[Short Report]

High Risk of the Formation of Milky White Rice Kernels in Cultivars with Higher Potential Grain Growth Rate under Elevated Temperatures

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Abstract: Our objective was to clarify whether rice cultivars with a higher grain dry-matter increase rate (GIR) under elevated temperature condition show a higher percentage of milky white rice kernels (MWK). The rate of MWK judged using a rice-quality selector significantly varied with the cultivar and experimental year. The spikes of the cultivars detached 5–7 days after heading were solution-cultured with an adequate nutrient supply for one week at 25, 28 and 33°C. GIR was overall the highest at 28°C, and GIR at this temperature was considered to reflect the potential GIR in all the grains tested. There was a close correlation between the rate of MWK and the GIR at 28°C under field conditions when temperatures during the grain-filling period were approximately over 25°C. It was suggested that cultivars with a higher potential GIR under elevated temperature conditions have a higher risk of the formation of MWK.

Key words: Cultivar, High temperature, Milky white rice kernel, Rice, Solution culture.

Rice quality has been decreasing in the last two decades in western Japan, and this decrease is now being seen in middle and northern Japan as well (Terashima et al., 2001; Morita, 2008). One of the most typical defects, milky white rice kernel, is caused by inadequate starch accumulation in whole grains (Hoshikawa, 1989; Terashima et al., 2001). Grains with a white base or back side of grains and cracked grains are also observed in immature grains. A high temperature during the grain-filling period causes the occurrence of milky white rice kernels (MWK), but the high temperature alone is not considered to directly increase MWK (Kobata et al., 2004; Morita, 2008). A lack of assimilate supply to grains plays a dominant role in increasing MWK, because the rate of MWK is higher in low radiant years (Terashima et al., 2001), plant thinning to increase assimilate supply to grains significantly reduces the rate of MWK (Kobata et al., 2004) and defoliation or shading to reduce the assimilate supply increases the rate of MWK (Nagato and Ebata, 1965; Nakagawa et al., 2008).

The assimilate supply to grain would be lower under a high temperature condition than under a cool condition, because the grain dry matter increase rate (GIR) increases but the assimilate supply does not change or rather decreases at a high temperature (Kobata and Uemuki, 2004). A low assimilate supply under the high temperature condition increased the rate of MWK (Kobata et al., 2004). Based on these findings, we hypothesized that cultivars with a higher potential GIR under high temperature conditions are more sensitive to a high temperature than other cultivars in developing MWK. The potential GIR can be estimated using the spike culture system, with an adequate nutritional supply (Kobata et al., 2001; Kobata et al., 2006). Our objectives were to clarify the relationship between the rate of MWK and the potential GIR in rice cultivars differing in sensitivity to the high temperature.

Materials and Methods

1. Plant materials

Five japonica rice cultivars, Koshihikari, Nipponbare, Hataboshi, Hanaechizen and Koshijiwase, were grown at the Shimane University experimental farm (9 m above sea level, 133°E, 35°N) in 2005 and 2006. The soil type was silty clay loam. The rate of MWK under a high temperature condition varies with the cultivar; Hataboshi is the most sensitive, Koshihikari and Nipponbare have intermediate sensitivity, and Koshijiwase and Hanaechizen are resistant...
to the high temperature causing MWK formation (Iida et al., 2002). Three-week-old seedlings were transplanted to paddy fields on 18 May 2005 and 24 May 2006. The seedlings were planted at a density of 22.2 hills m\(^{-2}\). In 2005, fertilizer N [as (NH\(_4\))\(_2\)SO\(_4\)], P (as CaHPO\(_4\)), and K (as KCl) were applied as a basal dressing at a rate of 4 g N, 8 g P and 4 g K m\(^{-2}\), respectively and 4.0 g N and 40 g K m\(^{-2}\) as top dressing at two weeks before heading. In 2006, the fertilizers N [as (NH\(_4\))\(_2\)SO\(_4\)], P (as CaHPO\(_4\)), and K (as KCl) were applied as a basal dressing rate of 4 g N, 10 g P and 10 g K m\(^{-2}\), respectively, and 3.0 g N m\(^{-2}\) as top dressing at two weeks after transplanting (except for Koshihikari) and two weeks before heading. In 2005 and 2006, each cultivar occupied 6.0 m\(^2\) and 4.3 m\(^2\) of the paddy field, with two and four replicates, respectively. Experimental fields where soil conditions and fertility have been carefully equalized and managed were used.

