Enhanced Nitrogen Uptake and Photosynthesis of Rice Grown with Deep and Permanent Irrigation Method: Possible Mechanism for Chalky Grain Reduction

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Abstract: Recently, the occurrence of chalky grain caused by high temperature stress at the ripening stage has been a global problem for rice. We previously showed that the deep and permanent irrigation method, which is the combination of the V-furrow direct seeding and deep-flood irrigation methods, reduced chalky grain occurrence. To study the possible physiological mechanisms for reduced chalky grain occurrence by the deep and permanent irrigation method, we conducted field experiments in 2008 and 2009 to examine the effects of the deep-flood treatment on plant nitrogen (N) content, stomatal conductance and photosynthetic rate especially at the ripening stage. Results showed that in the deep-flood treatment that maintained a 20 cm water depth, leaf N content was consistently and significantly higher than the control with only a 10 cm water depth. Except two measured days, the stomatal conductance under the deep-flood treatment was significantly higher than in the control. Furthermore, stomatal conductance and photosynthetic rate in the deep-flood treatment were always significantly higher than in the control in both years. Thus, the deep-flood treatment enhanced N uptake, and consequently photosynthetic activity, resulting in the reduction of chalky grain formation, as previously reported. Accordingly, the effects of deep-flood treatment on grain quality improvement in rice may possibly be attributed to the improvement in source activity.

Key words: Chalky grain, Deep-flood irrigation, Grain quality, High temperature, Nitrogen content, Photosynthesis, Rice.
leads to an increase in the photosynthetic capacity (Makino, 2003; Ookawa et al., 2003; Zhang and Kokubun, 2004). Tabata et al. (2008) predicted that reduction of photosynthetic rate at the ripening stage would increase the occurrence of chalky grain particularly the white-back grain. We therefore assumed that enhanced source ability through cultural management is needed to reduce the occurrence of chalky grain.

Deep-flood irrigation is one of the cultural methods that can reduce the occurrence of chalky grains (Sato et al., 2004; Chiba et al., 2009). Rice grown under deeper water level tended to produce less tillers but increased percentage of productive culms (Nishiki et al., 1987; Furuya et al., 1991; Ohe and Mimoto, 1998), and produce less number of spikelets per unit area (Kiriyama and Nakaya, 1987; Nishiki et al., 1987; Furuya et al., 1991) as compared with that under ordinary water management. These facts show that deep-flood irrigation is effective to control the sink size.

In contrast, Ohe and Mimoto (1999) found that deep-water treatment increased the dry matter production on the main stem of rice plants. Chiba et al. (2009) suggested that the positive effects of deep-flood treatment on grain quality may be attributed to the improvement in source activity. These facts suggest that the favorable effects of deep-flood irrigation on grain quality may be at least partly attributable to the promotion of photosynthetic activity. Won et al. (1999) showed that the N content of the flag leaf was higher in the plants grown with deep-flood irrigation than with ordinary water management. Based on the analysis of the amount of N in leaf blades at the full heading time, Chiba et al. (2011) suggested that deep-flood irrigation promoted photosynthesis at the ripening stage. These findings show that deep-flood irrigation enhanced N content of plant, especially leaf, which is expected to enhance photosynthetic activity. However, there has been no study that directly examined the photosynthetic activity of the plants grown with deep-flood water management.

We previously found that the deep and permanent irrigation method (DPI), a combination of deep-flood irrigation and the V-furrow direct seeding methods, which essentially requires to irrigate and maintain the water level at about 20 cm above soil surface (Hamada et al., 2007, 2008), reduced chalky grain occurrence (Hayashi et al., 2011). Hayashi et al. (2011) further showed that leaves became greener as expressed in higher SPAD value (leaf color) under deep-water treatment, suggesting that the DPI promoted N uptake as was also reported by Won et al. (1999).

