Effects of temperature during ripening on amylopectin chain-length distribution of ‘Koshihikari’ and ‘Ichihomare’

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ABSTRACT
Because of global warming, the ripening period of rice (Oryza sativa L.) is becoming hotter in Japan, and there is concern that eating quality will decline as a result. Amylopectin is the largest component of rice endosperm starch and its short-chain content has a considerable effect on the eating quality of cooked rice. Here we investigated the effect of high temperature during ripening on amylopectin chain-length distribution in ‘Koshihikari’, the leading cultivar in Japan, and in ‘Ichihomare’, released in 2017 and grown in Fukui prefecture, ripened in paddies over multiple years. The amylopectin short-chain content of degree-of-polymerization (DP) 6–13 of ‘Koshihikari’ grain ripened at high temperature was 3.03 points lower than that of grain ripened at low temperature. In contrast, that of ‘Ichihomare’ was only 0.26 points lower. For every 1°C increase in temperature during ripening, the short-chain content of DP 6–13 of ‘Koshihikari’ decreased linearly by 1.03 points whereas that of ‘Ichihomare’ did not decrease significantly. These results suggest that the amylopectin chain-length distribution of ‘Ichihomare’ might be more stable to temperature during ripening than that of ‘Koshihikari’. The reason is yet unknown, but this trait will contribute to breeding and production of cultivars with stable good eating quality under high temperatures during ripening.

Introduction
Eating quality of cooked rice (Oryza sativa L.) is one of the most important traits in rice production in Japan. Amylose content in rice endosperm starch is an essential index of eating quality (Okuno et al., 1983; Yano et al., 1988), and cultivars with low amylose content have been developed (Ando et al., 2010; Ise et al., 2001; Kunihiro et al., 1993). The annual average temperature over Japan have risen at rates of about 1.19°C per century (Japan Meteorological Agency, 2018a) the average temperatures in August in Fukui when

‘Koshihikari’ is ripening increased from 25.9°C in the 1950s to 27.9°C in the 2010s (Japan Meteorological Agency, n.d.b). High temperature during ripening decreases amylose content (Asaoka et al., 1985; Inatsu, 1988; Kato et al., 2019; Nishimura et al., 1985; Tateyama et al., 2005) but also reduces eating quality in ‘Koshihikari’ (Shimokobe et al., 2012), ‘Nipponbare’ (Oh-e et al., 2007), and ‘Akitakomachi’ (Kato et al., 2019). Nagahata & Kuroda (2004) reported decreases in amylose content and ‘Mido score’, the glossiness of
cooked rice, of 14 cultivars under high temperature during ripening. Kim et al. (2000) found that formation of chalky grains resulting from loose packing of starch granules under high temperature stress reduces eating quality. Morita et al. (2016) comprehensively reviews the recent trend in rice grain quality under climate change and showed the situation of heat-induced poor palatability of cooked rice. These reports suggest that eating quality of cooked rice ripened under high temperature cannot be explained by amylose content alone.

Amylopectin is another and the largest component of rice endosperm starch. Several studies indicated that amylopectin chain-length distribution is related to the texture of cooked rice. In particular, the amylopectin chain-length distribution of ‘Koshihikari’, the leading cultivar in Japan (Rice Stable Supply Support Organization, 2021), with strong stickiness of cooked rice (Kobayashi et al., 2018a), has been investigated. Kanemoto et al. (2019) showed that amylopectin short-chain content was higher in ‘Koshihikari’ than in other cultivars and that it contributed to the sticky texture of cooked rice. Ohya & Kawabata (1993) showed that ‘Koshihikari’ had more amylopectin short side chains than other japonica cultivars. Takahashi et al. (1998) reported that amylopectin from rice with favourable texture, including ‘Koshihikari’, had a smaller average amylopectin unit chain length than other cultivars. These reports suggest that amylopectin short chains contribute to the stickiness of cooked ‘Koshihikari’ rice. Furthermore, cooked ‘Ichihomare’ rice was stickier than ‘Koshihikari’ rice even though it has the same amylose and protein contents, on account of its richer content of amylopectin short chains (Kobayashi et al., 2018b).

