Genotypic Differences in Dry Matter Accumulation, Nitrogen Use Efficiency and Harvest Index in Recombinant Inbred Lines of Rice under Hydroponic Culture

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Abstract: To examine the possibility of breeding high-yielding cultivars with high nitrogen use efficiency for dry matter accumulation (NUE\textsubscript{d}) and to provide simple criteria for the selecting and breeding high-yielding cultivars with high NUE\textsubscript{d} as well as useful information for the mapping of quantitative trait loci (QTL) controlling NUE\textsubscript{d}, we cultured recombinant inbred lines (RILs) of rice hydroponically in 2000 and 2001. RILs with a higher grain yield tended to show greater total dry matter accumulation (TDMA) and higher harvest index (HI), while increasing TDMA resulted in a decrease in HI. The contribution ratio of the TDMA to grain yield (2000: 67.3\%, 2001: 68.2\%) was higher than that of HI in both 2000 and 2001. Even at the same high-yielding level, there was a significant difference in the TDMA and HI values. In both years, the contribution ratios of NUE\textsubscript{d} and total nitrogen absorption (NTA) to TDMA were about 62.0 and 38.0\%, respectively. The contribution ratio of the NUE\textsubscript{d} to grain yield was higher than those of the NTA and HI in both 2000 (41.6\%) and 2001 (42.9\%). These results suggested that the high-yielding rice plants generally displayed high TDMA and HI values. Further increase in rice grain yield should be based on the further increase in TDMA than in HI, and to increase TDMA leading to a high grain yield, the emphasis also should be put on improving NUE\textsubscript{d} in RILs.

Key words: Dry matter accumulation, Grain yield, Harvest index, Nitrogen use efficiency for dry matter accumulation, Recombinant inbred line.

Grain yield increase of rice can be achieved by increasing total dry matter accumulation (TDMA) and/or harvest index (HI) (Yoshida, 1981). It is controversial as to which component should be emphasized to improve yield potential of current cultivars (Ying et al., 1998). The improvement in grain yield in modern cultivars was attributed to the increase in HI rather than in TDMA (Takeda et al., 1983; Evans et al., 1984; 1993). When comparisons were made among modern cultivars, however, high yield was achieved by increasing TDMA (Jiang et al., 1988; Akita, 1989; Amano et al., 1993). Cui et al. (2000) reported a significantly positive correlation between HI and grain yield in the high-yielding cultivars bred in Asian countries, while not in the Japanese cultivars. TDMA is determined by two functional components: total nitrogen absorption (NTA) and nitrogen use efficiency for dry matter accumulation (NUE\textsubscript{d}). That is to say, grain yield can be improved by increasing NTA, NUE\textsubscript{d}, HI or all of them. However, these increases in rice production require an increase in applied chemical fertilizer, especially N fertilizer. However, heavy application of chemical fertilizers not only increases the cost of rice production, but also causes agro-environmental pollution such as eutrophication of rivers and lakes and nitrate pollution of surface and underground water. It also contributes to the greenhouse effect through the release of NO\textsubscript{x} gases from paddy soils (Galbally et al., 1987; Duxbury et al., 1993). Therefore, one solution to this problem is to breed high-yielding cultivars, which exhibit a high TDMA (NTA × NUE\textsubscript{d}), HI or all of them. However, breeding such cultivars in rice by conventional selection based on phenotype alone is time consuming and difficult.

Recent progress in molecular marking technology over the past decade enables us to efficiently locate genes controlling some important quantitative agronomic traits. For example, heading date (Li et al., 1995; Xiao et al., 1998), plant height (Li et al., 1995; Xiao et al., 1998; Cao et al., 2001; Zhang et al., 2004), grain yield and yield components (Li et al., 1997; Lou et al., 2001; Zhang et al., 2004), crop growth rate (Li et al., 2006) and seed dormancy (Wan et al., 2006) have been genetically dissected by quantitative trait loci (QTL) mapping approach in rice and nitrogen use efficiency in maize (Bertin and Gallais, 2000, 2001). However, few reports are available on the quantitative trait locus (QTL) analysis of TDMA, HI, NTA and NUE\textsubscript{d} in rice.
The rice materials used for QTLs were mostly hybrid populations derived from the cross of indica/japonica, which showed high genetic polymorphism and wide variation. At present, the hybrid rice combination of indica/indica or japonica/japonica has been commonly used in rice breeding projects. Since the hybrid rice combination of indica/japonica has some disadvantages such as the low seed-setting rate, low filling percentage of the grains and early senescence, hybrid rice derived from the cross of parents with a close genetic relationship for evaluation of traits or QTLs is considered beneficial for rice breeding. RILs derived from a cross between Zhenshan 97 and Minghui 63, parents of Shanyou 63, a popular F1 hybrid rice from China were used in the present study.

