Early Planting and Early Nitrogen Application Increase Stem Total Digestible Nutrient Concentration and Yield of Forage Rice in Southwestern Japan

Hiroshi Nakano, Ikuo Hattori, Kenzi Sato and Satoshi Morita

(National Agricultural Research Center for Kyushu Okinawa Region, Chikugo, Fukuoka 833-0041, Japan)

Abstract: The effects of planting time (early or normal), nitrogen (N) application rate (150 or 225 kg N ha\(^{-1}\)), and N application method (early, even, and late application of extra N) on total digestible nutrient (TDN) concentration and yield of forage rice (\textit{Oryza sativa} L.) in southwestern Japan were examined. For optimal forage rice production, it is important to maximize nutritional level in leaves and stems rather than panicles because the hull restricts cattle’s ability to digest rice grain. In particular, it is important to maximize the TDN in leaf sheath plus stems (stems) which are the major part of the crop rather than leaf blades (leaves). Stem TDN yield was higher with early planting (340 g m\(^{-2}\)) than with normal planting (217 g m\(^{-2}\)) irrespective of N application rate or method. The high stem TDN yield with early planting resulted from both the high DM yield and the high TDN and organic cellular content (OCC) concentration. Stem TDN yield was not affected by the N application rate. With both early and normal plantings, stem TDN yield was higher with early N application (374 and 226 g m\(^{-2}\), respectively) than with late N application (305 and 208 g m\(^{-2}\), respectively). With early planting, the high stem TDN yield with method 1 resulted from both the high DM yield and the high TDN and OCC concentration. Thus, to obtain high stem TDN concentration and yield of forage rice, early planting and early N application are recommended.

Key words: Forage rice, Nitrogen application method, Nitrogen application rate, Planting time, Total digestible nutrients.

In Japan, more farmers are producing forage rice to increase their self-sufficiency in forage production (Sakai et al., 2003; Kato, 2008). Since forage rice is generally supplied to cattle as whole-crop silage, it is important to understand how the culture method affects biomass production and quality. Total digestible nutrient (TDN) concentration is an important index of the nutritional value of forage. Whole plant TDN concentration of forage rice is lower than that of corn (Kondo et al., 1990). Total digestible nutrient concentration is generally higher in the panicles than in either leaves or stems because the panicle contains a large amount of starch. However, the toughness of the rice grain hull restricts the digestion as a food source (Hara et al., 1986; Nakui et al., 1988; Hosoda et al., 2005). This is a problem with forage rice unlike other feed crops (Takaki et al., 1984). In addition, combine harvesters lose a large amount of grain during harvesting (Nakano et al., unpublished data). It is therefore important to maximize nutritional level in leaves and stems rather than panicles in order to minimize the overall loss of nutrition from the crop. In particular, it is important to maximize the TDN in leaf sheath plus stems (stems) that account for major part of the harvested crop rather than in leaf blades (leaves).

Several forage rice cropping systems are currently practiced in southwestern Japan. These include: forage rice single cropping (very early planting), forage rice–Italian ryegrass (\textit{Lolium multiflorum} Lam.) cropping (early planting), forage rice–wheat (\textit{Triticum aestivum} L.) cropping, forage rice–barley (\textit{Hordeum vulgare} L.) cropping (normal planting), and forage rice–tobacco (\textit{Nicotiana tabacum} L.) cropping (late planting). We previously found that early planting of forage rice can provide a high DM yield and a high stem TDN concentration irrespective of the cultivar (Nakano et al., 2008). However, we tested only one N application rate and one N application method. Forage rice is grown intensively with the application of a large amount of cattle manure, a byproduct of the prosperous livestock industry (Hara et al., 2008), or with extensive cultivation. Thus, it is necessary to examine the TDN concentration and yield of the forage rice planted at...
different times under various N application rates and N application methods. Recently, we found that early application of a large amount of extra N is most effective to obtain a high DM yield of forage rice (Nakano and Morita, 2009). Our objective in the present study was to determine the effects of planting time, N application rate, and N application method on the TDN concentration and yield of forage rice and to develop a method to maximize TDN concentration of the leaves and stems rather than panicles.