In this study, we examined if the N uptake and consequently photosynthetic activity would be enhanced when rice was grown under deep flood conditions as a possible physiological mechanism for the reduction of chalky grain formation.

Moreover, several reports have shown that water and soil temperatures at the ripening stage affect the formation of chalky grains (Kasaneyma et al., 1999; Arai and Ito, 2001; Tsubone et al., 2008). Thus, we also examined the air, water and soil temperatures near the plant at the ripening stage to check whether difference in temperatures was influenced by water level.

Materials and Methods

1. Crop management

We used Koshihikari, the leading 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), Nagakute, Japan, in 2008 and 2009. The soil type is aeric, typic epiaquults.

The seeds were first disinfected with thiram water-dispersible powder (0.5% w/w) and then sown directly on well-drained paddy field with the V-furrow No-till Direct Seeding machine. This machine is attached to the tractor, which can make 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 m⁻². At the time of sowing, N fertilizer was applied to seeding furrow at the rate of 7.8 g m⁻². The N fertilizer used was a compound coated urea (controlled release urea, Chisso Co. Ltd., Tokyo, Japan) which is a blend of two kinds of linear type (MEISTER-10, MAISTER-15) and sigmoidal type (MEISTER-S12) urea at a ratio of 3 : 2 : 5 (Ikeda et al., 2001).

The water depth was maintained at 20 cm from the soil surface, which served as the DPI, and at 10 cm from the soil surface, which is the depth of water generally used when carrying out the V-furrow direct seeding methods in Aichi prefecture, as the control. The treatment and control plots were replicated four times with a randomized block design (plot size: 10 m × 5 m). The number of seedlings was 166 m⁻² in 2008 and 194 m⁻² in 2009.

The dates of sowing, herbicidal application and timing of waterlogging are shown in Table 1. We maintained the water level in each plot until the ripening stage. For weed control, glyphosate-isopropylammonium, cyhalofop-butyl-bentazon and pyrazolate were sprayed, before and after the emergence of rice seedlings, and after the start of water depth treatments. The above management practices almost perfectly controlled weeds, which are the essential components of DPI.

2. Measurements

For N content measurement, plants were sampled at the panicle formation stage (16 July 2008 and 10 July 2009), heading time (8 August 2008 and 10 August 2009) and ripening stage (12 September 2008 and 15 September 2009), from a 50 cm length of row in each plot. The samples were separated into leaf (leaf blade), stem (with
leaf sheath) and panicle, which were then oven-dried at 80°C for 72 hours for dry weight determination. After milling, N content (%) was measured with a MACROCORDER (JM1000CN, J-Science Lab Co. Ltd., Kyoto, Japan). Total N was calculated by multiplying N content (%) and plant dry weight per unit area (g m⁻²).

Protein contents of grain were measured by using near-infrared spectrum photometer (HON6400, NIRECO). The values were shown on dry weight basis.

Leaf stomatal conductance was measured with a leaf porometer (SC-1, DECAGON Devices Inc., Pullman, WA, USA), on 1, 14 and 21 August in 2008, and 15 July and 13, 20 and 26 August in 2009 in plants that showed average growth in each treatment. Flag leaves from ten plants in the treated and control plots were measured except 15 July 2009 when the next-to-the-uppermost expanding leaf was measured instead.

Leaf photosynthetic rate and stomatal conductance were measured with a portable photosynthesis system on a clear day (LI-6400, Li-Cor, Lincoln, Nebraska, USA) at about two weeks after heading time (22 August 2008 and 21 August 2009) in plants that showed average growth. Flag leaf from ten plants in each treatment was measured between 1115 and 1145 in 22 August 2008, and 1055 and 1120 in 21 August 2009, under a photosynthetic photon flux density of 1200 μmol m⁻² s⁻¹ artificially provided by red/blue light-emitting diodes. The leaf chamber temperature was maintained at 30°C, the reference CO₂ concentration was 400 μmol mol⁻¹, and relative humidity was 75.5%.

