A novel index to evaluate resource allocation pattern in panicles in Japanese rice cultivars

Shiori Yabe a,b, Hiroshi Yoshida c, Erina Fushimi c, Masanori Yamasaki a,d, Hideo Maeda a,e, Takeshi Hayashi a and Hiroshi Nakagawa c

ABSTRACT

The major impact of both genotypic and environmental factors on grain-filling efficiency of rice (Oryza sativa L.) makes evaluating cultivar’s grain-filling characteristics highly complicated. To assess grain-filling characteristics, the allocation index (Alli) was defined as a novel indicator representing the pattern of resource allocation in panicles. Alli was calculated as the ratio of source of yield utilized for producing well-filled grains to the total source consumed in a panicle, using estimated grain weight distribution data. We measured the Alli of 91 Japanese rice cultivars grown under nine environments involving multiple years, cropping seasons, three sites, and flag leaf clipping. Each cultivar’s stability in Alli was evaluated using the data of various sink–source balance conditions. As a result of integrated analysis of multiple cultivars, we observed a trade-off relationship between the stability of Alli and the stability of mean weight of well-filled grains (mu2). The popular high-yielding cultivars Hokuriku 193 and Takanari showed high stability of Alli and mu2 under various sink–source balance conditions. Among the 91 cultivars, Hokuriku 193 showed stable characteristics with a high sink-filling ratio. Our results demonstrate that the grain weight distribution and Alli could be used as novel indicator of grain-filling characteristics, and that the trade-off relationship between the stability of Alli and mu2 should be considered when we select cultivars for multi-environmental cultivation.

Introduction

Rice (Oryza sativa L.) grain yield is affected by environmental and genotypic factors, which makes the precise evaluation and improvement of genetic ability in grain yield an arduous task. Genes and quantitative trait loci control grain yield in rice by regulating the number of spikelets in a panicle, as demonstrated by Ashikari et al. (2005), who showed that Grain number 1a increased grain productivity. Influenced by growing conditions, high-yielding cultivars with heavy panicles often exhibit a low grain-filling ratio and many unfilled/poorly filled grains (Yang & Zhang, 2010; Yoshinaga et al., 2013).

CONTACT Hiroshi Nakagawa nakagawa16@affrc.go.jp

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Therefore, research focusing on production stability and grain-filling efficiency under various environments will contribute to rice cultivation and breeding.

During growth, rice plants change their phenotype according to their surrounding environments. Phenotypic plasticity can often be interpreted as an environmental adaptation mechanism. For example, rice plants respond to low irradiance by degenerating differentiated spikelets (Yao et al., 2000). After pollination, rice plants might have reduced proportion of filled grains and ceased ovary development as affected by the environment (Jiang et al., 2016; Nagato & Chaudhry, 1970). Under these circumstances, we can interpret that the levels and timing of environmental response differ among cultivars. Considering the process of ripening, when the source of yield is small relative to sink size, the strategy of source allocation would vary considerably among cultivars. As source allocation strategies, plants might allocate the limited source to a small proportion of grains, filling completely as many grains as possible, or allocate the source evenly among grains, sacrificing the average grain size. Hence, assessing strategies of source allocation among rice cultivars will contribute to breeding and cultivation initiatives aiming at the stable production of rice grain yield.

Here, we developed a novel index, allocation index (Alli), to evaluate the allocation pattern of source to grains in a panicle. To date, several indices of grain-filling status have been utilized (e.g. Kato, 2010; Liang et al., 2001; Tsukaguchi et al., 2016; Wang et al., 2008; Yamakawa et al., 2007; Yang & Zhang, 2010; Yoshida & Hara, 1977). The currently most used indices of grain filling are the percentage of filled grains and percentage of sink-filling based on grain number and grain weight, respectively. The percentage of filled grains needs arbitrary threshold for filled and unfilled grains. On the other hand, although the percentage of sink-filling does not require any arbitrary threshold, it cannot distinguish filled grains from unfilled ones. Here, we assessed grain weight distribution as an indicator for grain filling by estimating naturally-derived threshold, which separated filled grains from unfilled ones. Here, we assessed grain weight distribution as an indicator for grain filling by estimating naturally-derived threshold, which separated filled grains from unfilled ones. Here, we assessed grain weight distribution as an indicator for grain filling by estimating naturally-derived threshold, which separated filled grains from unfilled ones, without using arbitrary thresholds. Utilizing the method for describing grain weight distribution (Yabe et al., 2018; as shown in Figure 1(a)), we estimated the percentage of low-weight grains (p) and the Alli, as indicators of grain filling based on the grain number and the grain weight, respectively. We used 91 rice cultivars representing the diversity of Japanese varieties, and they were cultivated under different sink-source balance conditions.