Materials and Methods

1. Crop management

The study was conducted in 2004 and 2005 on a Lowland Paddy soil at the National Agricultural Research Center for Kyushu Okinawa Region (33°12’ N lat., 130°30’ E long., 10 m a.s.l.), Chikugo, Fukuoka, Japan. The previous crop grown in the field was rice, and the same field was used for the experiments in both years. Experimental treatments included two planting times, two N application rates, and three N application methods, and were arranged in a randomized complete block design with three replicates. The planting times were early and normal. Plants received either 150 or 225 kg N ha⁻¹ by means of one of three N application methods: 60% applied at transplanting, 20% at active tillering, 10% at 20 d before heading, and 10% at 10 d before heading (early N application); 40%, 20%, 20%, and 20% (even N application); and 20%, 20%, 30%, and 30% (late N application). The rice cultivar was Tachiaoba, which is normally grown for forage in southwestern Japan.

Germinated seeds were sown in nursery boxes in late April for early planting and in late May for normal planting. Seedlings were then transplanted into the paddy field by hand in mid-May and late June, respectively. The field received 30 kg N, 30 kg P₂O₅, and 30 kg K₂O ha⁻¹ in the form of synthetic fertilizer broadcast by hand 3 d before transplanting, and the fertilizer was incorporated into the soil to allow puddling. Plants received additional basal N just after transplanting and additional N at designated stages in the form of ammonium sulfate. These supplemental applications were broadcast by hand on the soil surface. After trimming, each plot was 1.5 m × 9.0 m with a mean of 22.2 hills m⁻² (three seedlings hill⁻¹, 30 cm × 15 cm for each hill).

Plants from 20 hills (0.9 m²) were harvested by hand at ground level at the yellow-ripe stage (late September in the early planting and mid-October in the normal planting). Two hills with an average number of tillers were separated into leaf blades (leaves), leaf sheaths plus stems (stems), and panicles, and were then dried at 80°C in a ventilated oven for 2 d with the plants from the other 18 hills to determine their dry weight. Dry matter yield was determined from the dry weight of these 20 hills.

2. Estimation of TDN

Total digestible nutrient concentration of forage rice can be accurately estimated by enzymatic analysis (Hattori et al., 2005). Each dried sample was ground in a Wiley mill (WT-100, Iketomo Scientific Technology, Tokyo, Japan) and passed through a 1-mm screen. About 500 mg of each sample was placed in a filter bag (F57, Ankom Technology, Macedon, NY), soaked in boiling water for 5 min, and then incubated in a digestion vessel (DAISY II Incubator, Ankom Technology) containing 2 L of sodium acetate-acetic acid buffer (pH 5.8) with 100 mg -amylase, 800 mg actinase, and 70 mg calcium acetate for 16 hr at 40°C. Each sample residue was double rinsed with boiling water for 5 min, and double rinsed with cold water for 5 min in a digestion vessel (ANKOM 200 Fiber Analyzer, Ankom Technology). Two subsamples of residues from each sample were prepared, and used for measurement of the low digestible fiber (Ob) concentration, which is resistant to cellulase treatment, and the organic cell wall (OCW) concentration.

For measurement of the Ob concentration, one sample residue was incubated in a digestion vessel (DAISY II Incubator) containing 2 L of sodium acetate-acetic acid buffer (pH 4.0) with 20 mg cellulase for 4 hr at 40°C, double rinsed with boiling water for 5 min, double rinsed with cold water for 5 min in a digestion vessel (ANKOM 200 Fiber Analyzer), soaked in acetone for 15 min, dried at 135°C for 2 hr to determine its dry weight, and then combusted at 600°C for 2 hr to determine its crude ash concentration. For measurement of OCW concentration, another sample residue was soaked in acetone for 15 min, dried at 135°C for 2 hr to determine its dry weight, and then combusted at 600°C for 2 hr to determine its crude ash concentration. TDN, organic cellular content (OCC), and high digestible fiber (Oa) concentration were estimated according to the method of Hattori et al. (2005), as follows:

\[
\text{TDN (g kg}^{-1}\) = 54.5 + 8.9 \times [\text{OCC (g kg}^{-1}\) + Oa (g kg}^{-1}\) + 4.5 \times \text{OCW (g kg}^{-1}\])
\]

\[
\text{OCC (g kg}^{-1}\) = 1000 - \text{OCW (g kg}^{-1}\) - \text{crude ash (g kg}^{-1}\)
\]

\[
\text{Oa (g kg}^{-1}\) = \text{OCC (g kg}^{-1}\) - Ob (g kg}^{-1}\)
\]

TDN yield was estimated as follows:

\[
\text{TDN yield (g m}^{-2}\) = DM (g m}^{-2}\) \times \text{TDN (g kg}^{-1}\) / 1000
\]