Previously (Ju et al., 2006), we referred to NTA and nitrogen use efficiency for grain yield (NUEg) and suggested the possibility of breeding high-yielding cultivars with high NUEg by using RILs derived from a cross between Zhenshan 97 and Minghui 63. A preliminary QTL analysis of NUEd using RILs of F12 generation (Shan et al., 2005) revealed a total of 12 QTLs using interval mapping with a LOD threshold of 2.0. Among the 12 QTLs, controlling nitrogen use efficiency for dry matter accumulation was located at the marker interval of Waxy-C1496 on chromosome 6, and the remaining 11 QTLs, associated with nitrogen concentration and accumulation in rice plant were positioned on chromosomes 1, 2, 4 and 6, respectively.

In the present study, we analyzed the genetic characteristics and correlations of TDMA (=NTA × NUEd) and HI with grain yield using the RILs of F12 and F13 generations in order to provide simple criteria for the selection and breeding of high-yielding cultivars with high TDMA and NUEd, and also to obtain useful information on the mapping of QTLs controlling grain yield, TDMA, and HI.

Materials and Methods

1. Rice materials

A total of 118 (F12) and 191 (F13) RILs were cultivated in 2000 and 2001, respectively. They were developed by the single-seed descent method from a cross between the cultivar Zhenshan 97 [ZX 97, an inbred indica and the female parent of Shanyou 63 (SY 63), the traditional hybrid rice grown in China] and the cultivar Minghui 63 [MH 63, an inbred indica and the male parent of Shanyou 63] generations (Ju et al., 2006).

2. Experimental design and growth conditions

The experiment was carried out in Yangzhou University, Yangzhou, China, during the summer of 2000 and 2001. Hydroponic culture was performed outdoors and the nitrogen supply and other nutritional factors were well controlled for uniformity throughout the growth period, because the phenotypic traits associated with nitrogen nutrition in rice can be greatly affected by imbalanced supply of nitrogen in culture media. Therefore, the data obtained from this study explained in more reliable genetic background in the RILs. Eight concrete ponds (880 cm × 135 cm × 50 cm in depth) were connected with each other by iron tubes (10 cm in diameter) running along the bottom. The ponds were covered with concrete planks (155 cm × 16.7 cm) each containing 14 holes (4 cm in diameter) for seedling fixation. One 2-leaf seedling per hole grown for 20 d in a soil seedbed, was fixed into the holes with a sponge on June 2 each year. In order to cultivate all the lines in a limited space, the plants were cultured at a high density (62 hills m-2).

Because nitrogen supply and other nutritional factors can be well controlled by plant growth conditions during the whole growth period in hydroponic culture, the high density did not lead to overluxurious growth. Four planks (56 seedlings) were prepared for each genetic material in a randomized complete design with two replicates.

The nutrient solution was formulated according to the method described by Yoshida et al. (1976). Ten mg L-1 nitrogen (Urea-N) with 250 mg L-1 Mg2+, 34 mg L-1 K+, 50 mg L-1 Fe2+, 2.86 mg L-1 BO3-, 0.22 mg L-1 Zn2+, 2.21 mg L-1 Mn2+, 0.08 mg L-1 Cu2+, 0.02 mg L-1 MoO42- and 41.86 mg L-1 Ca2+ were added to the ponds at the time of transplanting and replaced at 10-d intervals. The pH value in nutrient solution was monitored and adjusted to 4.5-5.5 once a day by adding diluted H2SO4.

