Reduction of Rice Chalky Grain by Deep and Permanent Irrigation Method; Effect on Growth and Grain Quality of Rice

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Abstract: Formation of chalky grains which is the main cause of the degradation of rice grain quality occurs frequently when the temperature during the 20 days after heading exceeds 27°C. In this study, we examined the grain quality, chalky grain formation, growth and yield of plants grown by the deep and permanent irrigation (DPI) method, which is a combination of the V-furrow direct seeding method and the deep-flooding irrigation method. Field experiments were conducted in 2008, 2009 and 2010. The DPI method in which the water level was maintained at about 20 cm above the soil surface, improved grain quality by decreasing the chalky grain ratio by about 5% and increasing the perfect grain ratio by about 10% as compared with the V-furrow direct seeding method in which the water level was maintained at about 10 cm above the soil surface. The DPI method decreased the number of tillers and panicles but increased the number of spikelets per panicle so that the two methods gave similar yields. DPI is a promising labor-saving method expected to reduce the formation of chalky grain due to a high temperature during ripening.

Key words: Chalky grain, Deep-flooding irrigation, Grain quality, High temperature, Rice, V-furrow direct seeding method.

Occurrence of chalky grains in rice substantially lowers the grade of grain quality and thus market value (He et al., 1999; Tan et al., 2000; Patindol and Wang, 2003; Cheng et al., 2005; Fitzgerald and Resurreccion, 2009; Ishimaru et al., 2009). Formation of chalky grain is enhanced by a high temperature during the ripening period, which is recently occurring more frequently due to global warming (Lin et al., 2005; Wakamatsu et al., 2007; Tsukaguchi and Iida, 2008; Lin et al., 2010). Many studies have been conducted about chalky grains. However, most of them were carried out for understanding the mechanism underlying the high-temperature-induced grain chalkiness (Patindol and Wang, 2003; Cheng et al., 2005; Lin et al., 2005; Lin et al., 2010), or genetic analysis (He et al., 1999; Tan et al., 2000).

Chalky grains are categorized into white-cored, milky-white, white-back, white-based, and white-belly types depending on the site of the chalky part in the grain (Nagato and Ebata, 1965; Tashiro and Wardlaw, 1991), and especially milky-white, white-back and white-based types are frequently found when the average temperature during the 20 days after heading exceeds 27°C (Wakamatsu et al., 2007).

Development of a method to effectively reduce chalky grains with little additional cost and labor is awaited. In Japan, several studies have been done to reduce the occurrence of chalky grains, such as fertilizer management (Nomura et al., 2004; Sakata et al., 2008), and water management (Arai and Itou, 2001), in addition to breeding (Watanabe et al., 1998; Hoshi et al., 2002; Yamaguchi et al., 2006). Deep-flooding irrigation is a simple and useful method of water management that requires only control of irrigated water level with no additional equipment or materials (Sato et al., 2004; Chiba et al., 2009). Such a culture method that reduces chalky grains occurrence needs to involve as few procedures and decision making processes as possible and at the same time must be laborsaving like direct seeding so that it becomes practical and easily accepted by the farmer.

The deep-flooding irrigation method was originally developed for protecting the seedlings and young panicles from cool weather damage (Kobayashi and Satake, 1979; Satake et al., 1988). Later, it was adjusted for use as a growth control method to suppress the development of non-productive and weak tillers, and promote that of productive tillers (Ohe and Mimoto, 1998). Ohe and Mimoto (2002) reported improvement in the productive tillers, yield and lodging tolerance when appropriate protocols were followed such as starting the deep-flooding...
treatment at the active tillering stage, and adjusting the water depth to the leaf sheath of the fully expanded leaf on the main stem. However, the panicle number per hill decreased when the deep treatment period was extended to a certain growth stage, (Nishiki et al., 1987; Watanabe et al., 2006) and the yield decreased (Nishiki et al., 1987; Watanabe et al., 2006), or the plants became more susceptible to lodging (Ohe and Mimoto, 1998). Deep-flooding irrigation effectively suppressed the development of non-productive and weak tillers, and promote that of productive tillers also under the conditions of direct seeding culture (Won et al., 1999; Ohe et al., 2010). However, the effects of deep-flooding irrigation on the grain quality have not yet been examined in direct seeding culture.