3. Statistical analysis

Statistical analyses were performed using a general linear model of SPSS (SPSS 17.0, SPSS Inc., Chicago, IL). Planting time, N application rate, and N application method were considered fixed effects. Year and replication were considered random effects. Analysis of variance (ANOVA) was conducted to test the effects of timing of first harvest, N application rate, and N application method on TDN yield, DM yield, TDN concentration, and components related to the estimation of TDN concentration. Significant treatment effects (P<0.05) were explored using...
Table 1. Mean total digestible nutrient (TDN) yield, dry matter (DM) yield, and TDN concentration at different planting times, N application rates, and N application methods.

| Planting time | N application rate | N application method | Estimated TDN yield | DM yield | Estimated TDN concentration |
|---------------|--------------------|----------------------|---------------------|----------|----------------------------|
|               | kg ha⁻³             | g m⁻²                | g kg⁻¹              |          | g kg⁻¹                     |
| Early         |                    |                      |                     |          |                            |
| Normal        | 150                | 828                  | 126                 | 217      | 485                        | 1506 | 299 | 519 | 687 | 547 | 419 | 457 | 709 | 550 | 422 | 418 | 706 |
| 225           |                    | 922                  | 148                 | 279      | 494                        | 1695 | 354 | 647 | 694 | 544 | 421 | 429 | 712 |                |
| Early         |                    |                      |                     |          |                            |
| Normal        | 150                | 906                  | 136                 | 278      | 491                        | 1637 | 322 | 625 | 691 | 553 | 424 | 442 | 711 |                |
| 225           |                    | 918                  | 148                 | 300      | 470                        | 1709 | 357 | 680 | 672 | 537 | 415 | 436 | 699 |                |
| Late          |                   |                      |                     |          |                            |
| LSD           |                    | 18                   | 7                   | 10       | 17                         | 30   | 14  | 17  | 24  | 6   | NS  | NS  | 6   |                |
| Early         |                    |                      |                     |          |                            |
| Normal        | 150                | 374                  | 806                 | 540      | 465                        |      |     |     |     |     |     |     |     |                |
| 225           |                    | 340                  | 731                 | 554      | 466                        |      |     |     |     |     |     |     |     |                |
| Late          |                    | 305                  | 690                 | 547      | 442                        |      |     |     |     |     |     |     |     |                |
| Normal        | Early              | 226                  | 554                 | 534      | 408                        |      |     |     |     |     |     |     |     |                |
| Even          |                    | 216                  | 518                 | 592      | 417                        |      |     |     |     |     |     |     |     |                |
| Late          |                    | 208                  | 486                 | 564      | 428                        |      |     |     |     |     |     |     |     |                |
| LSD           |                    | 15                   | 24                  | 8        | 12                         |      |     |     |     |     |     |     |     |                |

ANOVA

Planting time (PT) ** ** ** ** NS* ** ** ** NS NS NS ** NS
N application rate (NR) ** ** NS * ** ** ** NS ** NS ** **
N application method (NM) ** ** ** ** ** ** ** * ** NS NS **
PT×NR NS NS NS NS NS NS NS ** * NS NS NS NS NS NS NS NS NS NS
PT×NM NS NS ** NS NS NS NS * NS ** NS NS ** NS NS NS NS NS NS
NR×NM NS NS NS NS NS NS NS NS NS NS NS NS NS NS NS NS NS NS NS
PT×NR×NM NS NS NS NS NS NS NS NS NS NS NS NS NS NS NS NS NS NS NS

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
† Whole plant.
‡ Leaf blade.
§ Leaf sheath plus stem.
¶ Least significant difference at P<0.05.
# NS, not significant.
Fisher’s protected LSD.

Results

Table 1 shows the effects of planting time, N application rate, and N application method on TDN yield, DM yield, and TDN concentration. Whole plant, leaf, and panicle TDN yield were higher with early planting than with normal planting. Whole plant, leaf, and panicle TDN yield were higher with 225 kg N ha\(^{-1}\) than with 150 kg N ha\(^{-1}\). Whole plant TDN yield was higher with early and even N applications than with late N application. With 225 kg N ha\(^{-1}\), leaf TDN yield was highest with early N application. With early planting, stem TDN yield was highest with early N application, and with normal planting, it was higher with early N application than with late N application. Panicle TDN yield was lowest with early N application.

Whole plant, leaf, and stem DM yield were higher with early planting than with normal planting (Table 1). Whole plant and leaf DM yield were higher with 225 kg N ha\(^{-1}\) than with 150 kg N ha\(^{-1}\). With early and even N application, stem DM yield was higher with 225 kg N ha\(^{-1}\). Whole plant, leaf, and stem DM yield were highest with early N application. Panicle DM yield was lower with early N application than with late N application. The ratio of stem DM to panicle DM was higher with early planting. It was highest with early N application.