During the rice growth season, the weather was unstable across the two-year period. Mean temperature from July 1 to 10 and that from July 21 to 31 were higher in 2001, 31ºC and 32ºC, respectively, than in 2000, 28ºC and 29ºC, respectively, and vice versa from July 1 to 10 (30ºC in 2000 and 27ºC in 2001) and Aug. 1 to 20 (29ºC in 2000 and 27ºC in 2001). Sunshine hours were longer from June 21 to 30 (5.0 hr), Sep. 1 to 10 (7.5 hr) and Sep. 21 to 30 (7.3 hr) in 2001 than in 2000 (1.9, 5.7 and 1.6 hr, respectively), and vice versa from June 11 to 20 (10.0 hr in 2000 and 5.1 hr in 2001), July 11 to 20 (8.9 hr in 2000 and 5.6 hr in 2001), Aug. 1 to 10 (9.5 hr in 2000 and 3.4 hr in 2001) and Sep. 11 to 20 (10.1 hr in 2000 and 7.0 hr in 2001).

3. Sampling and measurements

Ten rice plants were sampled from each replicate at heading and maturity stages and divided into leaf blades, leaf sheaths plus stems, roots and panicles. Samples were oven-dried at 85ºC for measuring dry weight and nitrogen concentration by the Kjeldahl distillation method. At maturity, ten plants were sampled to determine the grain (unhulled) yield and yield components (Ju et al., 2006). Filled grains were selected using tap water with a gravity of 1.00 g cm-3 and the grain (unhulled) yield was adjusted to a moisture content (MC) of 13.5%.
4. Traits

The traits were defined as follows:

1. Grain yield (g m\(^{-2}\)) = unhulled grain weight at 13.5% of moisture content,
2. Total dry matter accumulation (TDMA, g m\(^{-2}\)) = plant dry-matter accumulation at maturity,
3. Pre-heading dry matter accumulation (Pre-DMA, g m\(^{-2}\)) = plant dry weight at heading,
4. Post-heading dry matter accumulation (Post-DMA, g m\(^{-2}\)) = TDMA minus Pre-DMA,
5. Total nitrogen absorption (NTA g m\(^{-2}\)) = nitrogen content of whole plant at maturity,
6. Nitrogen use efficiency for dry matter accumulation (NUEd, g DW g\(^{-1}\) N) = dry matter accumulation at maturity divided by NTA,
7. Harvest index (HI, %) = grain yield × 0.865 / dry matter accumulation × 100%.

5. Statistical analyses

The means of two replicates per genetic material were used in the data analysis for each trait. Hierarchical cluster analysis procedure was used to identify relatively homogeneous groups of cases (or variables) based on selected characteristics, using an algorithm that started with each case (or variable) in a separate cluster and combined clusters until only one was left (SPSS, Chicago, America, 1998).

All the data were subjected to one-way analysis of variance (ANOVA) to determine the differences among the clusters.

The calculation of the contribution ratio of each component was as follows: data analysis involved linearizing the multiplicative relationships by logs and then determining the contribution of each component trait to the sum of squares of the resultant trait. The sum of the cross products of each component trait by the resultant trait (\(\Sigma xy\)) divided by the resultant trait (\(\Sigma y^2\)) gave the relative contribution of each component variable to the resultant variable (Moll et al., 1982).

Results

1. Distribution of the mean values of total dry matter accumulation, nitrogen use efficiency for dry matter accumulation and harvest index

Table 1 shows the distribution of the mean values of total dry matter accumulation (TDMA), nitrogen use efficiency for dry matter accumulation (NUEd) and harvest index (HI). Significant genetic variations in these measured traits were detected among the parents in 2000 and among the RILs in 2000 and 2001, except for HI among parents. The values of TDMA, NUEd and HI in MH 63 were higher than those in ZX 97 and the differences in the TDMA and NUEd were significant (p < 0.05). The values of TDMA and HI in F1 hybrid (SY 63) were higher than those in either parent. Transgressive segregations of TDMA, NUEd and HI in the RILs in 2000 were also observed, and the mean values of these traits were closer to those in MH 63 than ZX 97, except for NUEd. Moreover, some of the RILs in 2000 showed higher values than SY 63.