For measuring air temperature, a sensor instrument (RTH-1120, ESPEC MIC Corp., Aichi, Japan) was set at 1.2 m above the paddy field. In addition, for measuring water and soil temperatures, sensor instruments (RTH-1120, ESPEC MIC Corp., Aichi, Japan) were set at soil surface and 5 cm beneath the soil surface. These microclimate sensors were connected to a thermo recorder (RT-12, ESPEC MIC Corp., Aichi, Japan) and values of each were recorded every 10 minutes. Temperatures were measured in all plots (2 × 4 points).

3. 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. As depth of water depth and inter-annual variation was explanatory variable, analysis of variance (ANOVA) was done. Comparisons of leaf stomatal resistance were tested by *t*-test.

### Table 1. Date of sowing, herbicide application and start of waterlogging in 2008 and 2009 field experiments.

| Year | Sowing date | Herbicidal application | Start of waterlogging |
|------|-------------|------------------------|-----------------------|
|      |             | Before the emergence of seedlings | After the emergence of seedlings | After irrigation |          |
| 2008 | 16 April    | 28 April               | 16 May                | 28 May          | 19 May   |
| 2009 | 9 April     | 23 April               | 15 May                | 27 May          | 19 May   |

Water level was maintained at 10 cm (control) and 20 cm (deep-flood treatment).

### Table 2. Effects of depth of water on plant nitrogen content.

| Year | Depth of water (cm) | Panicle formation stage | Heading time | Ripening stage | Protein content of grain | Total nitrogen (g m⁻²) |
|------|---------------------|-------------------------|--------------|----------------|--------------------------|-----------------------|
|      |                     | Leaf | Stem | Panicle | Leaf | Stem | Panicle | Leaf | Stem | Panicle | Leaf | Stem | Panicle | Heading time | Ripening time |
| 2008 | Deep                | 2.44 | 0.95 | 2.25    | 0.74 | 1.01 | 0.56    | 0.95 | 0.53 | 1.00    | 7.63 | 11.87 | 11.90   |              |
|      | Control             | 2.20 | 0.80 | 2.08    | 0.77 | 1.04 | 0.56    | 0.95 | 0.53 | 1.00    | 7.71 | 12.06 | 11.56   |              |
| 2009 | Deep                | 1.92 | 1.08 | 1.93    | 0.68 | 0.89 | 0.64    | 1.16 | 0.64 | 1.12    | 7.27 | 9.94  | 13.94   |              |
|      | Control             | 1.69 | 0.87 | 1.91    | 0.66 | 0.92 | 0.53    | 0.93 | 0.53 | 0.95    | 7.78 | 9.42  | 9.98    |              |

ANOVA

| Year(Y) | Depth of water(D) | Y × D |
|---------|-------------------|-------|
| **      | **                | ns    |
| **      | *                 | ns    |
| **      | **                | ns    |
| ns      | ns                |       |
| **      | ns                |       |
| ns      |                   |       |

Numerals in the table are Unit % (w/w). Heading time was 4 August in 2008 and 5 August in 2009. Sampling time was 16 July in 2008 and 10 July in 2009 as panicle formation stage, 8 August in 2008 and 10 August in 2009 as heading time, 12 September in 2008 and 15 September in 2009 as ripening stage. Nitrogen contents were evaluated with MACROCORDER (JM1000CN, J-Science Lab Co. Ltd., Kyoto, Japan). Total nitrogen was measured at heading time and ripening stage. Protein content of grain was measured by using a near-infrared spectrum photometer (HON6400, NIRECO). The values are shown on dry weight basis. **, * and ns shows significant difference at 1% and 5% probability level and no-significant difference respectively with ANOVA. Deep: water level was maintained at 20 cm from the soil surface; Control: water level was maintained at 10 cm from the soil surface.
Results

We previously found in a two-year study (Hayashi et al., 2011) that the percentage of perfect grains in the deep-flood treatment was always significantly higher than that in the control (68.5% versus 58.3% in 2008 and 81.4% versus 78.8% in 2009) while the white-based/white-back grains ratio was significantly lower (9.1% versus 13.5% in 2008 and 2.8% versus 4.1% in 2009) (Hayashi et al., 2011).

