosome (from IRGSP v. 1.0). The positions of sd1 and qGA8 are shown on the left side. Closed and opened regions: Tomohonami and Emi-no-kizuna alleles, respectively. Shaded arrows: Region thought to contain the indicated QTL.

Table 3. Several agronomic traits of near-isogenic lines (NILs).

| Heading date (month/day) | AT20 (°C) | PG (%) | MW (%) | BW (%) | WB (%) | CL (cm) | PL (cm) | PN (/plant) | SN (/panicle) | PTSN (/plant) | GW (g) | Putative yield (g/plant) |
|-------------------------|-----------|--------|--------|--------|--------|---------|----------|-------------|---------------|----------------|---------|-------------------------|
| NIL_1 8–3 26.2 74.9c 9.4b 8.3b 3.8b 72.8b 18.6a 16.2a 122.6a 1981a 19.6a 38.7a |          |        |        |        |        |         |          |             |                |               |         |                         |
| NIL_2 8–3 26.2 80.7b 4.5c 8.0b 2.4c 72.8b 19.4a 17.0a 128.2a 2175a 18.6b 40.4a |          |        |        |        |        |         |          |             |                |               |         |                         |
| Tomohonami 8–2 26.1 54.9d 13.1a 19.0a 8.7a 77.8a 19.4a 15.0a 114.4a 1728a 20.1a 34.9a |          |        |        |        |        |         |          |             |                |               |         |                         |
| Emi-no-kizuna 8–3 26.2 93.8a 2.1d 1.2c 0.7d 71.4b 17.8a 17.2a 90.4b 1551a 20.1a 31.2a |          |        |        |        |        |         |          |             |                |               |         |                         |

ANOVA *** *** *** *** ** ns ns *** ns *** ns

*** and **: Significant at the levels of 0.1 and 1%, respectively. ns: Not significant at the 5% level. Values followed by the same letters do not differ significantly at the 5% level according to the Tukey test.

Putative yield is defined as PTSN × GW/1000.

ANOVA, analysis of variance; AT20, the mean air temperature in the 20 days after heading; PG, perfect grain; MW, milky white grain; BW, basal white grain; WB, white back grain; CL, culm length; PL, panicle length; PN, panicle number; SN, spikelet number per panicle; PTSN, putative total spikelet number; GW, 1000-grain weight.
around the sd1 locus of Emi-no-kizuna. We suggest two possibilities. One is genetic linkage between sd1 locus and genes associated with GA, because QTLs associated with GA have been reported near sd1 locus (Dong et al., 2018; Zhu et al., 2018). The other is a pleiotropic effect of the sd1 gene. Moreover, the percentages of MWs and WBs of NIL_2 were significantly lower than those of NIL_1 (Table 3). These results suggest that GA could be further improved by the introgression of Emi-no-kizuna alleles in the qGA8 region, and that qGA8 reduces the incidence of not only WB but also MW. In addition, the CLs of the NILs were significantly shorter than that of Tomohonami (Table 3), indicating an improvement of lodging resistance. It would thus be possible to improve both GA and lodging resistance, especially by introgressing Emi-no-kizuna alleles in the region of the sd1 locus.

On the other hand, the GW of NIL_2 was significantly lower than that of NIL_1 and Tomohonami (Table 3). As an explanation for the detection of QTLs associated with GW in the same region as qGA8 (Figure 3, Table 2), the effect of qGA8 may be pleiotropic. Although the reduction of GW might be slight, because there was no significant difference in PTSN (and therefore in putative yield) between NIL_1 and NIL_2 (Table 3), further analysis of the relationship between qGA8 and yield performance is needed. As we cannot rule out a genetic linkage between genes associated with GA and GW, fine mapping in this region is desirable. Moreover, the concrete and additive effects of QTLs associated with GA, including heat tolerance, should be multilaterally investigated via the evaluation of NILs.

**Conclusion**

Introggression of Emi-no-kizuna alleles in the region of the sd1 locus could improve GA. In addition, introgression of

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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**References**

Asano, M., Kanno, H., & Endo, H. (2011). Characteristics of growth and yield component maintained quality of brown rice by number of unhulled rice. *Tohoku Agricultural Research, 64*, 33–34.*In Japanese.