The V-furrow direct seeding (VFDS) method developed by Aichi Agricultural Research Center (Hamada et al., 2008) is a technique requiring about 30% less labor than the conventional transplant cultivation (Aichi Agricultural Research Center, 2003) and is characterized by deep-flooding irrigation and heavy fertilizer application. Based on this study, as a new culture method, Hamada et al. (2008) proposed the deep and permanent irrigation method (DPI) that essentially requires the irrigated water level to be kept about 20 cm above soil surface as a combination of deep-flooding irrigation and VFDS. Since VFDS requires only the maintenance of water level until maturity without midseason drainage, they concluded that it can be combined effectively with deep-flooding irrigation, which may then save irrigation water and labor without yield penalty. DPI is a promising labor-saving method expected to reduce chalky grain formation, but its impact on grain quality needs to be evaluated.

Chiba et al. (2009) suggested that the effects of deep-flooding treatment on grain quality may be attributed to the improvement in source activity, i.e., photosynthetic activity in addition to the decrease in number of spikelets per unit area (sink size reduction). In addition, Won et al. (1999) showed that the nitrogen content in the flag leaf was higher in plants grown with deep-flooding irrigation than ordinary water management (shallower water depth). Since the high leaf nitrogen concentration is positively correlated to high photosynthetic activity (Ishihara et al., 1979a, 1979b; Ookawa et al., 2003; Zhang and Kokubun, 2004), the favorable effects of deep-flooding irrigation on grain quality may be at least partially attributable to the promotion of photosynthetic activity under high temperature conditions. However, there have been no studies showing clearly the high photosynthetic activity for the plants grown with deep-flooding irrigation.

In the present 3-year study, we evaluated the effects of DPI on the chalky grains, growth, yield and grain quality in comparison with the conventional method.

Materials and Methods

1. Crop cultivation

We used Koshihikari, a leading paddy rice variety of Japan, in this study. The experiments were carried out at the experimental farm of Aichi Agricultural Research Center (N: 35°9’46”, E: 137°4’5”, Alt. 90 m, Soil type is aeric, typic epiaquults), Nagakute, Japan.

Seeds were first disinfected with thiram water-dispersible powder (0.5% w/w). Seeds were direct seeded on well-drained paddy field with the V-furrow No-till Direct Seeding machine. This is an attachment of tractor which makes seeding furrow (width: 2 cm, depth: 5 cm, row distance: 20 cm) with disk to drive (Hamada et al., 2007). The sowing rate was 7 g m² and row distance was 20 cm. At the time of sowing, nitrogen fertilizer of compound coated urea (release controlled urea; Chisso Co. Ltd., Tokyo, Japan) was applied to seeding furrow at a rate of 7.8 g m².

In Experiment 1, the water level was maintained at 10 cm (control) and 20 cm (deep treatment). In Experiment 2 the paddy field was inclined. Refer to Materials and Methods for details.

| Experiment | Year | Sowing date Before the emergence of seedlings | After the emergence of seedlings | After irrigation | Start of waterlogging |
|------------|------|-----------------------------------------------|---------------------------------|-----------------|----------------------|
| 1          | 2008 | 16 April                                     | 28 April                        | 16 May          | 28 May               | 19 May               |
|            | 2009 | 9 April                                      | 23 April                        | 15 May          | 27 May               | 19 May               |
|            | 2010 | 19 April                                     | 30 April                        | 28 May          | 5 June               | 1 June               |
| 2          | 2008 | 30 April                                     | 5 May                           | 16 May          | –                    | 27 May               |
|            | 2010 | 19 April                                     | 30 April                        | 28 May          | –                    | 1 June               |
2. Experiment 1: Effects of deep and permanent irrigation method (DPI) on growth and grain quality

This experiment was carried out on a paddy field (100 m x 10 m) for three years in 2008, 2009 and 2010. There were two treatments for water depth; (1) deep (water depth was maintained at 20 cm above the soil surface), which served as the DPI, (2) Control (water depth was maintained at 10 cm above the soil surface). Each treatment was replicated four times with a randomized block design.