1. Nitrogen content (2008, 2009)

In both years, leaf N content in the deep-flood treatment with water level maintained at 20 cm was always significantly higher than in the control at all the sampling times (Table 2). Likewise, stem N content in the deep-flood treatment was significantly higher than that in the control at the panicle formation and ripening stages. In contrast, there was no significant difference in panicle N content between the deep-flood treatment and the control. Protein contents of grain in the deep-flood treatment were significantly lower than in the control, and this trend was more evident in 2009.

At heading time, there was no significant difference in the total N between the deep-flood treatment and the control in both years. In the deep-flood treatment, the total N at the ripening stage tended to be slightly larger than the control, and this trend was more evident in 2009.

2. Photosynthetic rate and stomatal conductance (2008, 2009)

To determine the right timing for the measurement, we observed diurnal change in stomatal conductance in 2008 (Fig. 1). A similar pattern was recorded in 2009 (data not shown). The results showed that stomatal conductance was at its peak between 1100 and 1200 but the difference in stomatal conductance between the deep-flood treatment and control was largest at 1100 in both years. Therefore, we used the data at 1100 for comparison between the deep-flood treatment and control.

In 2008, the stomatal conductance in the deep-flood treatment tended to be higher than in the control on all measurement dates but the difference was only significant on 21 August, around 17 days after heading (Fig. 2). In 2009, the stomatal conductance in the deep-flood treatment was always significantly higher than in the control.

Fig. 1. Effects of depth of water on diurnal changes of stomatal conductance in 2008.
Stomatal conductance was evaluated with a porometer (SC-1, DECAGON). Measurement was done on 21 August 2008. ○, deep-flood (water level was maintained at 20 cm from the soil surface); ●, control (water level was maintained at 10 cm from the soil surface). The stomatal conductance was measured for ten different plants. ** and * shows significant difference at 1% and 5% probability level with t-test, respectively. Vertical bars represent standard deviation.

Fig. 2. Effects of depth of water on stomatal conductance.
Stomatal conductance was evaluated with a porometer (SC-1, DECAGON). The stomatal conductance was measured for ten different plants. Measurements were done at 1100. The grey bars denote deep-flood (water level was maintained at 20 cm from the soil surface), the white bars denote control (water level was maintained at 10 cm from the soil surface). ** and * shows significant difference at 1% and 5% probability level with t-test, respectively. Vertical bars represent standard deviation.
control on all measurement dates.

Furthermore, photosynthetic rate and stomatal conductance in the deep-flood treatment were always significantly higher than in the control (Table 3) in both years. No significant interactions between water treatments and years were observed in any of the above parameters measured.

3. Relationship between depth of water and mean temperatures of air, water and soil at ripening stage (2008, 2009)

We measured the mean temperature of air, water and soil near the plant at the ripening stage and 20 days after heading in both years. Results showed that except for inter-annual variations in the mean air temperature, there was no significant difference in any of the temperatures between the treatment and year (Table 4).

Discussion

1. Effects of DPI on N content

Previously, we reported that the leaf color (SPAD value) of rice plants grown by DPI tended to be maintained at higher values, which implies that the DPI enhances N uptake (Hayashi et al., 2011). In fact, several studies showed that deep flood management made leaf color greener (Kiriyama and Nakaya, 1987; Nishiki et al., 1987; Furuya et al., 1991), because of the maintenance of relatively high plant N content (Kiriyama and Nakaya, 1987). In this study, we also found a consistently and significantly higher leaf N content and total N content in the deep-flood treatment than in the control at the ripening stage (Table 2). These results clearly indicate that the deep flood treatment enhanced N uptake at ripening stage.