High temperature during ripening also decreases amylopectin short-chain content. Umemoto et al. (1999) showed that it decreased contents in chamber-grown ‘Kinmaze’ and ‘IR36’. Yamakawa et al. (2007) showed that it enriched long-chain amylopectin contents in incubator-grown ‘Nipponbare’, ‘Koshihikari’, ‘Tentakaku’, ‘Sasanishiki’, and ‘Hatusubishi’. Kato et al. (2019) reported that cooked ‘Akitakomachi’ rice ripened in a high-temperature greenhouse was less palatable, which they attributed to the reduced amylopectin short chains. Takahashi et al. (2013) found a high negative correlation between amylopectin short-chain content and temperature during ripening of a brewing rice cultivar.

In that way, the amylopectin chain-length distribution is important in eating quality of cooked rice, and temperature during ripening severely affects amylopectin chain-length distribution. Although Kanemoto et al. (2019) analysed the amylopectin chain-length distribution of ‘Koshihikari’, they bought their samples on the market and so could not assess the effect of temperature during ripening. The effects of temperature on amylopectin chain-length distribution have also been evaluated by pot experiments in incubators or chambers (Jiang et al., 2003; Kato et al., 2019; Umemoto et al., 1999; Yamakawa et al., 2007) or by using purified starch in vitro (Ohdan et al., 2011). Response of the amylopectin chain-length distribution of ‘Koshihikari’ in the wide range of air temperatures during ripening has not been adequately studied under the paddy field conditions.

In this study, the response of amylopectin chain-length distribution to air temperatures during ripening was analysed using field-grown grains of ‘Koshihikari’ and ‘Ichihomare’ over several years. ‘Ichihomare’ has superior heat tolerance during ripening to ‘Koshihikari’ (Kobayashi et al., 2018b). We also analysed whether decline of grain appearance quality has any effect on changes in amylopectin chain-length distribution due to high temperature during ripening.

Materials and methods

Cultivation and sample preparation

We grew ‘Koshihikari’ from 2015 to 2020 in paddy fields at Fukui Agricultural Experiment Station (Fukui, Japan, 36°03′N, 136°16′E). In each year, plants were transplanted on different dates in different fields (A–F) so as to head at different times and thus experience different temperatures during ripening (Table 1). Hill spacing was 0.21 m × 0.23 m, with three or four seedlings per hill, in plots of 4.86 m². Nitrogen fertilizer was applied at 3 g N m⁻² as basal dressing and at 4 g N m⁻² as top dressing. In the 2016 F³ and 2017B⁺ plots, ‘Koshihikari’ was grown in an H-steel vinyl greenhouse placed in the paddy. The greenhouse was ventilated when the inside air temperature exceeded 35°C to prevent high-temperature-induced sterility. Water management and pest control followed our normal practices. The central 72 hills were harvested at maturity, then threshed, air-dried, and hulled. Percentage of perfect grains in brown rice was determined with 1000 kernels by a rice analyser (RGQ1108; Satake Co. Ltd., Hiroshima, Japan) in 2014 to 2016 and its successor model (RGQ190A; Satake Co. Ltd.) in 2017 to 2020. The brown rice was milled to 90% ± 0.5% in a one-pass mill (CBS550; Satake Co. Ltd.), and the resulting milled rice was ground into flour (Cyclotec 1093; Foss Japan, Tokyo, Japan).

‘Ichihomare’ was grown in the same way as ‘Koshihikari’ but with 5 g N m⁻² as basal dressing. It was grown in the same fields as ‘Koshihikari’ (except in 2017C and 2017D) and in the greenhouse in the 2016 F³ plot.
The air temperature at 30 cm above the panicles was measured by a data logger (Hobo U23 Pro v2 U23-002; Climatricc, Inc., Tokyo, Japan) with a force-ventilated radiation shield in the field and the greenhouse. The temperature during ripening was defined as the 24-h average temperature during the 20 days after heading.

**Starch properties**

The apparent amylose content in endosperm was determined according to the method of Juliano (1971). Milled rice flour (80 mg) was gelatinized by treatment with 4 mL of 0.5 N NaOH and 0.4 mL of 95% ethanol, homogenized, and stood for 24 h at room temperature. The apparent amylose content was measured in triplicate with an Auto Analyzer III (BL-TEC, Tokyo, Japan) calibrated with standard amylose from potato Amylose Type III (Sigma-Aldrich, Tokyo, Japan).

