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## A quantitative measure for assimilate partitioning efficiency in rice (*Oryza sativa* L.)



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### ABSTRACT

Grain filling in rice is determined by the balance between sink size and source supply. Partially filled or unfilled spikelets is produced when source supply per spikelet is insufficient. Cultivars with high assimilate partitioning efficiency, which produce fewer partially filled spikelets, would be preferable because the grains of partially filled spikelets have low market value. However, it is unclear whether there is a genotypic difference in assimilate partitioning efficiency, and there is no quantitative measure for this parameter. The objective of this study was to develop such a measure from the relation between grain-filling percentage (GFP) and percentage of filled spikelets (PFS) of cultivars with various grain-filling abilities. GFP is a dry-weight-based grain filling degree, i.e. the ratio of total hulled grain yield to the sink capacity. PFS is a spikelet-number-based grain filling degree, i.e. the proportion of the number of filled spikelets to the total number of spikelets per area. We investigated the factors associated with partitioning efficiency based on grain weight distribution during grain filling. We also determined the filling potential of empty spikelets, which are indistinguishable from unfertilized ones, under limited assimilate supply. Cultivars with a large (*indica*-dominant Takanari, *japonica*-dominant Momiroman) or moderate sink size (*indica* Kasalath, *japonica* Koshihikari) were grown in a paddy field and defoliated at full heading to limit assimilate supply. Grain weight distribution was investigated at 10, 20, and 30 days after anthesis. Yield, yield components, and shoot dry matter were measured at maturity. We found an exponential relation between GFP and PFS, which can be transformed to  $\text{Ln}(\text{PFS}) = B + A \times \text{GFP}$ , with regression coefficients  $A$  varying among cultivars. A lower regression coefficient shows a higher assimilate partitioning efficiency and production of fewer partially filled spikelets. In *indica* and *indica*-dominant cultivars, which have lower regression coefficients, the difference in the weight of fast- and slowly-growing grains was larger than in *japonica* and *japonica*-dominant cultivars, suggesting an association between assimilate partitioning efficiency and the initial grain growth rate of fast-growing superior spikelets. We found that empty spikelets caused by low assimilate supply were fertilized and had a filling potential. In conclusion, the results suggest the regression coefficient between GFP and  $\text{Ln}(\text{PFS})$  differs among cultivars with different grain filling abilities, and could be used as a quantitative measure of assimilate partitioning efficiency in rice breeding.

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### 1. Introduction

Grain filling is a key process that determines grain yield and quality of rice (*Oryza sativa* L.). Grain filling often limits grain yield, especially in rice cultivars with numerous spikelets per panicle (extra-heavy panicle type; Peng et al., 1999; Yang and Zhang, 2010). A large sink size or high sink activity does not seem to enhance

photosynthetic activity (Murchie et al., 2002; Ohsumi et al., 2011); therefore, a proportional increase in source capacity is necessary. Source supply is affected by environmental conditions such as solar radiation and air temperature and often does not match sink capacity.

Partially filled or unfilled spikelets is produced when source supply is insufficient. Grains of partially filled spikelets have low market value, and it would be preferable to have cultivars with a large sink size that efficiently partition assimilates to a limited number of spikelets to produce more fully filled spikelets under limited assimilate supply per spikelet. In this study, we regarded cultivars with higher assimilate partitioning efficiency as those that produce a fewer partially filled spikelets. Genotypic differences

Abbreviations: DAA, days after first anthesis; GFP, grain filling percentage; PFS, percentage of filled spikelets.

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in the production of partially filled spikelets have been reported (Jeng et al., 2006; Khush and Peng, 1996). However, partially filled spikelets may be affected by sink–source status and there is no quantitative measure for assimilate partitioning efficiency that is independent of assimilate availability per spikelet.

What plant traits are associated with partitioning efficiency? One of the key traits may be synchronous grain filling among spikelets in a single panicle. In general, earlier-flowering superior spikelets, located on apical primary branches, are filled fast and produce large and heavy grains, whereas later-flowering inferior spikelets, located on proximal secondary branches, are filled slowly and poorly and produce grains of low market value (Iwasaki et al., 1992; Mohapatra et al., 1993). Grain-filling rates of superior and inferior spikelets are positively correlated with the levels of cytokinins and abscisic acid, and are negatively correlated with ethylene levels (Yang et al., 2000, 2006). Growth and development of inferior spikelets are improved by application of gibberellic acids, kinetin (Patel and Mohapatra, 1992), or abscisic acid (Zhang et al., 2009). Ethylene inhibitors promote grain filling of inferior spikelets, whereas ethylene promoters have the opposite effect (Mohapatra et al., 2000; Naik and Mohapatra, 2000). Conducting tissues that supply superior spikelets are more developed than those that supply inferior spikelets (Chaudhry and Nagato, 1970; Nishiyama, 1983). Genotypic differences in the grain filling patterns of superior and inferior spikelets have been reported among various types of rice cultivars (Yamagishi et al., 1996; Yang et al., 2000), but it is unclear whether these differences are intrinsic or are affected by the changing environment (especially when the sink–source status changes), and whether these differences are associated with the grain-filling degree and assimilate partitioning efficiency.