With 225 kg N ha\(^{-1}\), whole plant and leaf TDN concentration were lower with 225 kg N ha\(^{-1}\) than with 150 kg N ha\(^{-1}\). With early N application, whole plant TDN concentration was lower with 225 kg N ha\(^{-1}\). With early N application, panicle TDN concentration was higher with 225 kg N ha\(^{-1}\). With early planting, whole plant TDN concentration was higher with early N application than with early N application, and with normal planting, it was highest with late N application. With 150 kg N ha\(^{-1}\), whole plant TDN concentration was higher with even and late N applications than with early N application, with 225 kg N ha\(^{-1}\), it was highest with late N application. With early planting, stem TDN concentration was higher with early and even N applications, and with normal planting, it was higher with late N application than with early N application. With 150 kg N ha\(^{-1}\), panicle TDN concentration was higher with early planting.

Figs. 2 and 3 show the effects of planting time, N application rate, and N application method on the concentration of components related to the estimation of TDN concentration in stems and panicles. Stem OCC concentration was higher with early planting than with normal planting (Fig. 2). It was lower with 225 kg N ha\(^{-1}\) than with 150 kg N ha\(^{-1}\). With early planting, stem OCC concentration was higher with early and even N applications than with late N application. Panicle OCC concentration was lowest with early N application. Panicle OCC concentration was highest with late N application. Stem Oa concentration was higher with early planting. With early and late N applications, stem Oa concentration was higher with 225 kg N ha\(^{-1}\). With early planting, stem Oa concentration was higher with early and even N applications, and with normal planting, it was higher with late N application than with early N application. With early planting, stem Oa concentration was higher with early and even N applications, and with normal planting, it was higher with late N application than with early N application. With early planting, stem Oa concentration was higher with early and even N applications, and with normal planting, it was higher with late N application than with early N application. With early planting, stem Oa concentration was higher with early and even N applications, and with normal planting, it was higher with late N application than with early N application. With 150 kg N ha\(^{-1}\), panicle Oa concentration was higher with even and late N applications.

Figs. 1 and 2 show the effects of planting time, N application rate, and N application method on the mean ratio of stem DM to panicle DM at different planting times, N application rates, and N application methods. Means with the same letters within a column do not differ by Fisher’s protected LSD (P < 0.05). Capital letter indicates multiple comparisons between lower side treatments. Lowercase letter indicates multiple comparisons between upper side treatments. PT, Planting time; NR, N application rate (kg ha\(^{-1}\)); NM, N application Method.
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The high stem TDN concentration with early planting was due to the high OCC concentration (Fig. 2). OCC concentration is positively related to the nonstructural carbohydrate (NSC) concentration, which comprises mainly starch and sugars (Nakano et al., 2008), because OCC was fractionated by α-amylase and actinase (Abe, 1988) and NSC by α-amylase and amyloglucosidase (Ohnishi and Horie, 1999). Rice plants decompose starch accumulated in the stem to sugar before heading and translocate the sugar to the panicles, resulting in further accumulation of starch in the stem (second starch accumulation) (Baba and Kitsutaka, 1953; Togari and Sato, 1954). The ratio of stem DM to sink size (number of grains per m²) at the start of the second starch accumulation period is positively correlated with the NSC concentration.

Discussion

We previously found that stem TDN concentration was increased by early planting irrespective of the cultivar (Nakano et al., 2008). Here, we examined the effects of N application rate and N application method in addition to planting time. Whole plant, leaf, and stem TDN yield were increased by early planting irrespective of N application rate or method (Table 1). The high whole plant and leaf TDN yield with early planting resulted from the high DM, whereas the high stem TDN yield with early planting resulted from both the high DM and the high TDN concentration irrespective of N application rate or method. The high stem TDN concentration with early planting was due to the high OCC concentration (Fig. 2). OCC concentration is positively related to the nonstructural carbohydrate (NSC) concentration, which comprises mainly starch and sugars (Nakano et al., 2008), because OCC was fractionated by α-amylase and actinase (Abe, 1988) and NSC by α-amylase and amyloglucosidase (Ohnishi and Horie, 1999). Rice plants decompose starch accumulated in the stem to sugar before heading and translocate the sugar to the panicles, resulting in further accumulation of starch in the stem (second starch accumulation) (Baba and Kitsutaka, 1953; Togari and Sato, 1954). The ratio of stem DM to sink size (number of grains per m²) at the start of the second starch accumulation period is positively correlated with the NSC concentration.
There is also competition between the sinks (i.e., panicles) and the sources (i.e., leaves and stems) for starch during the second starch accumulation period (Tsuno and Yu, 1988). In the present study, the ratio of stem DM to panicle DM was increased by early planting (Fig. 1). These results suggest that early planting increases the stem TDN and OCC concentration by increasing the ratio of source to sink size, and thereby increasing stem TDN yield.