The amylopectin chain-length distribution was determined by a modification of the fluorophore-assisted carbohydrate electrophoresis method (O’Shea et al., 1998) as described in Fujita et al. (2001). Milled rice flour (20 mg) was gelatinized and then the amylopectin was debranched with *Pseudomonas amyloferamosa* iso-amyrase (Hayashibara Biochemical Lab., Okayama, Japan). The reducing ends of each unit chain were labelled with fluorescent dye (8-amin-1,3,6-pyrenetri-sulfonic acid) in a Carbohydrate Labeling and Analysis Assay Kit 477600 (Beckman Coulter, Brea, CA, USA) and separated and quantified by the degree-of-polymerization (DP) of glucose chain-length in a capillary electrophoresis system PA800 (Beckman Coulter). Unit chains of amylopectin were quantified for DP 6–60 in duplicate. We did not convert amylose content based on its moisture content.

**Statistical analyses**

Testing the significance of the correlation coefficient was performed in Microsoft Excel 2010 as follows: find the correlation coefficient ($r$) using the corrr function, obtain the test statistic ($t$) from the correlation coefficient ($r$) and the sample size (n), and calculate the $P$-value using the tdist function. Covariance analysis was performed to compare the correlation coefficients ($r$) in Microsoft Excel 2010. We conducted Student’s t-test, which assumes that the two data sets came from distributions with the same variances using the t test function in Microsoft Excel 2010 to compare the content of short chain between ‘Koshihikari’ and ‘Ichihomare’.

**Results**

**Observed sample variations**

‘Koshihikari’ was transplanted from 1 to 25 May. The heading dates ranged from 21 July to 7 August, and the temperatures during ripening ranged from 26.0 to 29.2°C, with a mean of 27.6°C (Table 1). These are consistent with the 30-year (1991–2020) temperatures during ripening of ‘Koshihikari’ grown at our station (minimum 24.5°C, mean 27.6°C, maximum 29.9°C). The heading dates of ‘Ichihomare’ ranged from 27 July to 6 August (Table 2). The average temperature during ripening of ‘Ichihomare’ was 27.6°C.

The apparent amylose content of ‘Koshihikari’ averaged 14.8% (range, 12.6% to 17.3%), the sum of the peak areas of DP 6–13 gave an average amylopectin short-chain content of 46.6% (45.2% to 49.2%), and the percentage of perfect grains averaged 57.5% (8% to 78%; Table 1). The apparent amylose content of ‘Ichihomare’ averaged 15.3% (13.8% to 16.9%), the amylopectin short chain (DP 6–13) averaged 47.7% (47.1% to 48.3%), and the percentage of perfect grains averaged 66.6% (16% to 84%; Table 2).

**Annual variation in amylopectin chain-length distribution**

The average amylopectin chain length of ‘Koshihikari’ ($n = 20$) and ‘Ichihomare’ ($n = 8$) peaked at DP 11, at 8.47% and 8.53%, respectively, of total amylopectin (Figure 1a, b; Supplemental Tables S1 and S2).

Differences in peak areas (maximum minus minimum) ranged from 0.08 to 0.87 points, with an average of 0.26 points in ‘Koshihikari’, and from 0.03 to 0.51 points, with an average of 0.18 points, in ‘Ichihomare’ (Figure 1c, d; Supplemental Tables S1 and S2). The difference in ‘Koshihikari’ was greatest at DP 10 (Figure 1c). Its amylopectin chain-length distribution could be divided into three regions according to the magnitude of the variation: the short-chain region of DP 8–13 had the greatest variability (>0.5 points), followed by the short-chain of DP 6–7 and the medium-chain region of DP 14–27 (0.2–0.5 points) and then the long-chain region of DP ≥ 28
Table 1. Experimental design, temperature during ripening, and grain quality of ‘Kosshihikari’.