2. Grain yield

According to the hierarchical cluster analysis procedure, the grain yield of the RILs was classified into 6 clusters (A to F) from high to low (Table 2). The coefficient of variation (CV) of the lowest grain yield cluster (cluster F) was comparatively higher than that in higher grain yield cluster (cluster A-F) (Ju et al., 2006).

3. Dry matter accumulation, harvest index, total nitrogen absorption and nitrogen use efficiency for dry matter accumulation

Table 2 shows the TDMA and HI among the RILs for each cluster of grain yield. The TDMA was significantly different among the clusters (p < 0.05) in both years. The mean value of TDMA was 1558 g m\(^{-2}\) with a CV of 24.0% and 1515 g m\(^{-2}\) with a CV of 21.6% in 2000 and 2001, respectively. Generally, the higher the mean value of grain yield, the higher the mean value of
TDMA could be divided into two components, Pre-DMA and Post-DMA. Pre-DMA ranged from 776 to 1418 g m\(^{-2}\) (CV, 21.8%) with a mean value of 1049 g m\(^{-2}\) and from 918 to 1245 g m\(^{-2}\) (CV, 20.7%) with a mean value of 1068 g m\(^{-2}\) in 2000 and 2001, respectively (data not shown). Post-DMA ranged from 286 to 873 g m\(^{-2}\) (CV, 50.3%) with a mean value of 510 g m\(^{-2}\) and from 249 to 711 g m\(^{-2}\) (CV, 51.6%) with a mean value of 447 g m\(^{-2}\) in 2000 and 2001, respectively (data not shown). The contribution ratios of Pre-DMA and Post-DMA to TDMA were 68.5% and 31.5%, respectively, in 2000, and 71.6% and 28.4%, respectively, in 2001 (Table 3).

HI was significantly different among the clusters

### Table 2. TDMA, NUEd and HI in each cluster of the RILs grown in 2000 and 2001.

| Cluster | N  | Yield** | TDMA (g m\(^{-2}\)) | NTA** (g m\(^{-2}\)) | NUEd (g DW g\(^{-1}\) N) | HI (%) |
|---------|----|---------|---------------------|----------------------|-------------------------|--------|
|         |    | Mean    | CV (%)              | Mean                 | CV (%)                  | Mean   |
| 2000    |    |         |                     |                      |                         |        |
| A       | 7  | 1028 f  | 3.4                 | 2290 c               | 9.2                     | 25.1   |
| B       | 18 | 835 e   | 7.3                 | 1928 c               | 14.3                    | 21.0   |
| C       | 40 | 654 d   | 5.3                 | 1517 b               | 14.0                    | 16.4   |
| D       | 38 | 543 c   | 7.1                 | 1425 b               | 21.0                    | 15.3   |
| E       | 13 | 414 b   | 8.8                 | 1216 a               | 19.1                    | 13.7   |
| F       | 2  | 224 a   | 10.6                | 1253 ab              | 50.6                    | 13.3   |
| Total   | 118| 634 26.5|                     | 1558 24.0            |                         |        |

### Table 3. Results of multiple regression analysis and contribution ratios.

| Resultant trait | Component trait | R\(^2\) | Contribution ratio (%) |
|-----------------|-----------------|--------|------------------------|
|                 |                 | 2000    | 2001 | 2000 | 2001 |
| Y1 : Grain yield (g m\(^{-2}\)) | X1 : TDMA (g m\(^{-2}\)) | 0.976 | 0.966 | 67.3 | 68.2 |
|                 | X2 : HI (%)      |        |      | 32.7 | 31.8 |
| Y2 : TDMA (g m\(^{-2}\)) | X3 : Pre-DMA (g m\(^{-2}\)) | 1.000 | 1.000 | 68.5 | 71.6 |
|                 | X4 : Post-DMA (g m\(^{-2}\)) |        |      | 31.5 | 28.4 |
| Y3 : TDMA (g m\(^{-2}\)) | X5 : NTA (g m\(^{-2}\)) | 0.988 | 0.976 | 38.4 | 37.9 |
|                 | X6 : NUEd (g DW g\(^{-1}\) N) |        |      | 61.6 | 63.0 |
| Y4 : Grain yield (g m\(^{-2}\)) | X7 : NTA (g m\(^{-2}\)) | 0.971 | 0.952 | 25.8 | 25.2 |
|                 | X8 : NUEd (g DW g\(^{-1}\) N) |        |      | 41.6 | 42.9 |
|                 | X9 : HI (%)      |        |      | 32.7 | 31.8 |