Won et al. (1999) and Sato et al. (2004) suggested that the efficiency of utilizing indigenous soil nutrients was improved by deep flood treatment through the suppression of the formation of non-productive tillers. Our previous study also showed that the deep flood treatment suppressed tiller production while it increased SPAD value (leaf color) as mentioned above (Hayashi et al., 2011). Therefore, the enhancement of plant N content can be at least partly attributed to the inhibition of tiller production.

2. Effects of DPI on photosynthesis

Based on tiller production and yield components of plants grown under deep flood conditions, Chiba et al. (2009) suggested that the favorable effects of deep flood treatment on grain quality may be attributed to the improvement in source activity. Ohe et al. (2010) reported

### Table 3. Effects of depth of water on photosynthetic rate and stomatal conductance. Measurements were done on 22 August 2008 and 21 August 2009. Measurement times were 11:15 – 11:45 in 2008 and 10:55 – 11:20 in 2009. Leaf from each of 10 different plants was measured. Stomatal conductance and photosynthetic rate were evaluated with Portable Photosynthesis System (LI-6400, LI-COR).

| Year | Treatment | Photosynthetic rate (μmol m⁻² s⁻¹) | Stomatal conductance (mol m⁻² s⁻¹) |
|------|-----------|-----------------------------------|-----------------------------------|
| 2008 | Deep      | 21.6                              | 0.99                              |
|      | Control   | 15.2                              | 0.49                              |
| 2009 | Deep      | 26.4                              | 1.87                              |
|      | Control   | 17.5                              | 0.97                              |

ANOVA

| Year (Y) | ** | ** |
| Treatment (T) | ** | ** |
| Y × T | ns | ns |

The stomatal conductance was measured for ten different plants, ** and ns shows significant difference at 1% probability level and no-significant difference, respectively, with ANOVA. For deep and control treatments, refer to Table 2 for details.

### Table 4. Relationship between depth of water and mean temperatures of air, water and soil.

| Year | Treatment | Mean air temperature | Mean water temperature | Mean soil temperature |
|------|-----------|----------------------|------------------------|-----------------------|
|      |           | All period | 20 days | All period | 20 days | All period | 20 days |
| 2008 | Deep      | 27.5       | 29.1     | 25.5       | 26.5     | 25.6       | 26.1     |
|      | Control   | 27.5       | 29.0     | 25.7       | 26.7     | 25.4       | 25.8     |
| 2009 | Deep      | 26.0       | 26.5     | 25.7       | 26.5     | 25.2       | 25.8     |
|      | Control   | 26.0       | 26.4     | 25.5       | 26.3     | 25.2       | 25.8     |

ANOVA

| Year (Y) | ** | ** |
| Treatment (T) | ns | ns |
| Y × T | ns | ns |

Unit ºC. All periods: mean temperature throughout the ripening period. 20 days: mean temperature during the first 20 days after heading time. Air temperature was measured at 1.2 m from the soil surface. Water temperature was measured at soil surface. Soil temperature was measured at 5 cm beneath the soil surface. Temperature was measured in all plots (2 × 4 points). ** and ns shows significant difference at 1% probability level and no-significant difference, respectively, with ANOVA. For deep and control treatments, refer to Table 2 for details.
that the amount of N required to produce a unit weight of panicle was smaller in deeper water treatment than in control (shallower water level). Chiba et al. (2011) reported that deep-flood irrigation increased the amount of N in leaf blades at the full heading time as well as leaf area at the ripening stage. They only speculated, however, that deep-flood irrigation promoted photosynthesis at the ripening stage because they did not show whether the difference was caused by tiller restriction or improvement of photosynthesis.

Leaf stomatal conductance in the deep-flood treatment was expected to be higher than in the control and this has been fully shown in this study (Table 4).