| Year | Plot | Trans-planting date | Heading date | Temperature during ripening<sup>b</sup> °C | Amylose content % | Short chain content in amylopectin<sup>c</sup> % | Percentage of the perfect grains<sup>d</sup> % |
|------|------|---------------------|--------------|------------------------------------------|-------------------|-----------------------------------------------|---------------------------------------------|
| 2015 | A    | 5–25                | 8–7          | 26.0                                     | 17.3              | 49.1                                          | 70                                          |
|      | B    | 5–7                 | 7–27         | 27.1                                     | 16.7              | 48.2                                          | 68                                          |
|      | C    | 5–8                 | 7–26         | 27.2                                     | 14.0              | 46.3                                          | 78                                          |
| 2016 | A    | 5–9                 | 7–22         | 26.8                                     | 16.4              | 49.2                                          | 55                                          |
|      | B    | 5–10                | 7–24         | 26.9                                     | 15.6              | 47.0                                          | 78                                          |
|      | C    | 5–9                 | 7–26         | 27.0                                     | 15.0              | 46.3                                          | 75                                          |
|      | D    | 5–16                | 8–1          | 27.1                                     | 16.4              | 46.5                                          | 75                                          |
|      | E    | 5–23                | 7–30         | 27.1                                     | 15.6              | 45.8                                          | 74                                          |
|      | F<sup>*</sup> | 5–23              | 7–31         | 28.8                                     | 12.6              | 45.3                                          | 8                                           |
| 2017 | A    | 5–8                 | 7–22         | 27.3                                     | 14.3              | 47.0                                          | 61                                          |
|      | B<sup>*</sup> | 5–23               | 8–1          | 29.2                                     | 13.0              | 45.6                                          | 13                                          |
| 2018 | A    | 5–14                | 7–27         | 28.0                                     | 14.2              | 45.2                                          | 69                                          |
|      | B    | 5–7                 | 7–26         | 28.0                                     | 14.8              | 45.8                                          | 62                                          |
|      | C    | 5–7                 | 7–26         | 28.0                                     | 15.1              | 45.3                                          | 65                                          |
|      | D    | 5–1                 | 7–21         | 28.1                                     | 14.6              | 46.0                                          | 45                                          |
| 2019 | A    | 5–7                 | 7–26         | 28.8                                     | 13.7              | 46.6                                          | 40                                          |
|      | B    | 5–10                | 7–28         | 29.0                                     | 13.0              | 45.2                                          | 38                                          |
| 2020 | A    | 5–7                 | 7–30–27      | 27.3                                     | 14.0              | 46.6                                          | 61                                          |
|      | B    | 5–8                 | 7–30         | 27.3                                     | 15.7              | 47.8                                          | 65                                          |
|      | C    | 5–13                | 8–2          | 27.6                                     | 14.6              | 46.8                                          | 50                                          |
| Ave. |      | 5–11                | 7–27         | 27.6                                     | 14.8              | 46.6                                          | 57.5                                        |
| Max. |      | 5–25                | 8–7          | 29.2                                     | 17.3              | 49.2                                          | 78                                          |
| Min. |      | 5–1                 | 7–21         | 26.0                                     | 12.6              | 45.2                                          | 8                                           |
| SD   |      | 6.8                 | 4.2          | 0.8                                      | 1.3               | 1.2                                           | 20.0                                        |

<sup>a</sup>Grown in the greenhouse.
<sup>b</sup>24-h average temperature during the 20 days after heading.
<sup>c</sup>Content of DP 6–13 in amylopectin.
<sup>d</sup>Percentage of the perfect grains measured by a grain analyzer.

Table 2. Experimental design, temperature during ripening, and grain quality of ‘Ichihomare’.

| Year | Plot<sup>a</sup> | Trans-planting date | Heading date | Temperature during ripening<sup>b</sup> °C | Amylose content % | Short chain content in amylopectin<sup>c</sup> % | Percentage of the perfect grains<sup>d</sup> % |
|------|------------------|---------------------|--------------|------------------------------------------|-------------------|-----------------------------------------------|---------------------------------------------|
| 2015 | C                | 5–8                 | 8–6          | 26.0                                     | 16.9              | 47.8                                          | 84                                          |
| 2016 | D                | 5–16                | 8–5          | 27.2                                     | 15.9              | 47.1                                          | 82                                          |
| 2016 | F<sup>*</sup>    | 5–23                | 8–3          | 29.5                                     | 13.8              | 47.6                                          | 16                                          |
| 2017 | C                | 4–28                | 7–27         | 27.0                                     | 16.0              | 47.4                                          | 75                                          |
| 2017 | D                | 5–15                | 8–2          | 26.7                                     | 15.5              | 48.0                                          | 73                                          |
| 2019 | A                | 5–7                 | 8–1          | 28.7                                     | 14.0              | 48.0                                          | 59                                          |
| 2019 | B                | 5–10                | 8–4          | 28.4                                     | 14.6              | 47.5                                          | 66                                          |
| 2020 | A                | 5–7                 | 8–5          | 27.6                                     | 15.7              | 48.3                                          | 78                                          |
| Ave. |                  | 5–10                | 8–2          | 27.6                                     | 15.3              | 47.7                                          | 66.6                                        |
| Max. |                  | 5–23                | 8–6          | 29.5                                     | 16.9              | 48.3                                          | 84                                          |
| Min. |                  | 4–28                | 7–27         | 26.0                                     | 13.8              | 47.1                                          | 16                                          |
| SD   |                  | 7.5                 | 3.2          | 1.2                                      | 1.1               | 0.4                                           | 22.0                                        |