TDMA, total dry matter accumulation; Pre-DMA, pre-heading dry matter accumulation; Post-DMA, post-heading dry matter accumulation; NTA, total nitrogen absorption; NUEd, nitrogen use efficiency for dry matter accumulation; HI, harvest index.
The mean value of HI was 35.6% with a CV of 18.3% and 30.7% with a CV of 21.2% in 2000 and 2001, respectively. A wide variation of the HI values was observed in the RILs in the same cluster, especially in the cluster of low yield, and the higher the mean grain yield, the higher the mean value of HI. NTA was significantly different among the clusters (p < 0.01) in both years (Table 2). The mean value of NTA was 16.9 g m⁻² with a CV of 24.0% and 15.3 g m⁻² with a CV of 22.1% in 2000 and 2001, respectively. A wide variation of the NTA values was observed in the RILs in the same cluster, except for cluster A in 2000, and the higher the mean value of grain yield, the higher the mean value of NTA (Ju et al., 2006). There was a significant variation in the NUEd values among the clusters (p < 0.05), with a mean value of 92.6 g DW g⁻¹ N with a CV of 10.8% in 2000 and 100.0 g DW g⁻¹ N with a CV of 13.1% in 2001, though the difference was not significant between clusters in 2000. The range of variation among RILs in NUEd was comparatively smaller than those in TDMA and HI.

4. Relationships among grain yield, total dry matter accumulation, total N absorption, nitrogen use efficiency and harvest index

Table 4 shows the relationships among grain yield, TDMA, NTA, NUEd and HI in RILs. The TDMA was strongly and positively correlated with the Pre-DMA, Post-DMA, NTA, NUEd and grain yield and showed a significant negative correlation with HI in both years, although the coefficients of correlation were smaller in NUEd and HI than those in other traits. Pre- and Post-DMA showed significant positive correlations with NTA and grain yield in both years. Significant negative correlations were observed between NTA and NUEd in both years, although the correlation coefficient was not high. HI was negatively correlated with all the traits except for grain yield.

The results obtained from the multiple regression analysis indicated that 97.1 and 95.2% of the variation in grain yield in 2000 and 2001 was explained by NTA, NUEd and HI. The contribution ratios of these traits were 25.8, 41.6 and 32.7%, respectively, in 2000, and 25.2, 42.9 and 31.8%, respectively, in 2001 (Table 3).

Fig. 1 shows the relationships between the amount of TDMA and HI in relation to the grain yield in 2000 and 2001, respectively. TDMA had a significant negative correlation with HI (r = -0.261, p < 0.01 in 2000; r = -0.180, p < 0.05 in 2001, respectively). The correlation was higher at the same yielding levels. In the current study, for example, along the high iso-grain yield lines over 800 g m⁻² among RILs belonging to cluster B in 2000 and cluster A in 2001, the highest TDMA line showed a maximum TDMA of 2408 g m⁻².

Table 4. Correlation coefficients between grain yield or TDMA, Pre-DMA, Post-DMA, NTA, NUEd and HI of RILs grown in 2000 and 2001.