Recently, we found that applying a large amount of extra N early improves the DM yield of forage rice (Nakano and Morita, 2009). This technique differs from conventional techniques, and no information was available on the TDN concentration and yield of forage rice fertilized in this way. Whole-plant and leaf TDN yield were higher with 225 kg N ha\(^{-1}\) than with 150 kg N ha\(^{-1}\), but stem TDN yield did not differ between N application rates (Table 1). The high whole plant and leaf TDN yield with 225 kg N ha\(^{-1}\) resulted from the high DM, and the modest stem TDN yield with 225 kg N ha\(^{-1}\) resulted from the high DM and low TDN concentration. The low TDN concentration was due to the low OCC concentration (Fig 2). The ratio of stem DM to panicle DM did not differ greatly between N application rates (Fig. 1). This result contradicts with the findings of Sato (1956) that rice plants accumulate a large amount of excess starch in the stem when they have enough N during the later phase of ripening. However, our study differed from Sato’s study in terms of N application rate. Sato (1956) used rice plants that had been grown with low levels of N, but we used plants that had been grown with high
levels of N. When rice plants absorb N actively during the early growth stage, their photosynthetic products are preferentially used for protein synthesis and leaf production (Yoshida, 1981). As a result, the carbohydrate concentration of the stem tends to be lower with high levels of N. Thus, rice plants may not accumulate a large amount of excess starch in the stem when they have much more N during the later phase of ripening. With early N application, panicle TDN yield was higher with 225 kg N ha$^{-1}$ than with 150 kg N ha$^{-1}$ (Table 1). The high panicle TDN yield with 225 kg N ha$^{-1}$ resulted from the high DM and the high TDN concentration. The high TDN concentration with 225 kg N ha$^{-1}$ was due to the high OA (Fig. 2). However, we could not determine which factor increased OA.

Whole plant TDN yield was higher with early N application than with late N application, because whole plant DM yield was highest with early N application (Table 1). With 225 kg N ha$^{-1}$, leaf TDN yield was highest with early N application, because leaf DM yield was highest with early N application. With both early and normal plantings, stem TDN yield was higher with early N application than with late N application. With early planting, the high stem TDN yield with early N application resulted from both the high DM yield and the high TDN concentration. The high TDN concentration with early N application was due to the high OCC concentration (Fig. 2). However, with normal planting, the high stem TDN concentration with early N application resulted from only the high DM yield (Table 1). The ratio of stem DM to panicle DM was highest with early N application, particularly with early planting (Fig. 1). Thus, within early planting, the high ratio of stem DM to panicle DM would be advantageous for accumulating NSC in the stem, as described by Yamaguchi and Matsumura (2004). These results suggest that, within early planting, early N application increases the stem TDN and OCC concentration by increasing the ratio of source to sink size, and thereby produces a high stem TDN yield. Panicle TDN yield was higher with late N application than with early N application (Table 1). The high panicle TDN yield resulted from the high DM and the high TDN concentration. The high TDN concentration was due to the high OCC concentration (Fig. 3). N application from the panicle formation stage to heading increases the number of grains per panicle and the weight of individual grains (Yoshida, 1981). Thus, late N application might increase the panicle TDN and OCC concentration by increasing the ratio of panicle starch DM to panicle DM.

Panicle TDN concentration of forage rice is generally higher than the actual TDN concentration because the toughness of the rice grain’s hull restricts cattle’s ability to digest the food source (Hara et al., 1986; Nakui et al., 1988; Hosoda et al., 2005). In addition, combine harvesters drop a large amount of grain during harvesting (Nakano et al., unpublished data). It is therefore important to maximize TDN concentration and yield of stems rather than panicles in order to minimize the loss of nutrition from the crop. In the present study, early planting and applying a large amount of N produced a high stem TDN yield. In addition, we demonstrated that increasing stem DM, but not applying excess N, can effectively increase stem TDN concentration. Our new results suggest that this strategy will improve fodder quality.

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** In Japanese with English summary.
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