In the present study, we set the sink–source balance as an environmental covariate owing to its presumed impact on grain-filling characteristics. By using the novel index Alli, we aimed to (i) assess grain-filling parameters of 91 rice cultivars under various sink-source conditions, (ii) evaluate the stability of resource allocation pattern for each cultivar, and (iii) assess typical high-yielding varieties’ characteristics and factors contributing to their high performance.

Materials and methods

Field experiments

We used 91 Japanese rice cultivars, which were selected from all over Japan from Hokkaido (northern area in Japan) to Kyushu (southern area) to represent the wide diversity of the Japanese rice population. These cultivars included high-yielding varieties and varieties for whole crop silage. The list of cultivars is shown in Figure 2, in which the IDs were given according to their average days to heading. These cultivars were cultivated in nine environments, which consists of three sites, two years, and flag leaf clipping treatment (Table 1). The cultivation at each site was conducted using established management practices for field preparation and the control of pests and diseases in paddies. Flag leaf clipping was conducted on the heading date only to the stems that would be sampled after ripening in each plant.

Days to heading (the number of days from transplanting to heading) was recorded for each cultivar. For analysis of grain weight distribution and sink-filling ratio, one superior panicle was sampled from four mature plants per replicate, so that four panicles were used in a replicate. The total number of grains per panicle was measured. Unhulled grains were dried at 80°C for 72 h to measure grain weight. Single grain weight was measured using an automatic counting and weighing system (QWcalc; NK-Systems, Aichi, Japan). The temperature was obtained from a database ‘The Agro-Meteorological Grid Square Data, NARO’ (accessed 5 November 2018) (Ohno et al., 2016). Day length was calculated using the method proposed by Brock (1981).

Grain weight distribution

The method for describing grain weight distribution was proposed by Yabe et al. (2018) as a modification of the method proposed by Miyagawa (1980). We approximated the grain weight distribution using a mixture of gamma distributions (Figure 1(a)). The probability density of a single-seed weight x (mg) is represented as

$$f(x) = px^{a_1-1} \exp\left(-\frac{x}{\lambda_1}\right) + (1-p)x^{a_2-1} \exp\left(-\frac{x}{\lambda_2}\right)$$

(1)
where \( p \) is the mixing proportion, \( a_1 \) and \( a_2 \) are shape parameters, \( b_1 \) and \( b_2 \) are scale parameters of the two gamma density functions, and \( \Gamma(a) \) is the gamma function. The first (left-side) gamma distribution represents the distribution of low-weight grains, and the second (right-side) gamma distribution represents the distribution of high-weight grains. The distribution parameters were estimated using the expectation-maximization algorithm (Dempster et al., 1977) based on the method developed by Minka (2000).

Figure 1. Outline of the grain weight distribution using gamma distributions (a) and examples of two grain weight distribution with \( p = 0.3 \) (b). \( p \): the probability of low-weight grains, \( mode_1 \): mode of the low-weight grain’s distribution, \( skewness_1 \): skewness of the low-weight grain’s distribution, \( mu_2 \): mean of the high-weight grain’s distribution, \( var_2 \): variance of the high-weight grain’s distribution, \( Alli \): allocation index.
Figure 2. Distribution of parameters related to grain weight distribution in 91 cultivars grown under nine environments. The cultivars were sorted according to their cultivar-IDs, which are arranged based on their average days to heading. SinkFillingRatio: sink-filling ratio measured in each replication, $p$: the probability of low-weight grains, $mode1$: mode of the low-weight grain's distribution, $skewness1$: skewness of the low-weight grain's distribution, $mu2$: mean of the high-weight grain's distribution, $var2$: variance of the high-weight grain's distribution, Alli: allocation index calculated by each grain weight distribution.
The grain weight distribution parameters were estimated for each cultivar in each replication after single-grain weights of four panicles that belonged to a single replication were pooled, to avoid the effect of outliers and improve estimation accuracy in each replication. When the distribution was classified as unimodal, $p$ was defined as zero (i.e. all grains were partitioned to the distribution of high-weight grains).

To interpret the shape of the grain weight distributions, we used feature values of the distribution (mode of left-side distribution: $mode1 = (a_1 - 1)b_1$, skewness of left-side distribution: $skewness1 = 2/(a_1^{1/2})$, mean of right-side distribution: $mu2 = a_2b_2$, and variance of right-side distribution: $var2 = a_2b_2^2$; Figure 1). The parameter $p$ represents the percentage of low-weight grains.

Other grain-filling-related traits were also estimated based on grain weight distribution parameters. The total grain weight was calculated by multiplying the expected single-grain weight by the observed average number of grains per panicle, based on the integral of the estimated grain weight distribution function. The sink-filling ratio was calculated as the ratio of the total grain weight to the sink capacity. For the calculation of sink capacity, the value at the 95th percentile of the observed grain weight in each cultivar was multiplied by the number of grains because the maximum value of single weight would be under the influence of measurement errors. The boundary probability, which was the proportion of middle-weight grains, was calculated as the probability around the boundary of the two gamma distributions ($\pm 0.1$ mg; Figure 1(a)).

