Genotypic Variation in Nitrogen Uptake during Early Growth among Rice Cultivars under Different Soil Moisture Regimes

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Abstract: In order to enhance rice productivity under water-deficient conditions, it is essential to improve nitrogen (N) uptake ability or its use efficiency. The objectives of the present study were to examine the genotypic variation in N uptake ability and physiological N use efficiency (PNUE) among 70 rice cultivars, and to clarify its association with biomass production and water uptake ability. Plants were grown under three soil moisture regimes: flooded and two non-flooded (−0.10 and −0.52 MPa soil water potential) conditions. A substantial genotypic variation in the amount of aboveground N uptake was observed, and the amount bore a positive correlation with aboveground biomass production regardless of soil moisture regime, whereas PNUE showed a negative if any correlation with biomass production depending on soil moisture regime. A significant interactive effect on the amount of aboveground N uptake between cultivars and soil moisture regimes was observed; there existed cultivars that exhibited superior N uptake only under non-flooded conditions. N uptake ability was closely correlated with water uptake ability, while a substantial genotypic variation in N uptake per unit water uptake was found in the two non-flooded regimes. These results indicate that a substantial genotypic variation in N uptake ability under water deficient conditions exists among diverse rice genetic resources, and that the variation is associated with water uptake ability and biomass production under the water-limited conditions at the early growth stage.

Key words: Nitrogen uptake ability, Nitrogen use efficiency, Rice Diversity Research Set of germplasm, Soil moisture, Water uptake ability.

Water scarcity is becoming a serious global problem. About 1.2 billion people are living with absolute water scarcity, and the water-limited area is continuously expanding (FAO, 2010). About 70% of the global water resource is consumed by agricultural use. In Asia, about 90% of fresh water is used for agriculture, with more than 50% being used for rice production (Maclean et al., 2002). Water scarcity threatens stable rice production, which is strongly dependent on irrigation water.

Many techniques have been developed to maintain or increase rice production under conditions of limited water resources (Tuong et al., 2005; Bouman et al., 2007; Zhang et al., 2009). In addition to agronomical methods, the genetic improvement of adaptability to water-deficient conditions is one of the greatest challenges for sustainable rice production (Kamoshita, 2011; Serraj et al., 2011). For recent advances in molecular technology to be applied to practical breeding program, it is necessary to narrow the large gap between the knowledge of gene level and the plant performance in the field.

Under water-limited conditions, nitrogen (N) uptake was found to be closely correlated with water uptake; water stress reduces transpiration, leading to the reduction of nutrient uptake and biomass production by the plant (O’Toole and Baldia, 1982; O’Toole and Padilla, 1984). Regarding the relationship between N absorption and growth under water-deficient conditions, Kato et al. (2006) found, from a comparison of selected upland and lowland cultivars, that the amount of N uptake and growth decreased as soil moisture decreased, suggesting that N uptake capacity is a critical trait determining the growth under water-limited conditions. Previously, we compared upland New Rice for Africa (NERICA) cultivars and Japanese upland/lowland rice cultivars, and found a substantial genotypic difference in N uptake ability under rainfed upland conditions (Matsunami et al., 2009, 2010).
By improving N use efficiency in rice, we may reduce the amount of chemical fertilization which will contribute to environmental friendly production and reduction of production cost (Fageria et al., 2008).

Despite these findings, the information on N uptake ability and N use efficiency of rice plants under water-limited conditions is insufficient, because the range of cultivars employed in previous studies was limited. In this regard, we previously examined the genotypic variation in early growth of the rice diversity research set of germplasm (RDRS), which was developed by the National Institute of Agrobiological Sciences (NIAS, Tsukuba, Japan) under different soil moisture conditions, and revealed their wide genotypic diversity in biomass production and water uptake capacity (Matsunami et al., 2012). Genetic improvement of N uptake ability and N use efficiency will contribute to the efficient recovery of N input, and the amount of chemical N applied can be reduced and thereby alleviate environmental pollution. In the present study, we evaluated the genotypic variation in N uptake and N use efficiency in early growth among rice cultivars under different soil moisture regimes, and analyzed the association between biomass production and water uptake ability.