Date of sowing, herbicidal application and the start of waterlogging are shown in Table 1. We started the flooding treatment when the rice plants were at the three- to four-leaf stage, and maintained the water level in each treatment until the ripening period (about 5 days before the ripening). For weed control, glyphosate-isopropylammonium, cyhalofop-butyl-bentazone and pyrazolate were sprayed before and after the emergence of seedlings, and after the flooding treatment started. These applications gave almost perfect weed control, which is one of the essential components of this DPI method.

3. Experiment 2: Relationship between water level and grain quality

This experiment was conducted on an inclined paddy field (50 m long and the vertical difference in water surface between the two ends of the field was about 10 cm, The size of field was 85 m x 20 m) in 2008 and 2010. We set plots at three depths of water, 20, 15 and 10 cm in 2008, and four depths, 20, 16, 12 and 8 cm in 2010. For each depth, there were three replications with a randomized block design.

The date of sowing, herbicidal application and the start of waterlogging are shown in Table 1. As in experiment 1, we also started the flooding treatment when the rice plants were at the three- to four-leaf stage, and maintained the same water level in each treatment until the ripening period (about 5 days before ripening). For weed control, glyphosate-isopropylammonium was sprayed before the emergence of seedlings, and cyhalofop-butyl-bentazone and halosulfuron-methyl were sprayed after the emergence of seedlings.

4. Measurements

The number of seedlings that emerged was counted before the flooding treatment started. Plant height, tiller number and leaf color (chlorophyll meter value) were measured at 30 days after the onset of the flooding treatments, maximum tiller number stage and panicle formation stage. Leaf color was measured using a chlorophyll meter (SPAD502, Minolta Co. Ltd., Japan) on the next to the uppermost expanding leaf.

At the ripening stage, culm length, panicle length and panicle number were measured. For yield survey, plants within an area of 1.6 m\(^2\) (4 rows of 2 m each) were sampled from each plot. The weight and moisture content of grains were measured, and then grains were separated into two equal groups. The grains in one group were used for further investigation of yield components such as the number of filled and unfilled grains as well as 1000-grain weight. The filled grains were selected by using a sifter with a slit width of 1.85 mm, and grain weight (1000-grain weight) was measured for the filled grains that were adjusted to the moisture content of 14.5% w/w. Grains in the other group were used for the grain quality test.

5. Grain quality test

The appearance of fully matured grains was evaluated with a Rice Inspector (RGQ110, SATAKE) and grains were classified into perfect grains (PG), milky-white grains (MWG), and white-based/white-back grains (WBG). We calculated the ratio of the number of grains of each type to the whole grains per panicle.

6. Statistical analysis

Data were analyzed with the statistical package Excel Tokei 2008 (Social Survey Research Information Co. Ltd., Tokyo, Japan) and Excel 2003 for Win. In Experiment 1, as depth of water and annual variation were explanatory variables, analysis of variance (ANOVA) was done. Comparisons of means of plant height, tiller number and leaf color were made by the t-test. In Experiment 2, as depth of water was an explanatory variable, ANOVA was done. Comparisons of means were performed by Tukey’s method.

Results

1. Mean air temperature during the 20 days after heading

Headng time and mean air temperature during the 20 days after heading are shown in Table 2. In all the experiments, heading time was from August 2 to 4. The mean temperature during the 20 days after heading exceeded 27°C, except for 2009.

![Table 2. Heading date and mean air temperature during the 20 days after heading.](image)
2. Effects of deep and permanent irrigation method (Experiment 1)

In the three years, ratio of perfect grains in the deep treatment (20 cm water level) was always significantly higher than in the control (10 cm water level) while the WBG ratio was significantly lower (Table 3). There was no significant interaction between year and depth of water for the grain quality.

Plants in the deep treatment were significantly taller than control at 30 days after the onset of the flooding treatment (Fig. 1). This trend continued during vegetative growth. Tiller number in the deep treatment was less than that in the control (Fig. 2). The difference between the two treatments was largest on June 27 (around the maximum tiller number stage). Leaf color (SPAD value) tended to be darker in the deep treatment than in the control during vegetative growth (Fig. 3).

In 2009, plants were taller, had fewer tillers, and darker leaf color in the deep treatment than in the control (data not shown).

At ripening, the culm length was significantly longer, and panicle number was significantly less in the deep treatment than in the control while panicle length did not change significantly between treatments (Fig. 4).