In this study, we focused on the relation between the grain-filling percentage (GFP) and the percentage of filled spikelets (PFS). GFP is a dry-weight-based grain filling degree, i.e. the ratio of total hulled grain yield to the sink capacity. PFS is a spikelet-number-based grain filling degree, i.e. the proportion of the number of filled spikelets to the total number of spikelets per area. In cultivars with higher assimilate partitioning efficiency, which produce a fewer partially filled spikelets, a decrease in PFS with that in GFP is expected to be smaller. The objective of this study was to develop a measure of partitioning efficiency from this relation in cultivars that differ in grain filling; we used two cultivars with a large sink size and two with a moderate sink size under different assimilate supply conditions. We further tried to associate the grain-weight distribution in a panicle with partitioning efficiency. To characterize grain-weight distribution during grain filling, we investigated the differences in the intra-panicle distribution of grain weight in control and defoliated plants (i.e., plants with reduced assimilate supply).

In extra-heavy panicle type cultivars or in shaded plants, source capacity is insufficient, and many empty spikelets occur; such spikelets are indistinguishable from, and are sometimes counted together with, unfertilized spikelets (Kobata et al., 2006; Yoshinaga et al., 2013). To clarify whether fertilization failure is responsible for the increased number of empty spikelets under insufficient assimilate supply, we also examined the effect of panicle clipping on the percentage of empty spikelets.

## 2. Materials and methods

### 2.1. Plant materials

Field experiments were conducted at Ishikawa Prefectural University, Nonouchi, Japan (36°30'N, 136°35'E) in 2010 and 2012 in a Gray Lowland soil. Four cultivars were used: Koshihikari, Kasalath, Momiroman, and Takanari. Koshihikari (*japonica*) is a Japanese cul-

tivar with high eating quality, which is sensitive to lodging. Kasalath (*indica*) is an Indian landrace with low yield due to its sensitivity to lodging (Madoka et al., 2008). Momiroman and Takanari are Japanese high-yielding multi-purpose cultivars; Momiroman *japonica*-dominant and Takanari is *indica*-dominant (Yamamoto et al., 2010). Momiroman and Takanari are extra-heavy panicle type cultivars with large sink and Koshihikari and Kasalath have moderate sink.

Seeds were sown in a seedling nursery box and seedlings were transplanted to a paddy field. In 2010, 30-day-old seedlings (age of approximately 4.5–5.0 in leaf number) were transplanted on 20 May; in 2012, 25-day-old seedlings (age of approximately 4.5 in leaf number) were transplanted on 18 May (one seedling per hill, 15 cm between hills in rows 30 cm apart, or 22.2 hills m<sup>-2</sup>). Koshihikari and Kasalath received a total of 6 g nitrogen m<sup>-2</sup> (4 g m<sup>-2</sup> as basal dressing and 2 g m<sup>-2</sup> as topdressing) in 2010 and 4 g m<sup>-2</sup> (2 g m<sup>-2</sup> as basal dressing and 2 g m<sup>-2</sup> as topdressing) in 2012. Basal dressing was reduced in 2012 because of lodging in these cultivars in 2010. For Takanari and Momiroman, basal dressing was the same as for Koshihikari and Kasalath, but topdressing was 4 g m<sup>-2</sup> in both years. Nitrogen was applied as ammonium sulfate; basal fertilizer was applied at puddling, and topdressing was applied at 18–20 days before heading. Phosphorus (12 g m<sup>-2</sup> as calcium superphosphate) and potassium (15 g m<sup>-2</sup> as potassium chloride) were applied to all plots as basal fertilizers. Weeds, insects, and diseases were controlled with standard chemicals as necessary.

### 2.2. Defoliation treatment

At full heading (the day when 80% of all panicles headed), blades of all flag leaves were cut off. Plots were arranged in a split-plot design (with cultivars as main plots and defoliation treatment as subplots of 9 m<sup>2</sup>) with three replicates.

### 2.3. Dry matter and yield

At maturity, 12 plants were sampled for the determination of shoot dry matter from each subplot, oven-dried for 72 h at 80 °C, and weighed. Another 12 plants were harvested for the determination of yield and yield components. Panicles were counted, threshed, and spikelets were counted with an auto-counter. The proportions of spikelets with different degrees of filling were determined by using NaCl solutions of different specific gravities (SG = 1.20, = 1.13, or = 1.06). We regarded spikelets with SG of >1.06 as filled. Spikelets with SG of ≤1.06 were sorted into partially filled and unfilled by pressing with a finger. Unfilled spikelets were those in which swell of grains was not sensed; among them, those with the enlarged (but not of full length) remains of the ovary were considered as aborted and the rest as empty.

Single-filled-grain weight of filled spikelets was determined and adjusted to 14% moisture content. Sink capacity was defined as single-grain weight of filled spikelets multiplied by the total number of spikelets per area. GFP was calculated as the total hulled grain yield divided by sink capacity. PFS was the number of filled spikelets divided by the total number of spikelets per area.

### 2.4. Grain weight distribution

Panicles of main stems (or stems of similar sizes) in which the first anthesis was observed on the same day were tagged (one panicle per plant; 15 panicles per subplot). At 10, 20, and 30 days after first anthesis of the panicle (DAA), five panicles per subplot were harvested and dried at 80 °C for 48 h. Hulled grains of all spikelets, including the remains of ovaries of unfilled spikelets, were weighed.