### Allocation index ($Alli$)

$Alli$ assesses the pattern of source allocation to the grains in a panicle. $Alli$ was calculated as the ratio of the weight of the high-weight grains to the total grain weight based on grain weight parameters:

$$\text{Alli} = \frac{\text{weight of the high weight grains}}{\text{total grain weight}}$$

$$= \frac{\int_{-\infty}^{\infty} f(x)dx \times (\text{grain number})}{\int_{0}^{\infty} f(x)dx \times (\text{grain number})}$$

where $f(x)$ is the estimated grain weight distribution function represented in Eq. 1, and the boundary weight is the weight that shows the lowest density between two distributions (Figure 1(a)). In the distribution with $p = 0$, the boundary weight was set to 0 mg so that the $Alli$ was 1.0. $Alli$ can be interpreted as the tendency to produce high-weight grains using the available source of yield. Note that $p$ and $Alli$ were estimated based on the grain number and grain weight, respectively. Figure 1(b) illustrates the two types of grain weight distribution with $p = 0.3$. In this case, the shapes of the right-side distributions are same, and the shapes of the left-side distributions are different. $Alli$ differs according to the source of yield consumed for low-weight grains ($Alli = 0.93$ and 0.81 for upper and lower figure, respectively).

### Stability of grain weight distribution parameters and $Alli$

To evaluate the stability of the grain weight distribution parameters and $Alli$ in each cultivar under various environments, we measured the levels of change in grain
weight distribution parameters and Alli according to the sink-filling ratio, which was interpreted as the sink-source balance here. We supposed the sink-filling ratio to represent the sink-source balance in a panicle because it was calculated as the ratio of total grain weight to sink capacity, which could be interpreted as an approximate indicator of the ratio of available-source size to sink size. For Alli, a linear regression model was constructed for each cultivar using the data obtained from nine environments shown in Table 1, in which the explanatory and response variables were sink-filling ratio and Alli, respectively. The estimated regression coefficient (i.e. slope) was assessed as the stability in response to sink-filling ratio for each cultivar. To support the interpretation of stability, the precision of regression model and the variation of Alli were evaluated besides. The regression coefficients were calculated using the ‘lm’ function in R (R Core Team, 2020). We also calculated the regression coefficients of the linear regression model for grain weight distribution-related parameters ($p$, $mode1$, $skewness1$, $mu2$, $var2$, and boundary probability) in response to sink-filling ratio. The procedure of calculation was same as that of Alli. The estimated slope was treated as the stability of parameters in response to sink-source balance.

Statistical analysis

Parameter estimation and statistical analysis were conducted using R version 4.0.2 (R Core Team, 2020). In multiple comparison, Welch’s t-test was conducted with Bonferroni correction so that the significant level was set to p-value < 0.05. Correlation was calculated as Pearson’s correlation coefficient ($r$) and tested at p-value < 0.05. When we assessed cultivar’s grain weight distribution parameters ($p$, $mode1$, $skewness1$, $mu2$, $var2$, and boundary probability) and Alli, we used a median instead of mean in order to reduce impact of outliers (e.g. unimodal grain weight distributions).

Results

Genotypic and environmental effects on yield-related traits

The 91 cultivars showed large phenotypic variation in each environment as measured by days to heading, grain number, total grain weight per panicle, and sink-filling ratio (Table 2). Each cultivar also showed phenotypic variation as affected by the surrounding environments (Figure 2). In the same year in Ibaraki, in comparison with the early cropping season, the late cropping season had smaller value in days to heading. In Ibaraki, higher temperatures and longer day lengths were observed immediately after transplanting in the late cropping season compared with the early cropping season. Flag leaf clipping affected neither the total grain weight nor sink-filling ratio in 2016, whereas this treatment caused a significant decrease in both traits in 2017. The correlation ($r$) between environments were 0.93–0.99 in days to heading, 0.63–0.94 in grain number, 0.72–0.96 in total grain weight, and 0.15–0.90 in sink-filling ratio. The sink-filling ratio of Niigata-2017 showed correlation coefficients lower than 0.4 with five environments. The days to heading correlated positively with the grain number and total grain weight per panicle ($r = 0.37$ and 0.31, respectively; p-value < 0.05) and negatively with the sink-filling ratio ($r = −0.27$; p-value < 0.05). The grain number and total grain weight significantly correlated ($r = 0.90$; p-value < 0.05). The sink-filling ratio also correlated significantly with grain number and total grain weight ($r = −0.17$ and 0.11, respectively; p-value < 0.05).