### Table 3. Effects of deep and permanent irrigation method on grain quality (Experiment 1).

| Year | Treatment | PG  | MWG | WBG  |
|------|-----------|-----|-----|------|
| 2008 | Deep      | 68.5| 4.7 | 9.1  |
|      | Control   | 58.3| 6.0 | 13.5 |
| 2009 | Deep      | 81.4| 2.5 | 2.8  |
|      | Control   | 78.8| 2.0 | 4.1  |
| 2010 | Deep      | 69.8| 2.3 | 11.2 |
|      | Control   | 63.1| 3.3 | 14.0 |

Grain quality was evaluated with a Rice Inspector (RGQ10, SATAKE). Grains were classified into perfect grains (PG), milky-white grains (MWG), and white-based/white-back grains (WBG). **, * and ns show significant difference at 1% and 5% probability level and no-significant difference, respectively, with ANOVA. Deep, Water level was maintained at 20 cm above the soil surface; Control, Water level was maintained at 10 cm above the soil surface.
differ significantly in the three years (Table 4). Lodging was not observed in any of the treatments.

There was no significant difference in yield between the deep treatment and control consistently for three years (Table 4). In yield components, 1000-grain weight, percentage of ripened grains (PRG) and number of spikelets m$^{-2}$ (NSm-2) were not significantly different, but number of spikelets per panicle was significantly larger in the deep treatment than in the control. There was no significant interaction between year and depth of water for yield and yield components except 1000-grain weight.

3. Relationship between water depth and grain quality (Experiment 2)

In 2008, the ratio of perfect grains increased as the water depth increased, being significantly higher at a 20 cm water depth than at a 10 cm water depth, while the WBG ratio was significantly higher at a 10 cm depth than at a 10 cm depth. Such trends in perfect grains and white-based/white-back grains were also observed in 2010. The formation of MWG was not affected by the water depth (Table 5).

In 2008, similar to plant height, culm length tended to be longer in the order of 20-, 15- and 10-cm water depth, a significant difference being noted between 20- and 10-cm depths (Table 6). Panicle number tended to decrease as water depth increased and that at 10-cm was significantly larger than that at the other two depths. These trends in culm length and panicle number were observed in 2010 as well. In any of the treatments, lodging was not observed.

In 2008, water depth did not significantly affect yield and its components except Number of spikelets per panicle, which was significantly larger at a 20 cm than 10-cm treatment. In 2010, water depth significantly affected neither yield nor any of its components.

Discussion

DPI is a combination of deep-flooding irrigation and VFDS, and essentially requires only irrigated water level to be kept about 20 cm above soil surface (Hamada et al., 2008). In this study, we showed clearly that the DPI had an effect of reducing chalky grains occurrence, and this effect was stable over the years. Based on these results, we conclude that DPI is an effective method to reduce the occurrence of chalky grains.
1. Effects of DPI method on growth, yield and grain quality

Deep-flooding treatment increased the ratio of perfect grains but decreased the ratio of white-based/white-back grains consistently for three years (Table 3, Experiment 1). Wakamatsu et al. (2007) reported that chalky grains, especially white-back and white-based types, are frequently found when the average temperature during the 20-day period after heading exceeds 27ºC, which was the case in our study. Therefore, the measure against the degradation of grain quality due to the high temperatures during the ripening period should be to suppress the formation of white-back and white-based type chalky grains. In this study, the deep treatment decreased white-back/white-based grains (Table 3).

Chiba et al. (2009) reported that under deep-flooding treatment with transplanting culture, the occurrence of chalky grains, especially milky-white type, decreased. In this paper, we showed clearly that the occurrence of chalky grains, especially white-based/white-back type, was decreased by DPI. As stated earlier, VFDS on which DPI was based, can reduce labor by about 30% as compared with conventional transplant cultivation (Aichi Agricultural Research Center, 2003). Although the type of chalky grain was different, DPI also reduced the occurrence of chalky grains, and thus can be one of the measures against the degradation of grain quality caused by high temperatures during the ripening period.

Furthermore, we found that the DPI affected plant growth as well. Plant height was taller, and tiller number was less at deeper water level (Fig. 1 and 2). Ohe et al. (1994), using cv Sasanishiki under pot conditions, and Chiba et al. (2009), in transplanting culture, obtained results similar to this study that used cv Koshihikari with VFDS method under field conditions.