Materials and Methods

The plants used in the present study were the same as those used previously (Matsunami et al., 2012): RDRS developed by NIAS as a global rice core collection (NIAS Genebank, 2010) and a high drought-tolerant japonica cultivar Azucena (Sangam et al., 2010). RDRS consists of 69 cultivars, including 22 indica I types, 33 indica II types and 14 japonica types (personal communication with Dr. Ebana, NIAS) collected from 19 countries, and developed based on a genome-wide RFLP polymorphism survey of 332 accessions, which were selected on the basis of the passport data of all collections stored at the Genebank of NIAS, and holds 91% of the alleles identified in these accessions (Kojima et al, 2005).

Plants were grown in an environmentally regulated growth chamber (2 m × 2 m × 2.1 m height): 12 hr of fluorescent light with 450 μmol m⁻² s⁻¹ PPFD, day/night temperature of 25/20°C, and 70% relative humidity. The growth chamber was capable of containing about 80 pots at a time. We repeated the examinations several times for each cultivar/water regime, and three plants showing medium effects of the position were placed randomly with adequate spacing without mutual shading, and moved every day to minimize the effects of the position. Seeds were sown on a seedling tray then two-week-old seedlings were transplanted to 1/10,000 a Wagner pot, one plant per pot. Each pot contained 1.4 kg of Andosol soil. Mixed fertilizer was applied at a rate of 0.26, 0.26 and 0.26 g (N, P₂O₅, and K₂O, respectively) per pot. After transplanting, plants were grown under three soil moisture regimes: a flooded condition (43% (w/w)) and two non-flooded conditions (33% and 25%). The soil water potentials of each regime were -0.02, -0.10 and -0.52 MPa for the 43%, 33% and 25% soil moisture regimes, respectively. The water potential of the soil sample was measured using a dew point microvoltmeter (HR-33T, WESCOR, UT). The amount of evaporation from the soil surface was estimated from the weight reduction of pots which were not planted. The amount of water uptake was estimated from the daily reduction in pot weight and the amount of evaporation. Further details of cultural methods and soil properties were as described previously (Matsunami et al., 2012).

Three weeks after transplanting, the plants were sampled and separated into shoots and roots, then dried at 80°C for more than three days and weighed. For determination of the amount of N uptake of plants, the shoot samples were ground, and N concentration was analyzed by the Kjeldahl method (2300 Kjeltec Analyzer Unit, Foss TECATOR, Sweden).

The definitions of N use efficiency vary with the purpose of individual studies (Ladha et al., 1998; Fageria et al., 2008). In this study, physiological N use efficiency (PNUE) for shoot biomass production was calculated as follows:

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P_{\text{NUE}} = \frac{\text{aboveground dry weight}}{\text{aboveground N content}}
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Statistical analysis and cluster analysis were conducted by using JMP 8 software (SAS Institute, Cary, NC, USA). The dendrograms were calculated and drawn using the Ward hierarchical clustering method.

Results and Discussion

1. Genotypic variation in N uptake ability and its use efficiency

The primary object of the present study was to evaluate
the genotypic variation in N uptake ability and PNUE among 70 rice cultivars under different soil moisture regimes. When all the cultivars are pooled, significantly positive correlations were observed between the amount of aboveground N uptake under the flooded regime and that under the two non-flooded regimes (Fig. 1), suggesting that the cultivars with superior N uptake under the flooded regime generally exhibit superior N uptake under the non-flooded regimes as well.

Analysis of variance (ANOVA) revealed that cultivar and soil moisture had a significant effect on the amount of aboveground N uptake and PNUE among 70 rice cultivars. The summary of ANOVA is presented in Table 1.

Table 1. Summary of ANOVA for aboveground dry weight, the amount of aboveground N uptake and physiological nitrogen use efficiency (PNUE) of 70 rice cultivars subjected to three soil moisture regimes (flooded, −0.10 and −0.52 MPa).