Several cultivars had large variation in sink-filling ratio among the nine environments (Figure 2). Kinmaze (No. 70; sink-filling ratio ranged 0.37–0.72), Norin 18 (No. 90; 0.39–0.73), Hatsunishiki (No. 19; 0.39–0.73), Kamenoo (No. 65; 0.43–0.77), Tachiyaka (No. 62; 0.33–0.65), and Akisakari (No. 50; 0.40–0.72) were the six cultivars that showed the range of sink-filling ratio larger than 0.30 among all environments. In contrast, Matusribare (No. 72; sink-filling ratio ranged 0.49–0.55), Satojiman (No. 58; 0.67–0.74), Fukuhibiki (No. 29; 0.63–0.70), and Akitakomachi (No. 28; 0.67–0.74) had a small range of sink-filling ratio.

Grain weight distribution

The grain weight distributions of the 91 cultivars across environments varied greatly (Figures 2 and 3). Hoshiaoba (No. 55) showed larger $mu2$ than did the other cultivars (Figure 2). Hounen-wase (No. 13) and Tachiaoba (No. 91) exhibited large $p$ and a low sink-filling ratio. Takanari (No. 52) showed the smallest $var2$ compared with all cultivars, which appeared as a sharp distribution of high-weight grains (Figure 3). Contrastingly, Tsukinohikari (No. 63) showed the largest $var2$. When the grain weight distribution was classified to the unimodal distribution (one sample in Norin 22 [No. 69] and Matusribare [No. 72]; Figure 2), the estimated $var2$ was higher than that of the other samples because the high-weight grain’s distribution was responsible for all grains.

The response of grain weight distribution and its parameters to environment varied among cultivars. Hatsunishiki (No. 19), Kamenoo 4 (No. 23), Tachiyaka (No. 62), and Tachisuwak (No. 89) showed relatively
Table 2. Days to heading, grain number, total grain weight, and sink-filling ratio of 91 rice cultivars grown under nine environments.

| Site-Season | Ibaraki -Early | Ibaraki -Early | Ibaraki -Late | Ibaraki -Late | Ibaraki -Early | Ibaraki -Early | Ibaraki -Late | Niigata | Hyogo |
|-------------|----------------|----------------|--------------|--------------|----------------|----------------|--------------|---------|-------|
| Year        | 2016           | 2016           | 2016         | 2016         | 2017           | 2017           | 2017         | 2017    | 2017  |
| Treatment   | Flag leaf clipping | Flag leaf clipping | Flag leaf clipping | Flag leaf clipping | Flag leaf clipping | Flag leaf clipping | Flag leaf clipping |         |       |
| Days to heading | mean (s.d.) | 75.9 (15.0) | 62.5 (11.5) | 69.2 (13.7) | 57.4 (11.9) | 78.1 (16.5) | 64.6 (16.9) |         |       |
|             | minimum (daichino) | 43 (40) | 42 (42) | 42 (42) | 33 (33) | 42 (42) | 22 (22) |         |       |
|             | maximum (yukihikari) | 109 (87) | 102 (102) | 85 (85) | 115 (115) | 94 (94) |         |         |       |
| Grain number / panicle | mean (s.d.) | 112.6 (39.3) | 111.1 (34.6) | 101.4 (32.0) | 112.9 (32.9) | 101.3 (26.1) | 121.4 (36.1) |         |       |
|             | minimum (daichino) | 38.3 (43.3) | 24.0 (24.0) | - (24.0) | 36.3 (36.3) | 38.3 (38.3) | 46.5 (46.5) |         |       |
|             | maximum (takana) | 271.3 (246.0) | 266.0 (266.0) | - (266.0) | 236.8 (236.8) | 169.8 (169.8) | 228.6 (228.6) |         |       |
| Total grain weight (mg/panicle) | mean (s.d.) | 2515.0 (835.9) | 2351.0 (757.6) | 2432.4 (695.4) | 2288.6 (658.6) | 2226.6 (672.3) | 1900.3 (592.9) | 2335.5 (644.9) | 2311.0 (604.6) | 3114.3 (902.2) |
|             | minimum (torishima) | 1085.4 (958.4) | 1094.6 (968.1) | 968.1 (968.1) | 542.8 (542.8) | 322.7 (322.7) | 409.7 (409.7) | 719.5 (719.5) | 980.4 (980.4) |         |
|             | maximum (takana) | 5818.6 (5250.5) | 5397.3 (4854.3) | 4881.3 (4881.3) | 4418.6 (4418.6) | 4636.5 (4636.5) | 4333.5 (4333.5) | 6571.7 (6571.7) |         |       |
| Sink-filling ratio | mean (s.d.) | 0.68 (0.06) | 0.67 (0.06) | 0.65 (0.06) | 0.65 (0.06) | 0.64 (0.06) | 0.64 (0.06) | 0.64 (0.06) | 0.64 (0.06) | 0.68 (0.05) |
|             | minimum (takana) | 0.50 (0.49) | 0.51 (0.40) | 0.45 (0.45) | 0.37 (0.37) | 0.33 (0.33) | 0.49 (0.49) |         |       |
|             | maximum (takana) | 0.79 (0.78) | 0.77 (0.77) | 0.79 (0.79) | 0.78 (0.78) | 0.77 (0.77) | 0.79 (0.79) |         |       |