Dong, Q., Zhang, Z., Wang, L., Zhu, Y., Fan, Y., Mou, T., … Zhuang, J. (2018). Dissection and fine-mapping of two QTL for grain size linked in a 460-kb region on chromosome 1 of rice. *Rice, 11*, 44.

Fujita, D., Trijatmiko, K. R., Tagle, A. G., Sapasap, M. V., Koide, Y., Sasaki, K., … Kobayashi, N. (2013). NAL1 allele from a rice landrace greatly increases yield in modern indica cultivars. *Proceedings of the National Academy of Sciences, 110*, 20431–20436.

Iida, Y., Yokota, K., Kirihara, T., & Suga, R. (2002). Comparison of the occurrence of kernel damage in rice plants grown in a heated greenhouse and in a paddy field of high temperature year. *Japanese Journal of Crop Science; 71*, 174–177.*In Japanese with English abstract.

Kim, S., Ramos, J., Ashikari, M., Virk, P. S., Torres, E. A., Nissila, E., … Jena, K. K. (2016). Development and validation of allele-specific SNP/indel markers for eight yield-enhancing genes using whole-genome sequencing strategy to increase yield potential of rice, *Oryza sativa L. Rice, 9*, 12.
Kobayashi, A., Genliang, B., Shenghai, Y., & Tomita, K. (2007). Detection of quantitative trait loci for white-back and basal-white kernels under high temperature stress in japonica rice varieties. *Breeding Science, 57*, 107–116.

Kobayashi, A., Sonoda, J., Sugimoto, K., Kondo, M., Iwasawa, N., Hayashi, T., … Shimizu, T. (2013). Detection and verification of QTLs associated with heat-induced quality decline of rice (*Oryza sativa* L.) using recombinant inbred lines and near-isogenic lines. *Breeding Science, 63*, 339–346.

Kosambi, D. D. (1944). The estimation of map distance from recombination value. *Annals of Eugenics, 12*, 172–175.

Kuroda, A., Abe, H., Ishibashi, F., Hirano, M., & Murata, T. (1999). Varietal difference in the relationship between the number of panicles per hill and the number of spikes per panicle on the main stems and on primary and secondary tillers of rice. *Japanese Journal of Crop Science, 68*, 385–389.**In Japanese with English abstract.

Lander, E. S., Green, P., Abrahamson, J., Barlow, A., Daly, M. J., Lincoln, S. E., & Newburg, L. (1987). MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics, 1*, 174–181.

Monna, L., Kizawa, N., Yoshino, R., Suzuki, J., Masuda, H., Maehara, Y., … Minobe, Y. (2002). Positional cloning of rice semidwarfing gene, *sd-1*: Rice ‘green revolution gene’ encodes a mutant enzyme involved in gibberellin synthesis. *DNA Research, 9*, 11–17.

Morita, S. (2008). Prospect for developing measures to prevent high-temperature damage to rice grain ripening. *Japanese Journal of Crop Science, 77*, 1–12.**In Japanese with English abstract.

Nagaoka, I., Sasahara, H., Matsushita, K., Maeda, H., Fukuoka, S., Yamaguchi, M., … Miura, K. (2017). Quantitative trait loci analysis of grain appearance in *Oryza sativa* L. ‘Emi-nokizuna’. *Breeding Science, 67*, 421–426.

Nagato, K., & Ebata, M. (1965). Effects of high temperature during ripening period on the development and the quality of rice kernels. *Proceeding of the Crop Science Society of Japan*, 34, 59–66.***In Japanese with English summary.

Saka, N., Fukuoka, S., Terashima, T., Kudo, S., Shirota, M., Ando, I., … Inoue, M. (2010). Breeding of a new rice variety ‘Chubu 125’ with high field resistance for blast and excellent eating quality. *Research Bulletin of the Aichi Agricultural Research Center*, 42, 171–183.**In Japanese with English abstract.

Sasahara, H., Goto, A., Shigemune, A., Nagaoka, I., Komaki, Y., Yamaguchi, M., … Miura, K. (2018). A new variety for Sushi ‘Eminokizuna’. *Bulletin of the NARO Agricultural Research for Central Region, 5*, 1–18. ***In Japanese with English summary.

Sasaki, A., Ashikari, M., Ueguchi, M., Itoh, H., Nishimura, A., Swapan, D., … Matsuoka, M. (2002). A mutant gibberellin-synthesis gene in rice: new insight into the rice variant that helped to avert famine over thirty years ago. *Nature, 416*, 701–702.