### 2.5. Panicle-clipping experiment

In each subplot, another 10 panicles were tagged as in 2.4. The upper halves of five of them were clipped at 10 DAA and another five tagged panicles were left intact. At maturity, panicles were harvested, and the proportions of empty spikelets in the lower halves of the intact and clipped panicles were determined as described in 2.3.

### 2.6. Statistical analysis

Analysis of variance (ANOVA) was performed in SPSS v. 21 (SPSS Inc., Chicago, IL, USA) according to the split-plot design to assess varietal differences, the effects of defoliation, and the effects of cultivar  $\times$  defoliation interactions. In the panicle-clipping experiment, the significance of the difference between mean values was analyzed using Tukey's test ( $P < 0.05$ ) for each cultivar. The homogeneity of regression coefficients between GFP and Ln(PFS) was tested according to Gomez and Gomez (1976).

## 3. Results

The heading dates of Koshihikari and Kasalath were nearly the same, but the period between heading and maturity was 4–6 days longer in Koshihikari than in Kasalath in both years (Table 1). Momiroman headed 4–6 days later than Takanari and the period between heading and maturity was 6–10 days longer in Momiroman. Solar radiation during the filling period of Kasalath and Koshihikari was similar in both years, but was lower in the second half of the filling period of Takanari and Momiroman in 2010 than in 2012.

In both years, the number of spikelets averaged over treatments was 7%–10% larger in Takanari than in Momiroman, whereas single-grain weight was 10%–17% smaller, resulting in similar sink capacity (Table 2). The sink capacity in 2012 was markedly lower than in 2010, owing to reduced fertilizer. The PFS and GFP were decreased by defoliation. In both years, the PFS and GFP were higher in Takanari than in Momiroman, but the cultivar difference in PFS was mostly larger than that in GFP. Shoot dry weight and hulled grain yield were decreased by defoliation and there were no cultivar differences in these parameters averaged over treatments.

The number of spikelets was 45% larger in Kasalath than in Koshihikari, whereas single-grain weight was 30% smaller, resulting in a similar sink capacity in both years (Table 3). The PFS and GFP were decreased by defoliation. The PFS was higher in Kasalath than in Koshihikari, whereas there was no significant difference in GFP. Shoot dry weight and hulled grain yield were decreased by defoliation and there were no cultivar differences in these parameters.

In each cultivar, there was an exponential relation between GFP and PFS,  $PFS = B \times e^{A \times GFP}$ , which can be transformed to  $\ln(PFS) = B + A \times GFP$ , where  $A$  is a regression coefficient and  $B$  is a constant (Fig. 1). An exponential function fitted the relation between GFP and PFS better than a linear function. The  $A$  values varied widely among cultivars, from 0.0153 in Kasalath to 0.0281 in Koshihikari, and were lower in *indica* and *indica*-dominant cultivars than in *japonica* and *japonica*-dominant cultivars. A test for homogeneity of regression coefficients revealed a significant difference among cultivars ( $F$  value = 4.75;  $P < 0.05$ ).

The proportion of spikelets with  $SG > 1.13$  was larger in Takanari than in Momiroman (Table 4). There was a significant interaction between cultivar and defoliation treatment. The decrease in the proportion of spikelets with  $SG > 1.13$  caused by defoliation was stronger in Takanari than in Momiroman. The proportion of partially filled spikelets was higher and was increased by defoliation

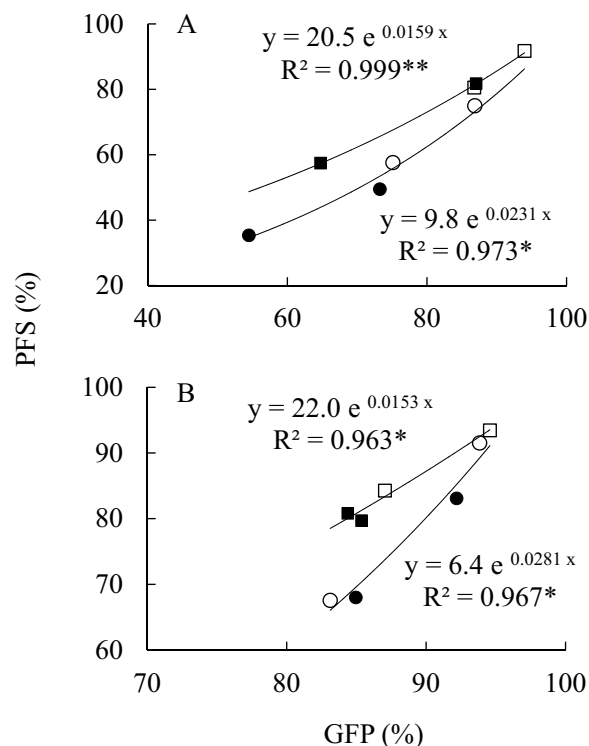


Fig. 1. Relation between the grain-filling percentage (GFP) and the percentage of filled spikelets (PFS) in (A) Momiroman (circle) and Takanari (square) and in (B) Koshihikari (circle) and Kasalath (square). Closed, 2010; open, 2012.

\*significant at the 0.05 level.

\*\* significant at the 0.01 level.

stronger in Momiroman than in Takanari, resulting in a significant interaction between cultivar and defoliation. The increase in the proportion of empty spikelets by defoliation was greater in Takanari than in Momiroman, resulting in a significant interaction between cultivar and defoliation in 2010.