The letter under the s.d. represents the results of Welch's t-test with Bonferroni correction (p-value < 0.05). s.d. = standard deviation.
large variation in the parameter $p$ in response to the surrounding environment (Figure 2). For six cultivars shown in Figure 3, Hatsunishiki (No.19) and Momiroman (No. 73) produced varying amounts of low-weight grains and grain weight distribution shapes. However, Hokuriku 193 (No. 64) homogeneously produced small amounts of low-weight grains without large changes in the distribution shape. Different replications of a single cultivar in a single environment resulted in a similar shape of grain weight distribution, except for a small percentage of samples.

Significant correlation (p-value < 0.05) was obtained between several grain weight distribution-related parameters ($p$, $mode1$, $skewness1$, $mu2$, $var2$, and boundary probability) and yield-related traits (days to heading, number of grains per panicle, total grain weight per panicle, and sink-filling ratio). The parameter $p$ correlated significantly with $mode1$ ($r = 0.15$), $skewness1$ ($r = -0.18$), boundary probability ($r = 0.71$), days to heading ($r = 0.22$), number of grains ($r = 0.23$) and sink-filling ratio ($r = -0.75$). Boundary probability also correlated significantly with $mode1$ ($r = 0.48$), $skewness1$ ($r = -0.30$), $mu2$ ($r = -0.21$), $var2$ ($r = 0.33$), days to heading ($r = 0.24$), number of grains ($r = 0.23$), and sink-filling ratio ($r = -0.47$).

**Allocation index (Alli)**

Alli varied greatly among all cultivars cultivated in the nine environments (Figure 2). Nipponbare (No. 66), Hayamasari (No. 2), and Toyonishiki (No. 32) showed median of Alli higher than 0.98, which means that less than 2% of the available source of yield was consumed to produce low-weight grains in these cultivars. However, Hounen-wase (No.13), Kusanohoshi (No. 88), and Tachiababa (No. 91) had a median of Alli lower than 0.90. Several cultivars had high variation in Alli among the nine environments (Figure 2). Tachiayaka (No. 62), Mizuhochikara (No. 74), Kamenoo 4 (No. 23), Norin 18 (No. 90), Kusanohoshi (No. 88), Momiroman (No. 73), Tachisuzuka (No. 89), Hatsunishiki (No. 19), Norin 22 (No. 69), and Domannaka (No. 27) were the ten cultivars that showed the highest variance in Alli among all environments. In contrast, Hoshinoyume (No. 7), Tsugaruroman (No. 17), Fukuhibiki (No. 29), Hokuriku193 (No. 64), and Millenishi (No. 54) had a lowest variance in Alli.

Alli correlated significantly (p-value < 0.05) with days to heading ($r = -0.17$), number of grains per panicle ($r = -0.19$), sink-filling ratio ($r = 0.69$), $p$ ($r = -0.95$), $mode1$ ($r = -0.13$), $skewness1$ ($r = 0.16$), $var2$ ($r = 0.09$), and boundary probability ($r = -0.59$).

**Stability of Alli and grain weight distribution parameters in response to sink-filling ratio**

All cultivars showed large variation in the regression coefficient (slope) of Alli in response to sink-filling ratio (Figure 4). The 25%- 50%- and 75%-quantile of the regression coefficients were 0.31, 0.44, and 0.62, respectively, in the 91 examined cultivars. For the accuracy of regression model, the 25%- 50%- and 75%-quantile in the root mean square error (RMSE) of regression models were 0.012, 0.016, and 0.022, and those in the standard errors of regression coefficients (SE of coef.) were 0.08, 0.10, and 0.13. Kusanohoshi (No.88) showed much larger RMSE than other cultivars with relatively large SE of coef. (RMSE = 0.056, and SE of coef. = 0.34), thus the evaluation of stability of Kusanohoshi seemed unreliable. The regression coefficients were positive, except for one cultivar Hoshinoyume (No. 7), although the value was almost zero (slope = -0.09). Among the cultivars with positive regression coefficient of Alli, Oborozuki (No. 9) had the lowest value (slope = 0.09). These cultivars with small regression coefficients showed relatively small variation in their Alli (Figure 2). Four cultivars had the regression coefficients higher than 1.0; Himenomochi (No.21) (slope = 1.00), Kamenoo 4 (No. 23) (slope = 1.11), Tachiayaka (No. 62) (slope = 1.22), and Mizuhochikara (No. 74) (slope = 1.24), which means that Alli changed more drastically than did the sink-filling ratio in these cultivars. Among the five cultivars shown in Figure 4(c), Momiroman (No. 73) had the highest regression coefficient (slope = 0.92), for which SE of coef. was under 75%-quantile.