<sup>a</sup>Grown in the greenhouse.
<sup>b</sup>24-h average temperature during the 20 days after heading.
<sup>c</sup>Content of DP 6–13 in amylopectin.
<sup>d</sup>Percentage of the perfect grains measured by a grain analyzer.
(<0.2 points). In contrast, the difference in ‘Ichihomare’ was smaller than that in ‘Koshihikari’ and had three peaks, at DP 8, 15, and 22 (Figure 1d).

Effect of temperature during ripening on amylopectin chain-length distribution and on apparent quality of brown rice

To examine the effect of temperature during ripening on the amylopectin chain-length distribution, we compared plots with higher temperature during ripening than 29°C (2017B* and 2019B for ‘Koshihikari’, 2016 F* for ‘Ichihomare’) and plots with lower temperature during ripening than 27°C (2015A, 2016A, and 2016B for ‘Koshihikari’ and 2015C and 2017D for ‘Ichihomare’). High temperature during ripening remarkably decreased the amylopectin short-chain content (DP 6–13) of ‘Koshihikari’: by 0.38 points on average and by 3.03 points in total relative to the lower temperature during ripening (Figure 1e, Supplemental Table S1). In contrast, it increased the mid-length chain content of DP 15–19: by 0.19 points on average and by 0.97 points in total relative to the lower temperature during ripening. It also increased the content of longer chains (DP ≥ 22), by 0.06 points on average and by 2.20 points in total (Supplemental Table S1). The short-chain contents (DP 6–7 and 9–13) were significantly and negatively correlated with temperature during ripening (Figure 1g). A spike of the coefficient was observed at DP 8. On the other hand, the longer-chain contents (DP 28–30 and 42–60) were significantly and positively correlated with temperature during ripening.

High temperature during ripening seemed to have a smaller effect on the amylopectin chain-length distribution of ‘Ichihomare’ than on that of ‘Koshihikari’. The decrease in the amylopectin short-chain content (DP 6–13) in ‘Ichihomare’ was only 0.03 points on average and 0.26 points in total (Figure 1f, Supplemental Table S2). The coefficients of the correlations between respective peak areas and temperatures during ripening were significant only for DP 6 and for DP 34, 35, and 37 (Figure 1h).

Endosperm starch properties of ‘Koshihikari’ changed with increasing temperature during ripening: a 1°C increase resulted in a 1.29-point decrease in amylose content (P < 0.01; Figure 2a) and a 1.03-point decrease in the short-chain content of DP 6–13 (P < 0.01; Figure 2c). For ‘Ichihomare’, a 1°C increase resulted in a 0.88-point decrease in amylose content (P < 0.01; Figure 2b) but no change in the short-chain content of DP 6–13 (Figure 2d).

In both ‘Koshihikari’ (Figure 2e) and ‘Ichihomare’ (Figure 2f), the percentage of perfect grains decreased significantly as the temperature during ripening increased (P < 0.01). Figure 2(g and h) shows the relationship between short-chain content (DP6–13) and percentage of perfect grains. The percentage of perfect grains of both cultivars did not significantly correlate with the short-chain content.

Discussion

We previously found that the short-chain content of ‘Ichihomare’ was larger than that of ‘Koshihikari’, using samples ripened at 26.0 to 27.2°C in 2015 and 2016 (Kobayashi et al., 2018b). In this study, the average short-chain content (DP 6–13) of ‘Ichihomare’ was 47.7% in the range of 26.0 to 29.5°C (Table 2), whereas that of ‘Koshihikari’ was 46.6% in almost the same temperature range (Table 1). It was confirmed that ‘Ichihomare’ had significantly larger content of short chains than ‘Koshihikari’ had even at higher temperatures during ripening by t-test (P < 0.05).