The proportion of spikelets with  $SG > 1.13$  was larger in Kasalath than in Koshihikari (Table 5). The proportion of partially filled spikelets was higher and was increased by defoliation stronger in Koshihikari than in Kasalath, resulting in a significant interaction between cultivar and defoliation. The increase in the proportion of aborted spikelets by defoliation was greater in Kasalath than in Koshihikari, resulting in a significant interaction between cultivar and defoliation in 2010. The proportions of empty spikelets were relatively low in these cultivars and there were no significant effects of cultivar or treatment.

Defoliation increased the proportion of empty spikelets by 5 percentage points in Momiroman and by 10 percentage points in Takanari in comparison with control plants (Fig. 2; similar results were obtained in 2012, data not shown). In both cultivars, defoliation plus panicle clipping reduced the proportion of empty spikelets in comparison with defoliation only. Panicle clipping marginally reduced the proportion of empty spikelets in control plants. There was no significant difference in the percentage of empty spikelets between the treatments in Koshihikari and Kasalath, although there was a slight increase in defoliated but not clipped Koshihikari plants (Fig. 2).

More grains attained  $>80\%$  of the single-grain dry weight of filled spikelets at 10 and 20 DAA and more grains remained at  $<10\%$  at 20 DAA in Takanari than in Momiroman (Table 6). Defoliation increased grains  $<10\%$  (although the difference was not significant at 10 DAA in 2010) and decreased grains  $>80\%$  at 20 DAA in 2012. There were no interactions between cultivar and treatment in these parameters. Grain weight distribution confirmed that there were

**Table 1**  
Dates of heading and maturity and meteorological data during the grain-filling period of four cultivars in 2010 and 2012.

	Heading (H)	Maturity (M)	Solar radiation		Mean temperature	
			Mj m <sup>-2</sup>		°C	
			H-20 DAH	21 DAH-M	H-20 DAH	21 DAH-M
2010						
Koshihikari	2-Aug	13-Sep	14.7	14.5	28.6	28.1
Kasalath	3-Aug	10-Sep	14.7	16.3	28.6	29.0
Takanari	6-Aug	17-Sep	14.3	12.7	28.5	26.5
Momiroman	10-Aug	1-Oct	14.6	11.1	28.6	25.6
2012						
Koshihikari	2-Aug	12-Sep	15.9	14.8	27.8	26.7
Kasalath	4-Aug	8-Sep	15.9	15.1	27.8	26.8
Takanari	4-Aug	15-Sep	15.9	14.7	27.8	26.3
Momiroman	10-Aug	25-Sep	15.3	14.1	28.3	24.7

H, heading; M, maturity; DAH, days after heading.

**Table 2**  
Shoot dry weight at maturity, hulled grain yield, and yield components in control and defoliated Momiroman and Takanari plants.

Cultivar	Treatment	Shoot dry weight g m <sup>-2</sup>	Sink capacity g m <sup>-2</sup>	No. of spikelets m <sup>-2</sup>	Percentage of filled spikelets (PFS) %	Single-grain weight mg	Hulled grain yield g m <sup>-2</sup>	Grain filling percentage (GFP) %
2010								
Momiroman	Control	1825	1126.6	44448	49.5	25.4	821.1	73.3
	Defoliated	1503	1197.2	47229	35.4	26.5	678.3	54.5
Takanari	Control	1702	1072.6	49796	81.8	21.6	926.2	87.0
	Defoliated	1454	1041.9	48358	57.4	21.5	665.1	64.8
Cultivar (A)		ns	ns	ns	b	a	ns	b
Treatment (B)		a	ns	ns	b	ns	b	b
A × B		ns	ns	ns	ns	ns	ns	ns
2012								
Momiroman	Control	1581	820.5	37063	75.0	22.2	712.6	86.9
	Defoliated	1386	770.2	34774	57.6	22.8	603.7	75.1
Takanari	Control	1499	810.1	38983	91.7	20.8	761.4	94.0
	Defoliated	1217	831.2	39972	80.5	19.8	687.8	86.8
Cultivar (A)		ns	ns	ns	b	b	ns	b
Treatment (B)		a	ns	ns	b	ns	a	b
A × B		ns	ns	ns	ns	ns	ns	ns

ns, not significant by ANOVA.

<sup>a</sup> Significant at the 0.05 level.<sup>b</sup> Significant at the 0.01 level.**Table 3**  
Shoot dry weight at maturity, hulled grain yield, and yield components in control and defoliated Koshihikari and Kasalath plants.