The regression coefficient of Alli significantly correlated with the regression coefficients of $p$, $mode1$, $skewness1$, $mu2$, and boundary probability ($r = -0.89$, -0.40, 0.33, -0.76, and -0.37, respectively; p-value < 0.05). It should be noted that the regression coefficient with a large absolute value (large value to both positive and negative directions) indicated unstable characteristics of the parameter. When the regression coefficient of Alli increased from zero to positive value (moved from stable to unstable genotypes), the regression coefficient of $p$ decreased from zero to negative, the regression coefficient of $mode1$ fluctuated from positive to zero and then to negative, the regression coefficient of $skewness1$ increased from zero to positive, the regression coefficient of $mu2$ decreased from positive to zero, and the regression coefficient of the boundary probability decreased from zero to negative.

The stability of $mu2$ showed a trade-off relationship with the stability of Alli (Figure 5). For both parameters, regression coefficients close to zero showed stable characteristics under varying sink-filling ratio. We
Figure 3. Grain weight distribution of Hatsunishiki, Koshihikari, Takanari, Hokuriku 193, Nipponbare, and Momiroman in 2017. Histograms show the measured single-grain weight. The solid line represents the estimated grain weight distribution function. Cropping environment is shown in the title of each plot. Due to small gap between replications, grain weight distribution was estimated for all replications together in each environment for this plot.
incorporated the simple partitioning of cultivars based on the stability of \( mu2 \) and \( Alli \). The most stable cultivars were defined to be partitioned in [slope of \( Alli \)] \( \leq 0.6 \) and [slope of \( mu2 \)] \( \leq 5 \). Next, the cultivars with secondary stable characteristics were defined to be allowed more unstable \( mu2 \), [slope of \( Alli \)] \( \leq 0.6 \) and [slope of \( mu2 \)] \( \leq 10 \). Yukihihaki (No. 3), Hatsunishiki (No. 19), and Hokuriku 193 (No. 64) showed stable characteristics for both parameters (Figure 5). Following these three cultivars, Akihikari (No. 15), Norin 1 (No. 18), Moemimori (No. 31), Haenuki (No. 36), Dontokoi (No. 41), Yumehitachi (No. 44), Akanezora (No. 46), Takanari (No. 52), Kameno (No. 65), Hiyokumochi (No. 79), and Tachiharuka (No. 86) showed relatively stable characteristics in both parameters with [slope of \( Alli \)] \( \leq 0.6 \) and [slope of \( mu2 \)] \( \leq 10 \). In contrast, the cultivar with high sink-filling ratio scattered throughout the plot of the regression coefficient of \( mu2 \) and \( Alli \) (Figure 5), which means that the stability of them less depends on sink-filling ratio. Among the three cultivars that showed stable characteristics in both \( Alli \) and \( mu2 \) (Yukihihaki, Hatsunishiki, and Hokuriku 193), Hokuriku 193 (Alli-slope = 0.36; \( mu2 \)-slope = 4.93) showed high sink-filling ratio (0.63–0.74; mean = 0.69), \( Alli \) (0.94–0.99; mean = 0.97), and \( mu2 \) (24.93–26.21 (mg); mean = 25.53 mg). Among the 11 cultivars that belonged to the secondary stable group, Akihikari (No. 15), Norin 1 (No. 18), Haenuki (No. 36), and Dontokoi (No. 41) had high sink-filling ratios.

Discussion

We assessed grain-filling characteristics of 91 rice cultivars under various conditions of sink–source balance. To this end, the allocation index (\( Alli \)) was defined as a novel index representing the amount of consumed source of yield for high-weight grains based on grain weight distribution (Figure 1). Tsukaguchi et al. (2016) and Okamura et al. (2018) reported that rice grain weight distribution is influenced by genotype, environment, and the position of grains in a panicle. Yabe et al. (2018) also showed the possibility of genomic prediction for grain weight distribution, which suggests that it is genetically controlled. By using the grain-weight-based index \( Alli \), we evaluated the genotypic variation and characterized typical cultivars in the population.