2. Yield and rice quality

At maturity, 5 plants in a 0.75 m row from each replication were harvested from the center of the plots and measured for yield and yield components as reported previously (Yoshida, 1981). After filled grains were selected by the gravitational method using specific gravity of a 1.06 \(\times 10^3\) kg m\(^{-3}\) salt solution, 300 − 400 spikelets were husked, and the quality of the brown rice was determined using a rice-quality selector (RGQI 10B, Satake Co. Hiroshima, Japan). MWK counted by this method included white core rice kernels (Satake Co., personal communication), although MWK does not include white core rice kernel in the quality classification of rice kernel (Hoshikawa, 1989). However, MWK selected by the selector would be generally accepted as the so-called MWK indicating a critical score of rice quality in diverse agricultural organizations that introduce the selector. It would not be easy to strictly distinguish between the white core rice kernel and MWK except for typical cases such as brewer’s rice. Therefore, MWK selected with the selector would be generally accepted as MWK in the broad sense in our research. The white based immature grain selected with this selector approximately coincides with white belly rice, and the white belly and backed immature rice selected with the selector includes white belly rice where most of the backed immature rice consists of white belly rice (Tanaka et al., 2010). Therefore, the scores with the selector differ from visual scoring in past research in the strict sense, while the objectivity of classification by machine counting would be higher than that by visual counting. Significant differences (0.05 level) by Tukey’s test were calculated from the analysis of variance for rice quality.

3. Methods of panicle culture

Panicles on the main stems or primary tillers that headed in the late morning were tagged, and the shoots were cut at the stem base in the early evening 5–7 d after heading, when flowering in the panicles was almost complete. The culture methods and procedures were as reported by Kobata et al. (2001). The stem base was immediately immersed in shallow water and the shoots were detached and enclosed in a plastic bag before transport to the laboratory, where each stem was cut off just below the node of the flag leaf in fresh water to protect against capillary discontinuity, and the flag leaf was removed. The stem below the neck node of the panicle was then sterilized with 25 g L\(^{-1}\) NaOCl solution for 10 min, and subsequently washed with water. The neck of the panicle was inserted through a small hole in a plastic cap into a test tube containing 45 mL of culture solution. The tubes had an interior diameter and height of 0.018 and 0.200 m, respectively, and the each test tube was then filled with half-strength Murashige & Skoog Plant Salt Mixture (Nippon Seiyaku Co., Tokyo) containing sucrose at 60 g L\(^{-1}\). This sucrose concentration is in the optimal range for grain dry-matter increase (Kobata et al. 2001; Kobata et al.
Two replications consisting of sub-samples of four spikes or four replications of one spike were used in 2005 and 2006, respectively.

The test tubes with panicles were housed in a refrigerator at 5ºC (Engel, Sawafuji Elec. Co., Tokyo) to prevent microbial contamination of the solution. The upper parts of the panicles were exposed to air in an incubator (NK Systems Biotron, Nippon Ika Co., Tokyo) at 25, 28 and 33ºC. Styrofoam plates were used to form partitions between the test tubes and upper parts of the panicles. The panicles were continuously exposed to fluorescent light at 84 \( \mu \text{mol m}^{-2} \text{s}^{-1} \). Between 0 and 7d after the start of culture, four panicles or one panicle per replication were harvested, dried in an oven at 80ºC for 48 h, divided into spikelets and other parts, and weighed. The grain dry matter increase was indicated as the filling percentage of grains (F%) because the cultivars used have different grain sizes (Kobata et al., 2006). The F% was defined as the ratio of the grain dry weight (G) to the potential grain weight (GP):

\[
F\% = \left( \frac{G}{GP} \right) \times 100
\]

where GP is calculated by multiplying the spikelet number by the dry weight of a single fully ripened brown rice grain at harvest, as derived from seeds selected by specific gravity.

Results

1. Meteorological conditions and rice quality at the field site

The average temperature was the highest around the first of August and then slowly decreased in both years (Fig. 1) (JMA, 2010). From September, the average temperature was lower in 2006 than in 2005. The heading date in Hanaechizen and Koshijiwase was 5 days earlier than in Hatsuboshi and Koshihikari, and that in Nipponbare was three weeks later than in others in 2005. In 2006, the heading date in four cultivars was almost similar to that in 2005 although that in Nipponbare was a week earlier than in 2005. The duration of bright sunshine slowly decreased from the first of August in both years, although it greatly varied with the day (Fig. 1). In 2005, the duration of sunshine at the beginning of August was higher than in 2006.