Cultivar	Treatment	Shoot dry weight g m <sup>-2</sup>	Sink capacity g m <sup>-2</sup>	No. of spikelets m <sup>-2</sup>	Percentage of filled spikelets (PFS) %	Single-grain weight mg	Hulled grain yield g m <sup>-2</sup>	Grain filling percentage (GFP) %
2010								
Koshihikari	Control	1681	690.4	31190	83.1	22.1	634.9	92.2
	Defoliated	1316	697.7	31507	68.0	21.6	576.0	85.0
Kasalath	Control	1384	687.9	44976	91.5	15.3	616.0	93.6
	Defoliated	1337	698.1	45661	79.7	15.4	594.2	85.4
Cultivar (A)		ns	ns	b	b	b	ns	ns
Treatment (B)		a	ns	ns	b	ns	a	b
A × B		ns	ns	ns	ns	ns	ns	ns
2012								
Koshihikari	Control	1121	559.2	25333	91.5	22.1	524.7	93.8
	Defoliated	1013	525.0	23785	67.6	21.2	418.6	83.1
Kasalath	Control	1233	570.6	36777	93.5	15.5	539.7	94.6
	Defoliated	1125	537.2	34646	84.3	15.1	457.1	87.0
Cultivar (A)		ns	ns	b	b	b	ns	ns
Treatment (B)		a	ns	ns	b	ns	b	b
A × B		ns	ns	ns	ns	ns	ns	ns

ns, not significant by ANOVA.

<sup>a</sup> Significant at the 0.05 level.<sup>b</sup> Significant at the 0.01 level.

**Table 4**  
Proportions of empty, aborted, partially filled and filled spikelets with different specific gravities (SG) in Momiroman and Takanari plants.

Cultivar	Treatment	Proportion of spikelets with SG > 1.13 (%)	Proportion of spikelets with SG ≤ 1.13 and >1.06 (%)	Proportion of partially filled spikelets (%)	Proportion of aborted spikelets (%)	Proportion of empty spikelets (%)
2010						
Momiroman	Control	11.4	38.0	35.1	1.8	13.5
	Defoliated	9.7	25.6	47.6	2.3	14.7
Takanari	Control	57.3	24.5	9.7	1.9	6.6
	Defoliated	39.1	18.4	14.3	8.0	20.2
Cultivar (A)		b	a	b	ns	ns
Treatment (B)		a	a	a	ns	a
A × B		a	ns	a	ns	a
2012						
Momiroman	Control	16.4	58.5	16.1	4.0	5.0
	Defoliated	15.9	41.7	30.9	4.3	7.2
Takanari	Control	83.3	8.5	3.5	1.1	3.6
	Defoliated	60.9	19.7	7.4	4.4	7.7
Cultivar (A)		b	b	b	ns	ns
Treatment (B)		a	ns	a	ns	a
A × B		a	ns	a	ns	ns

ns, not significant by ANOVA.

<sup>a</sup> Significant at the 0.05 level.

<sup>b</sup> Significant at the 0.001 level.

**Table 5**  
Proportions of empty, aborted, partially filled and filled spikelets with different specific gravities (SG) in Koshihikari and Kasalath plants.

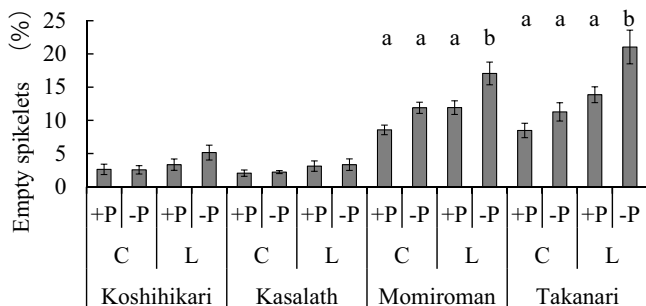
Cultivar	Treatment	Proportion of spikelets with SG > 1.13 (%)	Proportion of spikelets with SG ≤ 1.13 and >1.06 (%)	Proportion of partially filled spikelets (%)	Proportion of aborted spikelets (%)	Proportion of empty spikelets (%)
2010						
Koshihikari	Control	38.9	44.2	14.0	0.3	2.6
	Defoliated	29.8	38.2	25.1	1.4	5.5
Kasalath	Control	80.4	11.1	5.6	0.7	2.1
	Defoliated	69.4	10.3	10.6	6.6	3.1
Cultivar		c	c	b	ns	ns
Treatment (B)		a	ns	a	a	ns
A × B		ns	ns	a	a	ns
2012						
Koshihikari	Control	62.3	29.2	3.6	2.2	2.7
	Defoliated	32.9	34.7	24.5	5.2	2.7
Kasalath	Control	91.3	2.2	2.1	2.5	2.0
	Defoliated	78.1	6.1	5.3	7.9	2.5
Cultivar (A)		ns	ns	c	ns	ns
Treatment (B)		a	ns	a	a	ns
A × B		ns	ns	a	ns	ns

ns, not significant by ANOVA.

<sup>a</sup> Significant at the 0.05 level.

<sup>b</sup> Significant at the 0.01 level.

<sup>c</sup> Significant at the 0.001 level.



**Fig. 2.** The percentage of empty spikelets in the lower halves of primary rachis branches of control (C) and defoliated (L) plants with (+P) or without (-P) panicle clipping in 2010.

Values for each cultivar with the same letter are not significantly different ( $P=0.05$ , Tukey's test). Error bars represent standard errors ( $n=3$ ).

more heavy and light grains in Takanari than in Momiroman (Fig. 3; similar results were obtained in 2012, data not shown). In Takanari, fast-growing grains were heavier than those in Momiroman at 10 DAA in both treatments. There were two distinct peaks of heavy and light grains with few grains in between in Takanari at 20 and in defoliated Takanari at 30 DAA, whereas grain weight was distributed evenly at 20 DAA and the peak of heavy grains was lower at 30 DAA in Momiroman in both treatments in comparison with Takanari. Mean grain weight was consistently higher in Takanari than in Momiroman (Table 6). Mean grain weight was decreased by defoliation at 20 DAA. There was a significant interaction between cultivar and defoliation in this parameter at 20 DAA because the effect of defoliation was larger in Takanari than in Momiroman.