| Soil moisture regime | Source of variation | Aboveground dry weight | The amount of aboveground N uptake | PNUE |
|----------------------|---------------------|-----------------------|----------------------------------|-------|
| Flooded/−0.10 MPa    | Cultivar (C)        | ***                   | ***                              | ***   |
|                      | Soil moisture (S)   | n.s.                  | ***                              | ***   |
|                      | C × S               | *                     | ***                              |       |
| Flooded/−0.52 MPa    | C                   | ***                   | ***                              |       |
|                      | S                   | ***                   | ***                              |       |
|                      | C × S               | ***                   | ***                              |       |
| −0.10 MPa/−0.52 MPa  | C                   | ***                   | ***                              |       |
|                      | S                   | ***                   | ***                              |       |
|                      | C × S               | ***                   | ***                              |       |
| All regimes          | C                   | ***                   | ***                              |       |
|                      | S                   | ***                   | ***                              |       |
|                      | C × S               | ***                   | ***                              |       |

*, **, *** indicate significance at 0.05, 0.01 and 0.001 levels, respectively. n.s. indicates no significance.

Fig. 2. Genotypic variation in (A) the amount of aboveground N uptake and (B) physiological N use efficiency (PNUE) among 70 rice cultivars subjected to three soil moisture regimes (flooded, −0.10 and −0.52 MPa). Number of cultivars vs. the value of N uptake with a range of 20 mg plant⁻¹ and that of PNUE with a range 2 g g⁻¹ are shown. Triangles indicate the mean value of each moisture regime for indica I, indica II and japonica types. Means with different letters indicate significant differences at P < 0.05 according to Tukey’s means comparison test.
indicating that there exists a substantial genetic variation among the diverse genetic resources in these traits responding to different soil moisture conditions. In addition, the analysis revealed an interactive (cultivar × soil moisture) effect on these traits except on PNUE between –0.10 MPa and –0.52 MPa regimes; the interaction was significant on the amount of aboveground N uptake and PNUE between flooded and non-flooded regimes, while it was significant only on the amount of aboveground N uptake between the two non-flooded regimes (–0.10 and –0.52 MPa). These results suggest that N uptake capacity rather than PNUE contributes to genetic variation in aboveground dry weight under water-restricted conditions.  

Genotypic variation in the amount of aboveground N uptake and PNUE among 70 rice cultivars subjected to three soil moisture regimes are shown in Fig. 2. The amount of N uptake varied markedly regardless of soil moisture; it ranged from 25.3 to 112.8 mg plant$^{-1}$ in the flooded, 38.7 to 125.6 mg plant$^{-1}$ in the –0.10 MPa and 14.4 to 68.7 mg plant$^{-1}$ in the –0.52 MPa regimes, respectively (Fig. 2A). The frequency distribution patterns were similar under the flooded and –0.10 MPa regimes, whereas that under the – 0.52 MPa regime was confined to fewer classes, with a substantially lower mean. In contrast, genotypic variations in PNUE were small, although the distribution in the –0.10 MPa regime was slightly confined

### Table 2. Top 10 cultivars that exhibited superior N uptake under three soil moisture regimes and their physiological N use efficiency (PNUE).