The 91 examined rice cultivars, including improved varieties and landraces, were collected throughout Japan, so that the population represents a wide genetic diversity of Japanese rice. In the 91 cultivars studied here, 66 were selected from the 114 rice cultivars representing Japanese rice population (Yamasaki & Ideta, 2013), other 25 were selected to add variation in yield-related traits. In the present study, the cultivars showed large phenotypic variation (Table 2 and Figure 2). The cultivars with similar days to heading showed considerable variation in the sink-filling ratio in spite of negative correlation between them (Figure 2). The sink-filling ratio ranged from 0.79 to 0.33 in total, also showing variation in each cultivar (Figure 4(a)). In the present study, we also supposed the sink-filling ratio as the environmental covariate simply representing the sink–source balance. For modelling the environmental response of resource allocation in a panicle, sink–source balance could be interpreted as an environmental (external/previously decided) influence having large impact during ripening. We concluded that the variation of sink-filling ratio was adequate for evaluation of grain-filling patterns. Flag leaf clipping treatment decreased mean of total grain weight and sink-filling ratio though the effects were not significant in 2016 (Table 2). Rice flag leaves play an important role in the supply of the source of yield (Ishimaru et al., 2004; Nagato & Chaudhry, 1970). Translocation of assimilated substances between stems might have occurred as compensation for the large heterogeneity of nutritional condition during ripening, as reported by Sato (1961) and Kashibuchi et al. (1967), because our flag leaf clipping treatment was limited to a few stems in each plant.

Alli differed depending on the cultivar and environment, following the changing grain weight distribution (Figures 2 and 3). Showing high correlation between weight-based index \( Alli \) and number-based index \( p (r = -0.95; p-value < 0.05) \), the range of \( Alli \) (0.57–1.00) was narrower than that of \( p \) (0.02–0.76, excluding two data with unimodal distribution), which resulted from the slow decrease in \( Alli \) as compared to \( p \) (Figure 6(a)). As the balance of the amount of consumed source, \( Alli \) can be interpreted as the indicator of priority among grains compared under the same \( p \) (Figure 1(b)). In addition, the cultivars showed several patterns of change in grain weight distribution and \( Alli \) according to environments. Looking two cultivars with high \( Alli \) (Hokuriku 193 and Hinohikari) and other two cultivars with medium – low \( Alli \) (Hatsunishiki and Momiroman) under various conditions (Figure 6(b)), Hatsunishiki and Hokuriku 193 changed the distribution height (probability density; Figure 6(c) and 6(d)), while Momiroman and especially Hinohikari changed the position of right-side distribution (e.g. \( mu2 \); Figure 6(e) and 6(f)). When the consumed source decreased by a factor of \( k \), weights of all grains decreased by a factor of \( k \) if the priority levels are maintained among grains. In this case, grain weight distribution can be represented as \( g(y) = (1/k)f(y/k) \) with the single grain weight \( y \approx kx \) using Eq. 1, and then the \( Alli \) would not change. Hinohikari showed
Figure 4. Linear regression of Alli in response to the sink-filling ratio. (a) Regression results of all the 91 cultivars. Regression coefficients > 1.0 are shown as dashed lines. Each line represents a single cultivar. (b) Histogram of the regression coefficients of allocation index in response to sink-filling ratio for the 91 cultivars. (c) Examples of the linear regression results of Hatsunishiki, Koshihikari, Takanari, Hokuriku 193, and Momiroman.
this pattern. On the other hand, if superior grains are more prioritized under less source, inferior grains would decrease their weights more drastically. Hatsunishiki and Hokuriku 193 showed this pattern. In this case, stable Alli suggests that the prioritization to superior grains is occurred under unfavorable condition. Besides, since our experiment also included the sterile spikelet close to 0 mg, the values of Alli should be interpreted carefully.

To meet the demand of amount of filled grains at the available resource, high value in Alli would be required. Among the evaluated 91 cultivars, Nipponbare (No. 66), Hayamasari (No. 2), and Toyonishiki (No. 32) showed the quite high Alli (Figure 2). These cultivars would play an important role considering the production of filled grains under changing environments. On the other hand, the stability of Alli is also important to characterize the yield response to environment in each cultivar. That knowledge would help rice producers to decide cultivars to be cultivated. Besides, if serious relationship in stability with other yield-relating traits are found, it should be revealed for understanding the grain filling characteristics. To evaluate the stability of Alli and grain weight distribution parameters in each cultivar, we used the regression coefficients of these variables in response to sink-filling ratio, following methods such as Finlay-Wilkinson regression analysis (Finlay & Wilkinson, 1963). In the present study, the reliability of stability (i.e. estimation accuracy of linear model) should be lower when the variance of sink-filling ratio among nine environments was very small, in which the effect of sink-filling ratio on Alli or grain weight distribution parameters could not be estimated correctly. Among the 91 cultivars, No. 72, 28, 58, 29, 15, 17, 21, 59, 54, and 32 were top 10 cultivars with small variance in sink-filling ratio, thus we would note that the stability of these cultivars would harbor relatively low reliability. The 91 cultivars showed large variation in the stability of Alli (Figure 4). A trade-off relationship was found between the stability of Alli and mu2 (Figure 5). This result suggests that the cultivars maintaining constant Alli sacrificed mu2 (i.e. mean single filled-grain weight) under unfavorable