More grains attained >80% of the single-grain dry weight of filled spikelets at 10 and 20 DAA and more grains remained at <10% at 10 DAA in Kasalath than in Koshihikari (Table 7). Defoliation increased grains <10% at 10 and 20 DAA in both years and decreased grains

**Table 6**

Mean grain dry weight (GW) and the percentage of grains with dry weight of <10% or >80% of the GW of filled spikelets 10 and 20 days after first anthesis (DAA) in control and defoliated Momiroman and Takanari plants.

		10 DAA			20 DAA		
		<10%	>80%	mean GW	<10%	>80%	mean GW
2010							
Momiroman	Control	68.7	0.0	1.7	18.0	18.6	8.9
	Defoliated	84.1	0.0	1.6	27.3	16.8	8.5
Takanari	Control	51.0	1.4	4.5	27.7	52.9	11.6
	Defoliated	63.0	0.0	2.8	42.9	44.7	9.3
Cultivar (A)		a	ns	b	c	c	b
Treatment (B)		ns	ns	ns	c	a	a
A × B		ns	ns	ns	ns	ns	a
2012							
Momiroman	Control	65.7	0.0	1.8	15.8	20.5	9.4
	Defoliated	66.4	0.0	1.7	23.5	22.9	9.1
Takanari	Control	46.4	1.9	4.6	23.0	56.1	12.3
	Defoliated	55.1	1.1	3.8	39.8	48.3	10.0
Cultivar (A)		c	c	c	c	c	c
Treatment (B)		a	ns	ns	c	ns	a
A × B		ns	ns	ns	ns	ns	a

ns, not significant by ANOVA.

<sup>a</sup> Significant at the 0.05 level.

<sup>b</sup> Significant at the 0.01 level.

<sup>c</sup> Significant at the 0.001 level.

**Table 7**

Mean grain dry weight (GW) and the percentage of grains with dry weight of <10% or >80% of the GW of filled spikelets 10 and 20 days after first anthesis (DAA) in control and defoliated Koshihikari and Kasalath plants.

		10 DAA			20 DAA		
		<10%	>80%	mean GW	<10%	>80%	mean GW
2010							
Koshihikari	Control	28.3	0.0	5.0	11.7	53.4	13.6
	Defoliated	38.1	0.0	3.7	14.1	35.8	12.1
Kasalath	Control	33.5	11.3	5.5	10.2	70.3	12.0
	Defoliated	48.1	7.4	4.8	20.8	65.8	10.2
Cultivar (A)		b	c	a	ns	c	b
Treatment (B)		c	ns	b	b	a	b
A × B		ns	ns	ns	ns	ns	ns
2012							
Koshihikari	Control	23.6	0.0	5.4	10.0	60.3	14.9
	Defoliated	31.0	0.0	4.6	12.3	49.8	13.2
Kasalath	Control	30.2	18.8	5.9	3.7	70.6	12.1
	Defoliated	45.6	19.7	5.2	13.1	68.7	11.1
Cultivar (A)		b	c	ns	ns	c	b
Treatment (B)		b	ns	b	b	ns	b
A × B		ns	ns	ns	ns	ns	ns

ns, not significant by ANOVA.

<sup>a</sup> Significant at the 0.05 level.

<sup>b</sup> Significant at the 0.01 level.