| Soil moisture regime | Cultivar Type | The amount of aboveground N uptake (mg plant$^{-1}$) | PNUE (g g$^{-1}$) |
|----------------------|---------------|----------------------------------------------------|------------------|
| Flooded             | 1 Jaguary Japonica | 113 ± 10 | 23.0 ± 0.1 |
|                     | 2 Chin Galay Indica II | 103 ± 7 | 25.3 ± 1.6 |
|                     | 3 Hakphaynlay Indica II | 100 ± 7 | 25.9 ± 0.7 |
|                     | 4 Radin Goi Sesat Indica II | 95 ± 4 | 24.8 ± 1.1 |
|                     | 5 Qingyu (Seiyu) Indica II | 92 ± 26 | 22.2 ± 0.6 |
|                     | 6 Shwe Nang Gyi Indica II | 90 ± 9 | 27.7 ± 0.6 |
|                     | 7 Ryou Suisan Koumai Indica II | 89 ± 7 | 24.8 ± 0.2 |
|                     | 8 Vandaran Indica II | 89 ± 8 | 24.3 ± 0.6 |
|                     | 9 Lebed Indica II | 88 ± 19 | 23.7 ± 0.6 |
|                     | 10 Davao 1 Indica II | 87 ± 12 | 24.2 ± 0.7 |
| –0.10 MPa           | 1 Jaguary Japonica | 126 ± 6 | 21.8 ± 0.3 |
|                     | 2 Puluik Arang Indica II | 109 ± 2 | 23.8 ± 0.8 |
|                     | 3 Bei Khe Indica II | 105 ± 7 | 23.2 ± 0.9 |
|                     | 4 Chin Galay Indica II | 105 ± 18 | 23.4 ± 0.2 |
|                     | 5 Davao 1 Indica II | 102 ± 7 | 21.0 ± 0.6 |
|                     | 6 Dejjaohualuo Indica II | 101 ± 21 | 22.4 ± 0.4 |
|                     | 7 Bingala Indica II | 96 ± 7 | 25.0 ± 0.3 |
|                     | 8 Badari Dhan Indica I | 92 ± 17 | 24.8 ± 0.2 |
|                     | 9 Shuusoushu Indica II | 91 ± 12 | 22.6 ± 0.9 |
|                     | 10 Ryou Suisan Koumai Indica II | 91 ± 15 | 22.1 ± 0.1 |
| –0.52 MPa           | 1 Davao 1 Indica II | 69 ± 2 | 21.9 ± 0.8 |
|                     | 2 Puluik Arang Indica II | 64 ± 8 | 24.2 ± 0.4 |
|                     | 3 Jaguary Japonica | 62 ± 7 | 24.0 ± 0.1 |
|                     | 4 Bingala Indica II | 56 ± 1 | 23.5 ± 0.4 |
|                     | 5 Kemasin Indica II | 55 ± 12 | 23.6 ± 0.2 |
|                     | 6 Naba Indica II | 53 ± 4 | 24.7 ± 0.4 |
|                     | 7 Ryou Suisan Koumai Indica II | 53 ± 4 | 22.4 ± 0.3 |
|                     | 8 Khao Nok Japonica | 52 ± 7 | 24.9 ± 0.4 |
|                     | 9 Chin Galay Indica II | 51 ± 14 | 25.6 ± 0.3 |
|                     | 10 Bleiyo Indica II | 50 ± 10 | 24.1 ± 0.3 |

Means ± SE are shown. Underlined cultivars appear regardless of moisture regime.
Table 2 lists the top 10 cultivars that exhibited superior N uptake under –0.52 MPa regime; four out of 10 cultivars (underlined) are also evaluated as the top 10 under flooded and –0.10 MPa regimes (Table 2). Regardless of soil moisture regime, most indica II cultivars were top rankers.

The amount of N uptake was strongly correlated with the aboveground dry weight regardless of soil moisture regime (Fig. 3A). In contrast, the aboveground dry matter production and PNUE were not significantly correlated under the flooded and –0.10 MPa regimes (Fig. 3B). The correlation was significant under the –0.52 MPa regimes, but the correlation coefficient was substantially low (r = 0.319).

Many previous studies found variations of N uptake ability among genotypes and its correlations with biomass production (Miyama and Okabe, 1986; Ichii and Tsumura, 1989; Hasegawa, 1990; Wada et al., 2002; Haeifie et al., 2008; Katsura et al., 2010). However, these studies
produced inconsistent results regarding the association of PNUE with biomass production. For example, in a study where a variation in PNUE ((dry weight of N treatment–dry weight of non-N control)/(N content of N treatment–N content of non-N control)) among 31 cultivars was examined under varying N conditions, a significant cultivar difference in PNUE was found (Namai et al., 2009), although the definition of PNUE was different from our study. They suggested that the variation reflects the genetic and/or cultural backgrounds of the cultivars employed, including 20 indica and japonica types. In the present study, the cultivars with high PNUE did not always exhibit high N uptake and high biomass production, because PNUE and the amount of N uptake of a cultivar did not vary in parallel (Fig. 3). In contrast, in other studies in which N use efficiency for grain yield (grain yield per plant N uptake) was examined, N use efficiency was evaluated as an important parameter for efficient rice production (Tirol-Paddre et al., 1996; Mae et al., 2006; Samonte et al., 2006; Ju et al., 2009). Whether N use efficiency contributes to biomass production and/or grain yield under water-deficient conditions needs further study.