Table 1. Rice quality and yield in five rice cultivars under field conditions in 2005 and 2006. Rice quality was measured with a rice quality machine (RGQI20A, Satake co.).

| Cultivar    | Perfect rice grain (%) | Immature grain (%) | Damaged grain Immature grain (%) | Death grain (%) | Cracked grain (%) | Grain yield g m\(^{-2}\) |
|-------------|------------------------|--------------------|----------------------------------|-----------------|-------------------|------------------------|
|             | Milky white rice kernel | White based immature rice | White belly and backed immature rice | Others          | Broken grain       | Rasty and Malformed grain | Total          |                     |
| 2005        |                        |                     |                                  |                 |                   |                        |                 |                     |
| Hatsuboshi  | 39.0                   | 16.5               | 6.0                              | 6.7             | 19.1              | 48.3                   | 0.0            | 0.4                   |
| Nipponbare  | 59.4                   | 11.6               | 4.7                              | 3.2             | 12.8              | 34.4                   | 0.1            | 1.5                   |
| Koshihikari | 45.5                   | 9.0                | 10.7                             | 4.3             | 12.2              | 30.2                   | 0.3            | 0.4                   |
| Hanaechizen | 48.7                   | 5.3                | 4.1                              | 2.8             | 10.2              | 25.9                   | 0.8            | 0.7                   |
| Koshijiwase | 42.3                   | 4.5                | 3.9                              | 3.6             | 18.0              | 30.0                   | 0.6            | 1.8                   |
| LSD\(_{0.05}\) | ns                     | 8.6                | ns                               | ns              | ns                | 22.2                   | ns             | ns                    |
| 2006        |                        |                     |                                  |                 |                   |                        |                 |                     |
| Hatsuboshi  | 41.7                   | 8.9                | 10.3                             | 9.0             | 25.4              | 53.6                   | 0.2            | 0.7                   |
| Nipponbare  | 76.7                   | 3.2                | 1.0                              | 0.8             | 27.9              | 19.5                   | 0.4            | 0.8                   |
| Koshihikari | 38.8                   | 10.4               | 9.4                              | 3.2             | 19.1              | 50.9                   | 0.1            | 2.0                   |
| Hanaechizen | 79.1                   | 1.2                | 1.6                              | 0.8             | 14.5              | 14.9                   | 0.3            | 1.4                   |
| Koshijiwase | 64.5                   | 1.9                | 2.9                              | 0.6             | 11.3              | 25.7                   | 0.2            | 2.3                   |
| LSD\(_{0.05}\) | 16.0                   | 4.1                | 6.4                              | 5.3             | 9.8               | 15.1                   | ns             | ns                    |
| Year        | ns                     | ns                 | ns                               | ns              | ns                | ns                     | ns             | ns                    |
| Cultivar    | ns                     | *                  | *                               | *               | ns                | ns                     | ns             | ns                    |
| Year × Cultivar | *                     | ns                 | ns                               | ns              | ns                | ns                     | ns             | ns                    |

1. Each value is the average of two (2005) and four (2006) observations.
2. LSD is by Tukey’s test at 0.05.
3. *, ** Significant at 0.05 and 0.001 probability level, respectively; ns=not significant.
4. Grain yield is at 14% water content.
5. Milky white rice kernels include white core rice kernel.
6. White belly and backed immature rice includes white belly and backed rice kernels.
In 2006, while in the middle of September, it was higher in 2006 than in 2005.

Grain yield (14% water content) was between 448 to 576 g m\(^{-2}\) in 2005 and between 524 and 657 g m\(^{-2}\) in 2006 (Table 1). Spikelet number ranged from 23,000 to 37,000 in those two years, and the percentage of ripened grain was between 72 and 92%; 72% in Nipponbare in 2006 was the lowest, and the dry one-thousand grain weight was between 17 and 20 g (data not shown). The percentage of MWK significantly differed with the cultivar in both years and through two years, and the higher percentage resulted in an increase in the percentage of immature grains in Hatsuboshi and Koshihikari (Table 1). There was a close correlation between the rates of immature grain and MWK in all cultivars (r = 0.799, P < 0.01). The rate of white base immature grain in two years significantly differed with the cultivar, although the rate in 2005 and interaction between year and cultivar on both rates of the kernel did not (Table 1). Among other rice quality components, the rate of cracked rice significantly differed with the cultivar in 2006, and the years and the interaction of year × cultivar had significant effects on the rate of cracked rice. In both years the rate of MWK was highest in Hatsuboshi, lowest in Koshijiwase and Hanaechizen, and intermediate in Koshihikari and Nipponbare.