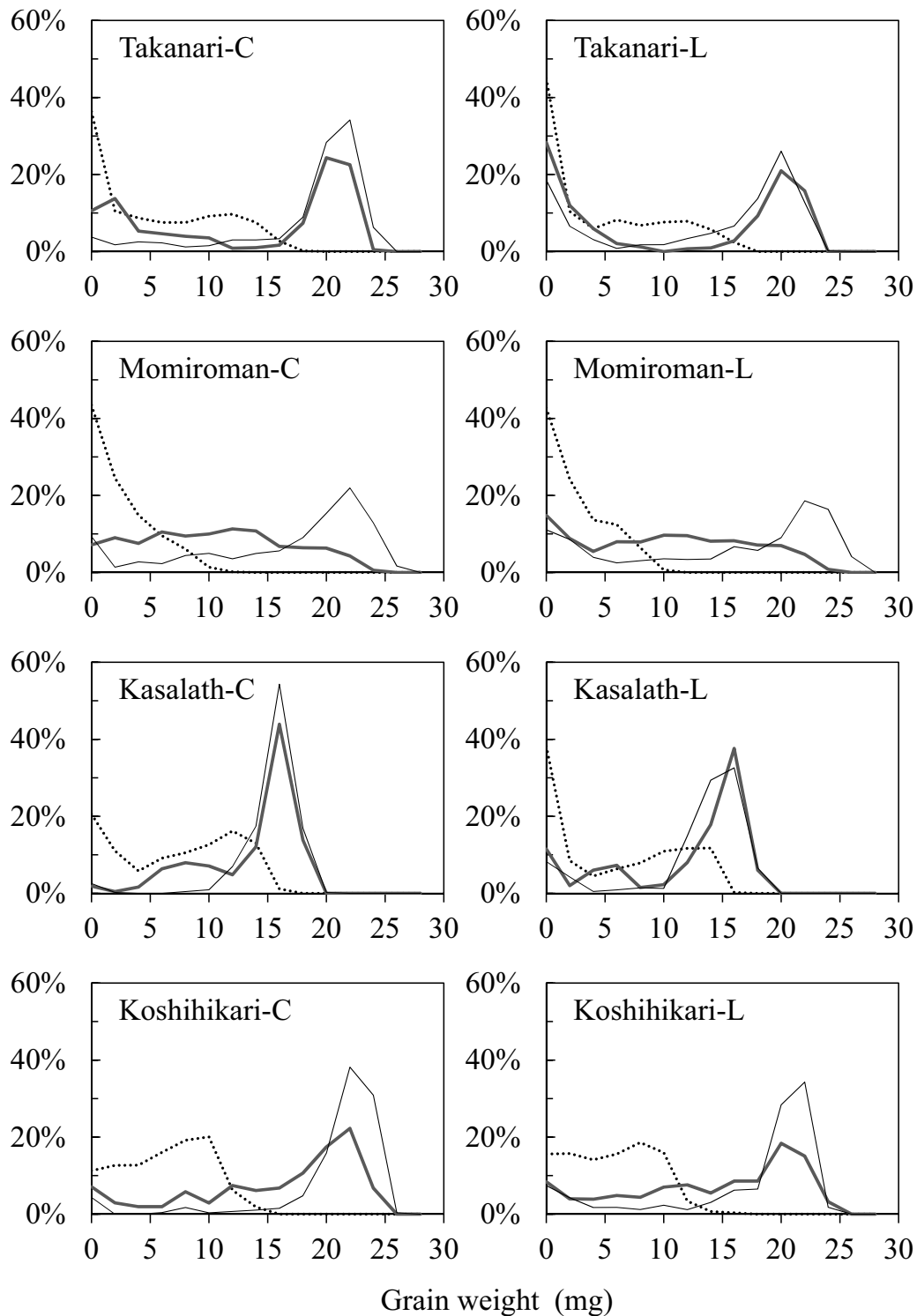
<sup>c</sup> Significant at the 0.001 level.

>80% at 20 DAA in 2012. There were no interactions between cultivar and treatment in these parameters. In Kasalath, fast-growing grains were heavier than those in Koshihikari at 10 DAA. The peaks of heavy grains were higher in Kasalath than in Koshihikari at 20 DAA in both treatments (Fig. 3). Mean grain weight was higher in Kasalath than in Koshihikari at 10 DAA (although the difference was not significant in 2012), but it was significantly lower in Kasalath than in Koshihikari at 20 DAA, possibly reflecting the lower final grain weight of Kasalath.

#### 4. Discussion

We found an exponential relation between GFP and PFS, with the regression coefficients between GFP and Ln(PFS) varying among cultivars (Fig. 1). This relation shows the degree of a decrease in PFS when GFP (which reflects assimilate supply per spikelet) decreases. A lower regression coefficient indicates a smaller decrease in PFS

with the same GFP decrease. Therefore, the cultivars with low regression coefficients, Takanari and Kasalath, preferentially allocate assimilates to a limited number of spikelets to efficiently produce filled spikelets, whereas those with high regression coefficients, Momiroman and Koshihikari, distribute assimilates to a larger number of spikelets and produce more partially filled spikelets when assimilate supply is low relative to sink capacity. These results were confirmed by the analysis of the proportions of spikelets with different specific gravity (Tables 4 and 5). The proportion of partially filled spikelets was low even in the defoliated plants of Takanari and Kasalath, cultivars with lower regression coefficient. The genotypic effects on the proportion of partially filled spikelets were reported (Khush and Peng, 1996; Jeng et al., 2006). However, our results revealed that the proportion of partially filled spikelets is highly affected by assimilate availability per spikelet, suggesting that this parameter as such may not be a stable measure for assimilate partitioning efficiency. The results



**Fig. 3.** Distribution of grain weight at 10 days after first anthesis (DAA, dotted lines), 20 DAA (solid gray lines), and 30 DAA (solid black lines) in control (C) and defoliated (L) plants of four cultivars in 2010. Data are the mean of three replications.

of this study suggest that assimilate partitioning efficiency is well represented by the regression coefficient between GFP and  $\ln(\text{PFS})$  which is unaffected by sink–source status and that it is higher in *indica* and *indica*-dominant cultivars than in *japonica* and *japonica*-dominant cultivars.

Kasalath (*indica*) and Takanari (*indica*-dominant), whose regression coefficients are lower than those of *japonica* and *japonica*-dominant cultivars, had higher proportions of spikelets

with  $\text{SG} > 1.13$  (Tables 4 and 5). A higher percentage of high-density grains ( $\text{SG} > 1.20$ ) was reported in *indica* cultivars than in *japonica* cultivars (Venkateswarlu et al., 1988; Kato et al., 2007; Kato, 2010). In our study, only Kasalath had high-density grains with  $\text{SG} > 1.20$  (data not shown), but Takanari showed a higher proportion of spikelets with  $\text{SG} > 1.13$  than the *japonica*-dominant Momiroman. However, the proportion of spikelets with  $\text{SG} > 1.20$  (data not shown) or  $> 1.13$  was reduced by defoliation and tended

to be lower in extra-heavy panicle type cultivars. In both years, the proportions of spikelets with  $SG > 1.13$  were similar between defoliated Takanari and control Koshihikari (Tables 4 and 5). Therefore, the proportion of spikelets with a specific SG alone may not be a good measure to compare cultivars with different sink–source status.