Ohe and Mimoto (1998) found that in transplanting culture, lodging tolerance decrease under long term deep-flooding treatment. In this study, however, despite the long-term deep-flooding treatment from seedling to the ripening period, lodging was not observed. Under DPI

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**Table 5. Relationship between depth of water and grain quality (Experiment 2).**

| Year | Depth of water (cm) | PG  | MWG | WBG  |
|------|---------------------|-----|-----|------|
|      |                     | 201 | 92  | 2 | 48 |
| 2008 | 20                  | 59.1 | 9.1 | 12.7 |
|      | 15                  | 55.1 | 8.8 | 19.1 |
|      | 10                  | 51.5 | 9.0 | 19.1 |
| 2010 | 20                  | 57.5 | 6.0 | 20.0 |
|      | 16                  | 55.1 | 7.6 | 21.0 |
|      | 12                  | 51.7 | 7.3 | 24.5 |
|      | 8                   | 51.7 | 7.5 | 24.5 |

Grain quality was evaluated with Rice Inspector (RGQ10, SATAKE). Grains were classified into perfect grains (PG), milky-white grains (MWG), white-based/white-back grains (WBG). Means followed by the same letter are not significantly different at 5% level by Tukey’s method.

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**Table 6. Relationship between depth of water and yield (Experiment 2).**

| Year | Depth of water (cm) | Culm length (cm) | Panicle length (cm) | Panicle number (m²) | Yield (g m⁻²) | PRG (%) | 1000-grain weight (g) | Grain number |
|------|---------------------|------------------|---------------------|---------------------|---------------|---------|-----------------------|-------------|
|      |                     |                  |                     |                     |               |         |                       |             |
| 2008 | 20                  | 101.1 b          | 19.2 a              | 413 a               | 588 a         | 85.5 a  | 21.6 a                | 31.8 a      |
|      | 15                  | 94.7 ab          | 18.1 a              | 438 a               | 583 a         | 83.2 a  | 21.7 a                | 32.4 a      |
|      | 10                  | 91.2 a           | 17.9 a              | 513 b               | 591 a         | 83.9 a  | 21.4 a                | 32.9 a      |
| 2010 | 20                  | 98.0 a           | 20.0 a              | 300 a               | 528 a         | 78.7 a  | 22.4 a                | 30.0 a      |
|      | 16                  | 95.0 ab          | 20.4 a              | 348 a               | 511 a         | 80.5 a  | 21.5 a                | 29.7 a      |
|      | 12                  | 92.0 b           | 19.6 a              | 399 a               | 495 a         | 77.1 a  | 22.4 a                | 28.8 a      |
|      | 8                   | 90.3 b           | 19.9 a              | 398 a               | 512 a         | 81.2 a  | 21.0 a                | 30.2 a      |

Yield and 1000 grain weight were determined with grains that were selected by using a sifter with slit width of 1.85 mm, and adjusted to the moisture content of 14.5%. PRG shows percentage of ripened grains, NSm2 number of spikelets per unit area (m²) and NSp1 number of spikelets per panicle. Means followed by the same letter are not significantly different at 5% level by Tukey’s method.
treatment, Hamada (2005) suggested that under VFDS, plants tend to be more tolerant to lodging because its seeding depth (about 5cm) is deeper than in other culture methods.

The deep-flooding appeared to have no significant effects on yield since the number of spikelets per panicle was increased although tiller number was decreased (Table 4). This result agrees with those reported by Hamada et al. (2008) and transplanting culture with deep-flooding treatment (Nishiki et al., 1987; Chiba et al., 2009).

Generally, a trade-off relationship exists between the number of spikelets per panicle and percentage of ripened grains; if the number of spikelets per panicle increases, the percentage of ripened grains tends to decrease. In this study, such a relationship was not observed. These facts strongly suggest that dry matter production of each panicle was increased by deep-flooding treatment. Ohe and Mimoto (1999) found that the dry matter growth rate on the main stem in plants grown under deep-flooding exceeded that in the control (shallower water depth). Koide et al. (1997) reported that under VFDS, grain weight on main stem was heavier than on other stems. Therefore, deep flooding is expected to further improve the efficiency of VFDS.