2. Relationships between N uptake, biomass production and water uptake

Water uptake has been found to be closely associated with N uptake. In rice, soil water deficit results in a reduction of transpiration of plants, thereby limiting N uptake (O’Toole and Baldia, 1982; O’Toole and Padilla, 1984; Yamzao and O’Toole, 1984). Our results confirmed this relationship; the reduction of soil moisture resulted in the reduction of water uptake and N uptake (Fig. 4). The values of correlation coefficient between water uptake and N uptake were in the following order: flooded regime > −0.10 MPa regime > −0.52 MPa regime.

The distribution patterns of N uptake per unit water uptake are shown in Fig. 5. The distributions of the amount of N uptake per unit water uptake were wider in the two non-flooded regimes than that in the flooded regime; they ranged from 0.14 to 0.24 mg g⁻¹ H₂O plant⁻¹ in
Fig. 6. Classification of 70 rice cultivars by cluster analysis based on aboveground and root dry matter production, the amount of aboveground N uptake, PNUE and the amount of cumulative water uptake. The plants were subjected to three soil moisture regimes (flooded, −0.10 MPa and −0.52 MPa). The dendrograms were calculated and drawn using the Ward hierarchical clustering method. The information on subgroups (J, japonica (black); I, indica I (blue); II, indica II (red)) and the origin of individual cultivar are shown in parentheses.
the flooded regime, 0.17 to 0.30 mg g⁻¹ H₂O plant⁻¹ in the –0.10 MPa regime and 0.13 to 0.34 mg g⁻¹ H₂O plant⁻¹ in the –0.52 MPa regime, respectively. A comparison of the trait among the three groups found that indica I exhibited a lower average under the flooded regime, whereas indica II exhibited a higher average than other groups under the –0.52 MPa regime. This superior capacity of N uptake per unit water uptake of indica II group appears responsible for superior N uptake capacity over a wide range of soil moisture in this group (Table 2). Regarding the dependence of N uptake on water uptake capacity of rice cultivars, Ju et al. (2006) examined the cultivar variation in N uptake capacity and exudation rate, which may be associated with root activity, and observed the association of root activity with N uptake capacity of a cultivar.

The N uptake capacity is also affected by N metabolic processes in which N transporters and enzymes that synthesize N-transport amino acids play critical roles (Yamaya and Oaks, 2004). Recent advances in molecular approaches will facilitate better understanding of mechanisms regulating N uptake and N metabolism, leading to the identification of physiological traits responsible for superior capacity of N uptake under stressed conditions, although further studies are required to apply these approaches into practical breeding programs.

Cluster analysis based on aboveground and root dry matter production, the amount of N uptake, PNUE and the amount of cumulative water uptake of the cultivars subjected to three soil moisture regimes resulted in grouping 70 cultivars into several clusters (Fig. 6). The cultivar classification varied with soil moisture regime; for example, Basilanon, Kalo Dhan, Shoni, Tupa121-3 and Asu were classified into the same group as Nipponbare under –0.52 MPa regime, while these cultivars were classified in the most remote group under flooded regime. It is notable that the cultivars with superior N uptake capacity regardless of soil moisture (Jaguary, Chin Galay, Ryou Suisan Koumai and Davao 1) were classified into the close groups, suggesting that these cultivars possess a close genetic background.

Since our study focused on the early vegetative stage under environmentally-regulated conditions, it is necessary to further evaluate the cultivars at later growth stages under different environmental conditions for providing information for practical breeding programs. Specifically, studies to clarify root function and root to shoot communication which are associated with N uptake, transfer and metabolism under water-deficient conditions are needed to improve N utilization of rice cultivars under adverse soil conditions where availability of water, nutrients and fertilizers is limited.

In conclusion, our results indicated that a substantial genotypic variation in N uptake ability under different soil moisture conditions existed among diverse rice genetic resources, and that the variation was associated with water uptake ability. The cultivars that exhibited superior N uptake under flooded conditions generally exhibited superior N uptake under non-flooded conditions as well. Specifically, indica II cultivars were identified to possess superior N uptake capacity over a wide range of soil moisture. Under rain-fed conditions where soil moisture fluctuates during growing season, the greater adaptability of this group may be of great advantage. In contrast, there were a few cultivars that exhibited superior N uptake only in water-restricted soil conditions (for example, Pululik Arang). This type of cultivar may have adaptability to scarcer soil moisture conditions.

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