Defoliation increased the proportion of empty spikelets in the cultivars with large sink size (especially in Takanari in 2010). The effect of defoliation on the proportion of empty spikelets was eliminated by panicle clipping (Fig. 2), which was conducted at 10 DAA (when flowering was over) and would increase assimilate supply to the remaining spikelets. Therefore, it is evident that empty spikelets caused by defoliation had been fertilized and had a filling potential (although they were indistinguishable from unfertilized spikelets) but did not grow. This result suggests that a certain proportion of empty spikelets might be fertilized and have a filling potential in intact plants of Momiroman and Takanari. Therefore, the “unfertilized spikelets” of rice cultivars with extra-heavy panicles (Kobata et al., 2006; Yoshinaga et al., 2013) may actually include a considerable proportion of fertilized spikelets with a filling potential.

There were more heavy and light grains in Takanari than in Momiroman at 20 DAA (Table 6 and Fig. 3) and in Kasalath than in Koshihikari at 10 DAA (Table 7 and Fig. 3). What causes the difference in grain weight distribution among cultivars? The heavy and light spikelets probably corresponded to superior and inferior spikelets, respectively (Yang et al., 2006). The difference in the initial grain-filling rate between these spikelets reflects the difference in their sink strengths (Marcelis, 1996), which may be due to different activities of enzymes (such as sucrose synthase, ADP-glucose pyrophosphorylase, or starch synthases) involved in sugar metabolism and starch synthesis (Liang et al., 2001; Naik and Mohapatra, 2000; Umemoto et al., 1994). The expression of corresponding genes is delayed by several days in inferior spikelets (Ishimaru et al., 2003, 2005). After the removal of superior spikelets, inferior ones are filled like superior ones (Ishimaru et al., 2003, 2005), indicating that superior spikelets delay sink-strength acquisition by inferior spikelets. Our findings suggest that this effect is larger in *indica* and *indica*-dominant cultivars.

The association between assimilate partitioning efficiency and the difference in grain growth between superior and inferior spikelets can be easily explained as follows. The higher grain growth rates of superior spikelets of *indica* and *indica*-dominant cultivars might considerably delay sink-strength acquisition by inferior spikelets, especially when assimilate supply is limited. By the time flowering of the inferior spikelets is over and they are ready to grow, the superior spikelets are already rapidly accumulating assimilates, leaving little assimilates available for the inferior spikelets and consequently they cannot start growing. Most inferior spikelets are filled after superior spikelets (Fig. 3), but some eventually lose viability and remain unfilled. Therefore, defoliation increased the proportion of unfilled spikelets in Takanari and Kasalath. In *japonica* or *japonica*-dominant cultivars, when inferior spikelets are ready to grow, the growth rate of superior spikelets is low and enough assimilates are left for inferior spikelets to start growing, which may cause a competition for assimilates between spikelets. If assimilate supply is decreased by defoliation and cannot meet the sink demand, more spikelets end up partially filled.

This scenario explains well the difference in grain weight distribution among cultivars. However, previous studies showed higher sugar content in inferior than in superior spikelets, indicating that the ovary sugar content does not directly regulate initial grain growth (Ishimaru et al., 2005; Yang et al., 2006). Various phytohormones play roles in heterogeneous grain filling in rice (reviewed by Mohapatra et al., 2011). The plant assimilate status may affect grain growth via phytohormones, but further study is required to elucidate this mechanism.

A cultivar-specific relation has been reported between nitrogen availability during grain filling and grain protein content, suggesting a large cultivar difference in grain protein accumulation ability (Tsukaguchi et al., 2016). The higher rates of initial grain filling in *indica* and *indica*-dominant cultivars suggest their higher ability to attract and accumulate assimilates in grains in comparison with *japonica* and *japonica*-dominant cultivars. The large differences in GFP and PFS between Momiroman and Takanari (Table 2) are unlikely to be explained by the difference in sink–source status because the sink capacity and the shoot dry weight were at the same level in both cultivars. Grain filling degree may be affected by the ability to attract and accumulate assimilates in grains especially in extra-heavy panicle type cultivars. If this is the case, selection based on assimilate partitioning efficiency would select higher ability to attract and accumulate assimilates in grains and thus higher grain filling ability; further study is required to test this hypothesis.

In conclusion, we found an exponential relation between GFP and PFS. The regression coefficients between GFP and  $\ln(\text{PFS})$  differ among cultivars and can be used as a quantitative measure for assimilate partitioning efficiency: a lower regression coefficient shows higher assimilate partitioning efficiency to produce filled spikelets. In *indica* and *indica*-dominant cultivars, which have lower regression coefficients, the difference between the weight of fast-growing and slowly growing grains was larger than in *japonica* and *japonica*-dominant cultivars, suggesting an association of the initial grain growth rate of fast-growing superior spikelets with partitioning efficiency. Our data also show that empty spikelets caused by low assimilate supply are fertilized and have a filling potential.

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