2. Relationship between water depths and grain quality

To confirm the effects of DPI on the reduction of chalky grains, we conducted another experiment for two years using an inclined field to examine whether the ratio of perfect grains increases as the depth of water increases (Table 5, Experiment 2). We also examined the effect on the grain protein content. Wakamatsu et al. (2008) showed a negative correlation between grain protein content and the occurrence rates of white-based/white-back grains under high temperature conditions during the ripening. Our study further showed that leaves became a deeper green showing higher SPAD values under deeper water levels, suggesting that DPI promoted nitrogen uptake, resulting in high ratio of perfect grains and low ratio of white-based/white-back grains.

The plants grown by the DPI methods were taller and had fewer tillers as described above (Experiment 1), and Experiment 2 showed that such characteristics became more pronounced as water depth increased (Table 6). Ohe et al. (1994) found that deep-flooding promoted leaf blade growth while inhibited the growth of tiller buds during the vegetative stage, which well explains the less tillering characteristics of plants grown by the DPI method in our study.

3. Advantages of DPI

The unbalance between the sink and source ability in terms of carbohydrate metabolism, which is caused by the high temperature during the ripening period, has been considered to be the main cause of chalky grains (Morita, 2008), and technical development for reducing chalky grains has been targeted on this aspect, such as fertilization management. Sakata et al. (2008) reported that chalky grains decreased by using release-controlled fertilizer as topdressing at the panicle formation stage. Furthermore, Nomura et al. (2004) reported that chalky grains were reduced by increasing the quantity of nitrogen fertilizer. In contrast, DPI requires only the control of the water level, without an increase in fertilizer.

However, there are a few problems with incorporating deep-flooding method into conventional transplanting culture. First, if the deep treatment period was prolonged to a certain growth stage, the panicle number per hill decreased than that in the conventional irrigation due to restricted tiller number (Nishiki et al., 1987; Watanabe et al., 2006), and yield (Nishiki et al., 1987; Watanabe et al., 2006) or lodging tolerance decreased (Ohe and Mimoto, 1998). Diligent water management is required to avoid yield penalty. Second, midseason drainage is practiced for transplanting culture, and thus a larger amount of irrigated water will be drained than in conventional transplanting culture. Therefore, it would be difficult for deep-flooding irrigation to be used on a large-scale farm from the viewpoint of efficient use of water resources. One of the important technical reasons to practice midseason drainage is to harden the clayey soil prevailing in Japan so that heavy machinery can be used for the harvest. Hamada et al. (2008) reported that in VFDS the water level is maintained from the beginning and does not require the midseason drainage. However in VFDS, the soil remains hard enough to support heavy machinery operation during harvest because of no tillage. For this reason, the DPI can be quite labor-saving as well as water saving mainly due to the advantages of VFDS culture. Furthermore, Hamada et al. (2008) stated that yield is decreased by deep-flooding irrigation if precise protocol of water management is not followed in conventional transplanting culture while DPI does not affect yield. Our study supported such advantages of DPI and further showed that it substantially improved grain quality as well.

In DPI, the water level is maintained at a depth of 20 cm from the start of waterlogging until ripening and does not require the adjustment of water depth with plant growth proposed by Ohe and Mimoto (2002). Neither yield decrease nor lodging was observed with DPI in this study. These results suggest that the DPI method is a practical and labor-saving method to reduce the occurrence chalky grain.

Furthermore, Arai and Miyahara (1956) reported that maintaining the water depth at 15 cm from transplanting time can control weeds. Sasaki et al. (1994) suggested that with the deep-flooding treatment, the deeper water level and more prolonged period could control weeds more
effectively. In this study, deep-flooding treatment effectively reduced the fresh weight of the weeds to about 1% of that in the control.

In summary, we found that DFI is a laborsaving method requiring less input of resources, with reduced chalky grain occurrence and good weed control. We therefore conclude that it is a practical method to improve rice grain quality, which can be adopted by large-scale rice farmers. In 2010, DFI is being practiced on about 250 ha in Japan and the area is expected to increase in the future.

Acknowledgement
This study was supported in part by a Grant-in-Aid from the National Agricultural and Food Research Organization (NARO).

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* In Japanese with English abstract.

** In Japanese with English summary.

*** In Japanese.