|                      | 2000                | 2001                |
|----------------------|---------------------|---------------------|
|                      | TDMA (g m⁻²)       | Pre-DMA (g m⁻²)    | Post-DMA (g m⁻²) | NTA (g m⁻²) | NUEd (g DM g⁻¹ N) | HI (%) | Grain yield (g m⁻²) |
|                     | 1.000              | 0.740**            | 0.799**          | 0.875**     | 0.262**           | -0.261** | 0.725**            |
| TDMA (g m⁻²)        |                    | 1.000              | 0.187 ns         | 0.016 ns    | 0.368**           | -0.133 ns | 0.598**            |
| Pre-DMA (g m⁻²)     | 0.740**            | 1.000              |                    | 0.620**     | -0.226*           | -0.263** | 0.524**            |
| Post-DMA (g m⁻²)    | 0.799**            | 0.016 ns           | 1.000             | 1.000       |                   | -0.130 ns | 0.722**            |
| NTA (g m⁻²)         | 0.875**            | 0.368**            | 0.620**           | 1.000       |                   | -0.226*  | -0.040 ns          |
| NUEd (g DW g⁻¹ N)   | 0.262**            | -0.226*            |                   |             | -0.346**          | 0.109 ns | 0.655**            |
| HI (%)              | -0.261**           | 0.109 ns           |                   |             |                   | 1.000    |                  |
| Grain yield (g m⁻²) | 0.725**            | 0.598**            | 0.524**           | 0.722**     | 0.443**           | 0.271**  | 1.000              |

TDMA, total dry matter accumulation; Pre-DMA, pre-heading dry matter accumulation; Post-DMA, post-heading dry matter accumulation; NTA, total nitrogen absorption; NUEd, nitrogen use efficiency for dry matter accumulation; HI, harvest index; * and ** indicated significant at p < 0.05 and 0.01, respectively; ns, not significant at p < 0.05.
with a minimum value HI of 31.2% in 2000; these values were 2488 g m$^{-2}$ and 30.1%, respectively, in 2001. In contrast, the lowest TDMA line showed a TDMA of 1469 g m$^{-2}$ and a HI of 44.9% in 2000; these values were 1628 g m$^{-2}$ and 43.1%, respectively, in 2001.

### Discussion

It is generally considered that the analysis of agronomic traits, for example, the grain yield and yield components or TDMA and HI, and so on should be based on these data in the paddy field, because the growth condition of rice in the paddy field is close to the practice. In the present study, we also examined the nitrogen use efficiency under controlled nutritional conditions. This phenotypic trait in rice can be greatly affected by imbalanced supply of nitrogen in culture media and this may be why information on QTL mapping of such traits under field condition is lacking.

1. **Differences in total dry matter accumulation, nitrogen use efficiency for dry matter accumulation and harvest index among RILs**

Genotypic variations in TDMA, NUEd and HI were significant among the RILs under hydroponic culture. Many authors confirmed significant genotypic variations in TDMA (Higuchi and Yoshino, 1986; Amano et al., 1993; Tirol-Padre et al., 1996; Singh et al., 1998; Peng et al., 1999; Cui et al., 2000; Inthapanya et al., 2000; Peng et al., 2000; Yang et al., 2002; Hasegawa 2003), NUEd (Higuchi and Yoshino 1986; Tirol-Padre et al., 1996; Koutroubas et al., 2003) and HI (Tirol-Padre et al., 1996; Singh et al., 1998; Ying et al., 1998; Inthapanya et al., 2000; Cui et al., 2000; Peng et al., 2000; Hasegawa 2003; Mae et al., 2006). However, such wide genotypic differences in TDMA (CV in 2000 was 24.0% and that in 2001 was 21.6%) and NUEd (CV in 2000 was 10.8% and that in 2001 was 13.1%) and HI (CV in 2000 was 18.3% and that in 2001 was 21.2%) observed in the present study was not recorded. This might be because of the lack of selection on these traits among RILs. Superior genotypes for rice production can be selected from the differences in genotype of TDMA, NUEd and HI among RIL lines. The differences in these values among the RILs should be clarified under different levels of nitrogen application in future studies (Seino, 1975).