The amylopectin chain-length distribution of ‘Koshihikari’ and ‘Ichihomare’ varied among years by an average of 0.26 and 0.18 points, respectively, per peak area (Supplemental Tables S1 and S2). The short chains in amylopectin of ‘Koshihikari’ had larger annual variation than those of ‘Ichihomare’ (Figure 1c, d). These differences suggest that the amylopectin chain-length distribution of ‘Koshihikari’ is more susceptible to temperature during ripening than that of ‘Ichihomare’. The short chains of DP 8–13 of ‘Koshihikari’ showed more variation than the long-chain region (Figure 1c), and the contents of DP 6–7 and 9–13 were significantly correlated with temperature during ripening (Figure 1g). Thus, the higher variability of the short-chain content of ‘Koshihikari’ in response to temperature during ripening resulted in the large annual variation in amylopectin chain-length distribution.

Previous studies have also shown that high temperature during ripening decreased the content of short chains of DP 6–7 and 9–13 and increased that of longer chains of DP > 19 of ‘Akita-Sake-Komachi’ (Takahashi et al., 2013) in a temperature range of 22 to 26°C (Δ = 4°C); it decreased short chains of DP 6–13 and increased mid to long chains of DP 20–27 and 44–54 of ‘Kinmaze’ in a temperature range of 18/13°C (day/night) to 32/27°C (Δ = 14°C; Umemoto et al., 1999); it decreased short chains of DP 6–7 and 10–19 of ‘Nipponbare’ in a temperature range of 25/20°C to 33/28°C (Δ = 8°C; Yamakawa et al., 2007), and it decreased short chains of DP 6–15 of ‘Akitakomachi’ in a temperature range of 24/22°C to 29/27°C (Δ = 5°C; Kato et al., 2019). Here, the
Figure 1. Amylopectin chain-length distribution of ‘Koshihikari’ (a, c, e, g) and ‘Ichihomare’ (b, d, f, h) grown and ripened in 2015 to 2020. (a, b) Peak areas. Bars are means (n = 20 and n = 8, respectively). Error ranges indicate the lowest and highest values. (c, d) Dots show differences in peak area between highest and lowest values of each degree-of-polymerization. (e) Difference in relative peak areas of ripened grains between average of the two highest temperatures (≥29°C) and average of the three lowest temperatures (<27°C) of ‘Koshihikari’. (f) Difference in relative peak areas of ripened grains between the highest temperature (29.5°C) and average of the two lowest temperatures (<27°C) of ‘Ichihomare’. (g, h) Dots indicate correlation between peak area and temperature during ripening of ‘Koshihikari’ (n = 20) and ‘Ichihomare’ (n = 8). Horizontal lines show significance at 5% level.
Figure 2. Relationships between temperature during ripening and starch and grain properties of ‘Koshihikari’ (a, c, e, g, n = 20) and ‘Ichihomare’ (b, d, f, h, n = 8). (a, b) Amylose content. (c, d) Amylopectin short-chain content (DP 6–13). (e, f) Percentage of the perfect grains. (g, h) Relationship between short-chain content (DP6–13) and grain quality. **: significant at 1% level.
short-chain content (DP 6–13) of ‘Koshihikari’ decreased by 3.03 points in a temperature range of 26.0 to 29.2°C ($\Delta = 3.2^\circ$C; Table 1, Figure 1e). Our results show that even this small difference at high temperature range substantially changed the amyllopectin chain-length distribution of ‘Koshihikari’. On the contrary, the content of short chains of DP 6–13 of ‘Ichihomare’ was not significantly decreased by high temperature during ripening (Figures 1f and 2d), and the contents of DP 7–13 were not significantly correlated with temperature during ripening (Figure 1h).

The increase in DP 15–19 in ‘Koshihikari’ may be due to reduced activity of BEIib. This enzyme excises short chains of DP 6–7 from chains of DP 14–21 (Ohdan et al., 2011), and high temperature significantly decreases its activity (Jiang et al., 2003; Ohdan et al., 2011; Takahashi et al., 2013; Yamakawa et al., 2007). Tanaka et al. (2004) showed that even a slight alteration in BEIib activity can dramatically change starch properties. So it is possible that the 29°C achieved here significantly reduced BEIib activity of ‘Koshihikari’, and thus the amyllopectin side chains of DP 14–21 remained unaltered, showing as an increase in DP 15–19. Such an increase in DP 15–19 was not observed in ‘Ichihomare’ (Figure 1f). Difference in the response of BEIib activity to air temperatures between two genotypes needs to be further analysed.