3. Physiological mechanisms for the reduced chalky grain formation

Wakamatsu et al. (2008) showed a negative correlation between grain protein content and the occurrence of chalky grain, white-back and white-based grain, under high temperature conditions at the ripening stage. Takata et al. (2010) showed that enhancing plant N content at the ripening stage reduced the occurrence of chalky grain. These results indicate that occurrence of chalky grain is regulated by the plant N content at the ripening stage. Moreover, Tabata et al. (2008) found that the occurrence of chalky grain, particularly that of the white-back grain was promoted when the photosynthetic rate declined at the ripening stage by partial excision of the root system. In our study, however, photosynthetic rate increased while the occurrence of chalky grain decreased when rice plants were grown under deep flood conditions (Hayashi et al., 2011).

Generally, the photosynthetic rate is closely related with the leaf N level (Makino, 2003; Ookawa et al., 2003; Zhang and Kokubun, 2004; Hirasawa et al., 2010), and is substantially regulated by stomatal conductance (Ishihara et al., 1979a, 1979b). Further, the stomatal conductance is known to be greatly affected by leaf N content (Ishihara et al., 1979a; Makino et al., 1987; Hirasawa et al., 2010). Based on the above studies and also from our previous study, we conclude that deep flood treatment enhanced N uptake, and consequently photosynthetic activity, resulting in the reduction of chalky grain formation. Thus, the effects of deep flood treatment on grain quality can be attributed to the enhancement of source activity in addition to the reduction of number of spikelets per panicle (sink size reduction) (Hayashi et al., 2011).

On the other hand, there was another possibility that the difference in depth of irrigated water may have affected water and soil temperatures. In fact, there have been reports that water and soil temperature at the ripening stage also influenced the occurrence of chalky grain. Kasaneyama et al. (1999) and Tsubone et al. (2008) reported that the occurrence of chalky grain was increased by warm water irrigation of about 35°C at the ripening stage. By contrast, Arai and Ito (2001) reported that flow irrigation dropped water temperature by 2.4°C, and decreased the occurrence of chalky grain at the ripening stage. Arai-Sanoh et al. (2010) showed that the occurrence of chalky grain was increased by high soil temperature of about 37°C. However, our study showed that the mean air, water and soil temperatures near the plant at the ripening stage were not affected by water depth (Table 4) indicating that higher N uptake and photosynthetic rate of rice plants grown under deep flood treatment was not due to the cooling effects of deep flood.

Sugiura et al. (2007) reported that leaf N content at the ripening stage was positively correlated with the protein content of grain. However, grain protein content was reported to have negative effects on eating quality (Ishima et al., 1974). Thus, the enhanced N uptake in the deep-flood treatment may adversely affect the grain eating quality. In the present study, however, the protein content of grains was significantly lower in the deep-flood treatment than in the control whereas that in leaf and stem showed an opposite trend (Table 2). Therefore, the cultural method we propose in this study using deep flooding is expected to improve grain quality without sacrificing eating quality.

We previously showed that there was no significant difference in yield between DPI and conventional water management in the V-furrow direct seeding method (Hayashi et al., 2011) under the conditions of conventional sowing rate (7 g m⁻²) and N fertilizer application (7.8 g m⁻²). Moreover in our previous paper, we showed that a tradeoff relationship between percentage of ripened grains and number of spikelets per panicle was not observed under DPI. Furthermore, the combination of deep flood management and high N fertilizer application was reported to increase grain yield (Won et al., 1999), the amount of N required to produce 1 g of panicle weight was smaller in deep flood management than in ordinary water management (Ohe et al., 2010). In addition, when the number of productive tillers exceeded 440 m⁻², deep flood management increased grain yield relative to ordinary water management (Chiba et al., 2009). These facts strongly suggest that, with high N fertilizer applications and many numbers of productive tillers deep flood management including DPI is the cultural method that can reduce chalky grains occurrence with high yield potential.

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* In Japanese with English abstract.
** In Japanese with English summary.
*** In Japanese.