2. **Total dry matter accumulation and harvest index in relation to grain yield.**

Grain yield could be increased by increasing total dry matter accumulation (TDMA) and/or harvest index (HI) (Yoshida, 1981). The contribution of biomass production and HI to genetic gains in grain yield potential varied from study to study (Peng et al., 2000). Comparisons between semidwarf and traditional rice cultivars attributed to the improvement in yield potential to the increase in HI rather than to the biomass production (Takeda et al., 1983; Evans et al., 1984, 1993). When comparisons were made among the improved semidwarf cultivars, however, a high yield was achieved by increasing biomass production (Jiang et al., 1988; Akita, 1989; Amano et al., 1993). In the present study, the contribution ratio of TDMA to the total variation in grain yield was higher than...
that of HI (Table 3). Song et al. (1990) and Yamauchi (1994) reported that hybrid rice have about 15% higher yield than inbred rice mainly due to an increase in biomass production rather than in HI. Ying et al. (1998) also reported that the further improvement in rice grain yield depended more on the ability to increase dry matter production than on increase in HI, by using different ecotypes rice cultivars. Peng et al. (2000) also reported that further increase in rice grain yield should be based on the further increase in dry matter accumulation. Because a strong compensation mechanism exists between TDMA and HI at the same grain yield level, an increase in one component will not necessarily result in an overall increase in grain yield. Grain yield would be increased by selecting cultivars for a high TDMA only if the comparative high HI were maintained. In the present study, some high-grain yielding lines showed a high TDMA with comparatively high HI (Fig. 1).

TDMA could be divided into two components, Pre- and Post-DMA. Yoshida (1972), Murata and Matsushima (1975) and Ntanos and Koutroubas (2002) reported that Pre-DMA may contribute 20–40% of the final crop yield depending on cultivar, reflecting its importance for attaining higher grain yields. Yoshida (1981) reported that increased dry matter accumulation after heading would be greatly beneficial to attain higher grain yield because 60 to 100% of the yield comes from assimilates produced during the grain-filling period. In the current study, the contribution ratio of Pre-DMA and Post-DMA to the total variation in TDMA was 68.5 and 31.5%, respectively, in 2000 and 71.6 and 28.4%, respectively, in 2001 (Table 3). These results indicated that Pre-DMA was more important than Post-DMA for increasing the TDMA consequently for increasing grain yield. The discrepancy from the report by Yoshida (1981) may be due to high planting density (62 hills m\(^{-2}\)) in the present study. Wada (1969) stated that with increase of planting density, the amount of carbohydrate and the percentage of the grain yield come from carbohydrate before heading increased. Murata and Iyama (1958) indicated that high planting density increased the dry matter accumulation before the middle of the plant growth duration, compared with the normal planting density.

3. Total nitrogen absorption and nitrogen use efficiency for dry matter accumulation in relation to total dry matter accumulation

The above results indicated that TDMA was more important to increase grain yield than HI. TDMA also could be increased by increasing either NTA and/or NUEd. Genetic variations in TDMA among the RILs at the same N level were also attributed to the variability in NTA and NUEd. Negative correlations between NTA and NUEd were observed in the present study (Table 4). The contribution of NTA and NUEd to the total variation in TDMA was 38.4 and 61.6%, respectively, in 2000 and 37.0 and 63.0%, respectively in 2001. These results suggested that more attention should be paid to NUEd for improving TDMA among RILs.

Since the grain yield =TDMA × HI and TDMA =NTA × NUEd, the grain yield could be determined by NTA × NUEd × HI. In the previous report (Ju et al., 2006), we have suggested that more attention should be paid to NUEg for improving grain yield. In the present study, the contribution ratio of NUEd to the total variation in grain yield was highest among all the functional components, accounting for 41.6% in 2000 and 42.9% in 2001 (Table 3). These result suggested that more attention should also be paid to NUEd for improving TDMA consequently for improving grain yield, because improving NUEd could reduce not only environment pollution but also agricultural cost.

The present findings revealed significant differences in TDMA, NUEd and HI among RILs that would help select superior genotypes for rice production. The higher-yielding rice plants generally showed higher TDMA and HI values. Further increase in rice grain yield should be based on the further increase in TDMA than HI. To increase TDMA leading to higher grain yield, we should concentrate on improving NUEd, compared with NTA and HI. We have conducted a preliminary study on QTLs of NUEp using RILs of the F\(_{12}\) generation (Shan et al., 2005). Further study on QTLs of grain yield, TDMA and HI and QTL analysis of NUEd will be done using RILs of the F\(_{12}\) and F\(_{13}\) generations. The present study showed the possibility of breeding higher-yield cultivars with higher NUEd and provided simple criteria for breeding on some quantitative characters.

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