Figure 5. Distribution of the regression coefficient of Alli and mu2 in response to the sink-filling ratio. Each point represents a cultivar. Point size represents the mean value of the sink-filling ratio for each cultivar. Gray points represent cultivars with average sink-filling ratio larger than the 70%-quantile of the 91 cultivars (0.68). Points with an asterisk represent the cultivars with the regression coefficient (slope) of allocation index ≦ 0.6 and the slope of mu2 ≦ 5. The number represents the ID of cultivars shown in Figure 2
conditions, as Hinohikari shown in Figure 6(f). In Hinohikari (Figure 6(f)), although Alli was maintained at higher level in all environments, mu2 much largely fluctuated compared with the variation of the sink-filling ratio (Figure 2, 5 and 6(b)). It is suggested that Hinohikari sacrificed mu2, then the demand of source for filled grains became smaller even if p was fixed, so that the Alli was maintained even under somewhat ill sink-source balance. We investigated the common characteristics among the cultivars with stable mu2 and
fluctuating Alli (e.g. No. 23, 62, 74, and 69). These cultivars produced relatively large amount of partially-filled grains under unfavorable conditions. Considering the adaptability of cultivars in multiple locations, the stability of both Alli and mu2 is an important feature, which positioned close to the origin of the coordinate axes in Figure 5. They tended to exhibit small boundary probability and mode1 relatively close to 0 mg under unfavorable conditions (e.g. No. 3, 19, 64, and 65 were in the top 20 showing low boundary probability under their lowest sink-filling ratio). The relationship of the stability in Alli and mu2 with the small values of boundary probability and mode1 under unfavorable conditions also suggests that the clear prioritization of source has affected to the balance between the stability of Alli and mu2.

We incorporated a simple partitioning for cultivars based on the stability of mu2 and Alli. The threshold in the slope of Alli was set at 0.6 firstly even arbitrarily, which was under the 75% quantile of the slope of Alli in 91 cultivars. As two levels of criteria for slope of mu2, 5 and 10 was adopted. They meant that the mu2 decreased by 1 mg and 2 mg, respectively, when the sink-filling ratio decreased by 0.2. Here, we used these two thresholds for mu2, considering the impact on 1000 kernel grain weight. Among the 91 cultivars, Yukihikari (No. 3), Hatsunishiki (No. 19), and Hokuriku 193 (No. 64) showed high stability in both Alli and mu2 (Figure 5). Hokuriku 193 exhibited satisfactory and stable Alli, mu2, and sink-filling ratio. Contrastingly, Yukihikari and Hatsunishiki showed relatively poor Alli, mu2, and sink-filling ratio. Regarding the expected total grain yield, Hokuriku 193 might be one of the optimal cultivars for cultivation in different environments among the examined cultivars. Hokuriku 193 is a high-yielding indica-dominant variety owing to its higher grain-filling ability than that of the japonica-dominant high-yielding varieties such as Momiroman (Yoshinaga et al., 2013). Likewise, in the present study, Momiroman (No. 73) showed unstable Alli and relatively low sink-filling ratios (Figure 2 and 4(c)). One of the most popular and well-studied high-yielding cultivar included in the present study is Takanari, which has a high performance under various conditions (Kato et al., 2009; Taylaran et al., 2009; Yoshinaga et al., 2013). With exhibiting small var2, Takanari (No. 52) was one of the 14 most stable cultivars in both Alli and mu2 ([slope of mu2] = 9.10; [slope of Alli] = 0.54; Figures 2, 3, and 5), although the stability was inferior to Hokuriku 193. Meanwhile, Takanari showed large mean grain number per panicle (196.4; the fourth largest value in 91 cultivars) but relatively low mean sink-filling ratio (0.64) and mu2 (22.38 mg). Takanari’s high yield performance would be associated with its large sink size owing to large grain number (Taylaran et al., 2009; Yoshinaga et al., 2013). Our results support previous reports on Takanari’s stability and the factor of high yield. Hence, our results show that Alli, grain weight distribution parameters, and their stability are useful parameters to evaluate the grain-filling characteristics of rice cultivars.

We assessed grain-filling parameters of 91 rice cultivars under nine different environments. Alli might suit a purpose to infer the pattern of source allocation among grains in a panicle. Although we observed a trade-off relationship between the stability of Alli and mu2, several cultivars showed relatively higher stability for both parameters. Our results demonstrate the grain-filling characteristics of high-yielding cultivars based on Alli and its stability. It is known that several genetic factors are linked to the grain-filling process, such as abscisic acid activity, sucrose concentration, and sucrose synthesis relating enzyme so far (Tang et al., 2009). Panicle structure is another factor that might affect the allocation of nutrients in a panicle (Seki et al., 2015). The method proposed in this study can be applied to future genetic and causal analysis to reveal the mechanism of grain-filling efficiency.

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