We wonder whether heat-induced grain appearance quality decline is involved in the observed alteration in amyllopectin chain-length distribution due to temperature during ripening. The tolerance to heat-induced grain appearance quality decline is ‘slightly weak’ in ‘Koshihikari’ (Kobayashi et al., 2018a) and ‘slightly strong’ in ‘Ichihomare’ (Kobayashi et al., 2018b). Here, in both cultivars, percentage of the perfect grains decreased significantly as the temperature during ripening increased ($P < 0.01$). A $1^\circ$C increase in temperature during ripening resulted in about a 19-point decrease in the percentage of perfect grains of ‘Koshihikari’ (Figure 2e) and a 16-point decrease in ‘Ichihomare’ (Figure 2f), which difference was not significant. However, the percentages of perfect grains at 28°C, for example, estimated from their regression lines were 50.5% in ‘Koshihikari’ and 60.5% in ‘Ichihomare’, supporting their difference in tolerant levels. Both ‘Koshihikari’ and ‘Ichihomare’ have tolerance alleles at QTLs associated with heat-induced grain appearance quality decline, $q\text{WB}_3$, $q\text{WB}_4$, and $q\text{WB}_6$, which are involved in the occurrence of back-white grains due to high temperature during ripening (Kobayashi et al., 2018b). Therefore, these QTLs are likely not related to the differences in amyllopectin chain-length distribution between ‘Koshihikari’ and ‘Ichihomare’. Other QTLs responsible for the difference in the tolerance to heat-induced grain appearance quality decline due to high temperature during ripening between these two cultivars needs to be clarified.

It is reported that amylose content decreases by 0.8–1.1 points per $1^\circ$C increase in temperature during ripening in ‘Tsugaru-Roman’ (Tateyama et al., 2005), 0.77 points in ‘Takanemini’ and 0.65 points in ‘Akitakomachi’ (Fujisawa & Takahashi, 1995), 0.82 points in ‘Taichung 65’ (Fwu, 1994). In this study, amylose content decreased by a 1.29-point and a 0.88-point per $1^\circ$C increase in temperature during ripening in ‘Koshihikari’ (Figure 2a) and ‘Ichihomare’ (Figure 2b), respectively, which correlation coefficients did not significantly differ by covariance analysis. However, the bigger correlation coefficients between both short-chain and amylose content and temperature during ripening of ‘Koshihikari’ also suggest that its starch properties are susceptible to temperature during ripening than ‘Ichihomare’ and other cultivars. Further study is needed.

The short-chain contents (DP 6–7 and 9–13, but not DP 8) of ‘Koshihikari’ were significantly and negatively correlated with temperature during ripening (Figure 1g). The content of DP 8 of ‘Ichihomare’ increased at high temperature during ripening (Figure 1f). The exception of DP 8 of ‘Ichihomare’ was also observed in ‘Akitakomachi’ (Kato et al., 2019) and in ‘Aki-Sake-Komachi’ (Takahashi et al., 2013). It could be due to high activity of starch synthase I (SSI) in the background of a reduction of starch branching enzyme Iib (BEIib) activity under high temperature: high temperature during ripening activates SSI and inactivates BEIib (Jiang et al., 2003; Ohdan et al., 2011; H. Takahashi et al., 2013; Yamakawa et al., 2007); by subtraction, these effects are observed as an increase in DP 8, as discussed in Kato et al. (2019).

In this study, there was no significant correlation between short-chain content and percentage of perfect grains for both ‘Koshihikari’ and ‘Ichihomare’ (Figure 2g, h). Also, Ishimaru et al. (2018) found no consistent difference in the amyllopectin chain-length distribution between milky-white grains and perfect grains of ‘Koshihikari’ grown under high temperature and low solar radiation. The relationship between amyllopectin chain-length distribution and eating quality of cooked rice ripened under high temperature needs to be further investigated. The differences in amyllopectin chain-length distribution among other cultivars, especially in the effects of temperature during ripening, need to be clarified in detail. The
knowledge will contribute to breeding and production of cultivars with stable amylopectin chain-length distribution and high eating quality even under the higher temperatures during ripening predicted under global warming (IPCC, 2013).

Disclosure statement

No potential conflict of interest was reported by the author(s).

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