Shiotsu, F., Liu, J., Bian, J., Toyota, M., & Kusutani, A. (2008). Studies on varietal difference of ripening ability in rice – relations of morphological characteristics to ripening ability. *Japanese Journal of Crop Science, 77*, 183–190.**In Japanese with English abstract.

Sunohara, Y., & Horisue, N. (1995). Rice breeding manual; V.1. Testing methodology of yield in transplanting culture. *Miscellaneous Publication of the National Agricultural Research Center, 30*, 97–100.*In Japanese.

Tabata, M., Hribayashi, H., Takeuchi, Y., Ando, I., Iida, Y., & Ohsawa, R. (2007). Mapping of quantitative trait loci for the occurrence of white-back kernels associated with high temperatures during the ripening period of rice (*Oryza sativa* L.). *Breeding Science, 57*, 47–52.

Tabata, M., Iida, Y., & Ohsawa, R. (2005). Genetic analysis of occurrence of white-back rice and basal-white rice associated with high temperature during ripening period of rice. *Breeding Research, 7*, 9–15.**In Japanese with English abstract.

Takai, T., Adachi, S., Taguchi, F., Sanoh, Y., Iwasawa, N., Yoshinaga, S., … Yamamoto, T. (2013). A natural variant of NAL1, selected in high-yield rice breeding programs, pleiotropically increases photosynthesis rate. *Nature Scientific Reports, 3*, 2149.

Takita, T. (1985). Inheritance of grain size and the relationship between grain size and other characters in rice. *Bulletin of the National Agricultural Research Center, 3*, 55–71.***In Japanese with English summary.

Terao, T., & Hirose, T. (2015). Control of grain protein contents through SEMIDWARF1 mutant alleles: *Sd1* increases the grain protein content in *Dee geo woo gen* but not in *Reimei*. *Molecular Genetics and Genomics, 290*, 939–954.

Terashima, K., Saito, Y., Sakai, N., Watanabe, T., Ogata, T., & Akita, S. (2001). Effects of high air temperature in summer of 1999 on ripening and grain quality of rice. *Japanese Journal of Crop Science, 70*, 449–458.**In Japanese with English abstract.

Umehara, M., Hanada, A., Yoshida, S., Akiyama, K., Arite, T., Takeda, N., … Yamaguchi, S. (2008). Inhibition of shoot branching by new terpenoid plant hormones. *Nature, 455*, 195–200.

Wada, T., Miyahara, K., Sonoda, J., Tsukaguchi, T., Miyazaki, M., Tsubone, M., … Yano, M. (2015). Detection of QTLs for white-back and basal-white grains caused by high temperature during ripening period in japonica rice. *Breeding Science, 65*, 216–225.

Wakamatsu, K., Sasaki, O., Uezono, I., & Tanaka, A. (2007). Effects of high air temperature during the ripening period on the grain quality of rice in warm region of Japan. *Japanese Journal of Crop Science, 76*, 71–78.**In Japanese with English abstract.

Wang, S., Basten, C. J., & Zeng, Z. B. (2006). Windows QTL Cartographer 2.5. Raleigh, NC: Department of Statistics, North Carolina State University. Retrieved form [http://stat.gen.ncsu.edu/qtlcart/WQTLCart.htm](http://stat.gen.ncsu.edu/qtlcart/WQTLCart.htm).

Yagi, H. (2016). The Japanese paddy farming in transition and burgeoning large-scale farms. *Bulletin of the Nippon Agricultural Research Institute, 29*, 65–94.*In Japanese.

Zhang, G. H., Li, S. Y., Wang, L., Ye, W. J., Zeng, D. L., Rao, Y. C., … Qian, Q. (2014). *LSCHL4* from * japonica* cultivar, which is allelic to NAL1, increases yield of *indica* super rice 93–11. *Molecular Plant, 7*, 1350–1364.

Zhu, A., Zhang, Y., Zhang, Z., Wang, B., Xue, P., Cao, Y., … Cao, L. (2018). Genetic dissection of *qPCG1* for a quantitative trait locus for percentage of chalky grain in rice (*Oryza sativa* L.). *Frontiers in Plant Science, 9*, 1173.