2. Grain dry matter increase in cultured panicles

When panicles from the five cultivars were cultured for one week after heading at 23, 28 and 33ºC, the F% increased with the increase in temperature, attained a ceiling, or decreased after the increase, although the F% from 23 to 28ºC in Hanaechizen (2005) and from 28 to 33ºC in Nipponbare (2006) scarcely changed (Fig. 2). The panicle culture started earlier in 2006 than in 2005 after flowering because the F% at the start of panicle culture was much smaller in 2006 than in 2005.

Discussion

The percentage of MWK in total ripened grains increased when the average temperature in the grain-filling period exceeds 23−25ºC in Koshihikari (Kobata et al. 2004) as other experiments suggested (Nishio and Hashimoto, 1997; Arisaka, 2001; Furudoi, 2002). In our two-year field experiments, the average temperature until the first of September covering a dominant grain-filling period in four cultivars except Nipponbare in 2006 was over 25ºC (Fig. 1). As a result, there was a clear cultivar difference in the rate of MWK among the five cultivars examined, although the rate in Nipponbare in 2006 was low due to the lower temperature in the grain-filling period (Table 1). The trend in cultivar differences of the rate of MWK coincides well with the results of the
incubated temperature in 2005 should be appropriate to the estimation of the potential GIR more than in 2006, because the F% in diverse rice cultivars was the highest between 20 and 70% in the middle of the grain filling period (Kobata and Uemuki, 2004; Kobata et al., 2006) and the result of solution culture in 2005 well covered the range. However, in 2006 spike culture in several cultivars started from an F% much lower than 20% and hence these F% would be affected by lower rate in the first phase of the grain-filling. The decrease in F% at 33ºC, suggested that an uncommon high average temperature [the highest average temperature in August in Matsue was 29.3ºC for the past 70 years (JMA, 2010)] inhibit starch accumulation due to such factors as the inhibition of starch synthesis (Inaba and Sato, 1976), although the exact reason cannot be determined from our results alone.

Increases in the F% at 28ºC during the culture term (one week) were calculated from the F% one week after the start of culture. The increase in F% during the one week highly correlated with the percentage of MWK that formed under the field condition in the five cultivars in 2005 (Fig. 3). In 2006, a high correlation was observed in four cultivars, except Nipponbare, because the average temperature during the grain-filling period was lower (Fig. 1). These results suggest that in cultivars showing a higher GIR under higher temperature conditions, the rate of MWK is higher. The higher requirement of the GIR under high temperature conditions causes a shortage of the assimilate supply to grains, thereby increasing the rate of MWK (Kobata et al., 2004). Hence, cultivars that have a lower potential GIR performance under high temperature conditions will have a lower rate of MWK. Furthermore, for resulting stability of the responses of GIR to temperature, appropriate culture conditions such as incubating temperature and timing (panicle stage) of the start of culture should be investigated to increase reliability as cultivar selection criteria.

The rate of F% after culture at high temperature conditions would be the conventional criterion for distinguishing cultivars that are at high risk of developing MWK. However, not only the GIR but also the source capacity (assimilation and reserved assimilate in straws) under higher temperature conditions might affect the rate of MWK (Kobata et al., 2004). Therefore, the performance of assimilation or reserved assimilate during the grain filling period as well as grains under high temperature conditions should be investigated to find an effective target for the development of resistant cultivars in MWK.

The potential GIR was estimated from the F% at a 28ºC spike solution culture, which was the highest among three temperature ranges in most of the cultivars (Fig. 2). The trend was clearer in 2005 than in 2006. Responses of F% to screening test in an elevated temperature greenhouse (Iida et al., 2002). Hence, the cultivars used, except for Nipponbare in 2006, showed a general trend of cultivar difference in the rate of MWK under the field conditions. Rates of MWK and white base immature rice significantly differed with the cultivar in both years (Table 1) and hence they showed stable cultivar performances for rice quality. MWK is more dependent on the environment than the white base kernel (Iida et al., 2002) although the white base kernel and the white base immature kernel are not the same. If environmental variance between years is small, these differences between both qualities are possible to be disappeared.

Fig. 3. Relationships between percentage of milky white rice kernels under field conditions and increase in filling percentage of grain during one week in panicles cultured at 28ºC. Each point is the mean of two (2005) or four (2006) observations.

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