

# Genetic Variations in Dry Matter Production, Nitrogen Uptake, and Nitrogen Use Efficiency in the AA Genome *Oryza* Species Grown under Different Nitrogen Conditions

Norimitsu Hamaoka<sup>1</sup>, Yuri Uchida<sup>1</sup>, Masayoshi Tomita<sup>2</sup>, Etsushi Kumagai<sup>3</sup>,  
Takuya Araki<sup>4</sup> and Osamu Ueno<sup>1,5</sup>

<sup>1</sup>Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan;

<sup>2</sup>Graduate School of Technology Education, Fukuoka University of Education, Akama, Munakata 811-4192, Japan;

<sup>3</sup>National Agricultural Research Center for Tohoku Region, Akahira, Shimokuriyagawa, Morioka 020-0198, Japan;

<sup>4</sup>Faculty of Agriculture, Ehime University, Tarumi, Matsuyama 790-8566, Japan;

<sup>5</sup>Faculty of Agriculture, Kyushu University, Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan)

**Abstract:** To clarify the genotypic variation of nitrogen (N) response in the AA genome *Oryza* species, we investigated dry matter production, N uptake, N and water use efficiencies (NUE and WUE), bleeding sap rate (BR), and root morphological traits at vegetative stage in 6 cultivars and 4 strains of 6 species (*O. sativa*, *O. glaberrima*, *O. barthii*, *O. nivara*, *O. meridionalis*, and *O. rufipogon*) grown under standard N (SN) and low N (LN) conditions. Some wild *Oryza* strains and *O. glaberrima* showed high dry matter production under both N conditions. In most plants, total dry weight decreased and root dry weight increased under the LN condition, resulting in decreased top-root ratio. In *japonica* cultivars of *O. sativa*, however, these traits were unaffected by the N condition. There were no significant differences in WUE with plant species or N conditions. In all plants, however, NUE was higher in the LN than SN condition, and was conspicuously high in most wild *Oryza* species and *O. glaberrima*. Some of them showed increased capacity of nitrate-N ( $\text{NO}_3\text{-N}$ ) uptake under the LN condition. In cultivars and strains with a high NUE, root dry weight, root surface area, and BR were also higher under the LN condition. These results suggest that a high NUE is associated with the development of a root system, increased BR, and probably increased capacity of  $\text{NO}_3\text{-N}$  uptake. This study revealed the presence of superior wild *Oryza* strains for growth under LN that may be a promising genetic resource for low N-input agriculture.

**Key words:** AA genome, Dry matter production, Nitrate-N, Nitrogen deficiency, Nitrogen use efficiency, *Oryza* species, Root system.

High inputs of nitrogen (N) fertilizers and agrochemicals have contributed to the increase in rice yield in Asian countries (Evans, 1993). However, a part of N is lost in the atmosphere or leached into groundwater, lakes, and rivers, which causes severe environmental pollution (Jarvis, 1996; Shi et al., 2009). Despite that major crops can utilize only 30 – 40% of the applied N (Raun and Johnson, 1999), the application of N fertilizer is one of the major economic cost for rice farmers in developing countries. Therefore, it is necessary to produce rice cultivars with high N use efficiency (NUE), which would be useful for low N input cultivation system (Glass et al., 2003; Kumagai et al., 2009).

N is a major essential nutrient for plants and affects photosynthesis, dry matter production, and yield of crops. Therefore, it is very important for crop cultivation to

understand the N uptake trait and NUE. The root system of crops plays an important role in the acquisition of water and nutrients (de Dorlodot et al., 2007).

So far, many studies have been carried out on the uptake and assimilation of N in rice plants (Hirel and Gadal, 1980; Kronzucker et al., 2000; Tabuchi et al., 2007). In paddy field, the main source of N in rice is ammonium-N ( $\text{NH}_4\text{-N}$ ) rather than nitrate-N ( $\text{NO}_3\text{-N}$ ) (Wang et al., 1993). However, some reports have indicated that rice plants enhance the capacity of  $\text{NO}_3^-$  absorption from roots, N allocation to shoot, NUE, and yield, when both  $\text{NH}_4^+$  and  $\text{NO}_3^-$  were applied as N source, as compared with application of  $\text{NH}_4^+$  alone (Youngdahl et al., 1982; Raman et al., 1995; Kronzucker et al., 1999; Duan et al., 2006, 2007; Shimono and Bunce, 2009).

Table 1. The AA genome *Oryza* species examined in this study.

	Species	Subspecies	Cultivar and Strain	Abbreviation	Origin
Cultivated	<i>O. sativa</i>	<i>Japonica</i>	Akenohoshi	AKN	Japan
		<i>Japonica</i>	Nipponbare	NPB	Japan
		<i>Indica</i>	IR24	IR24	Philippine
		<i>Indica</i>	Kasalath	KSL	India
	<i>O. glaberrima</i>		IRGC 10377	GL-1	Senegal
			IRGC 10403	GL-2	Mali
Wild	<i>O. barthii</i>		IRGC 10410	BRT	Chad
	<i>O. meridionalis</i>		W1625	MDN	Australia
	<i>O. nivara</i>		IRGC 10571	NVR	Cambodia
	<i>O. rufipogon</i>		W106	RPG	India

It is thought that the abilities of N uptake and assimilation and the extent of root development are concerned with NUE of rice plants (Shi et al., 2010). A previous study revealed a large genotypic difference in NUE among cultivars of *O. sativa*, and the *indica* cultivar, Kasalath, showed the highest N sensitivity (Namai et al., 2009). Duan et al. (2007) also confirmed that, when  $\text{NO}_3^-$  were applied together with  $\text{NH}_4^+$ , cultivars with high NUE showed higher N accumulation, dry matter production, and yield than those with low NUE and suggested that there was a close relationship between  $\text{NO}_3^-$  application and NUE.

Plants increase their N uptake ability by regulating some physiological and biochemical traits and changing root morphology under N-deficient conditions (Huang et al., 2004; Miller and Cramer, 2004). In rice plants, inhibition of seminal root elongation caused by an increase in exogenous  $\text{NH}_4^+$  concentration was more conspicuous in cultivars with high adaptability for heavy manuring than those with low adaptability (Tanaka et al., 1993). Amin et al. (2002) showed that the root growth under low N conditions was greater in local cultivars than in improved cultivars of the *indica* type. This result implies that the improved cultivars have been selected for cultivation under high N application and lost some traits responsible for growth under low N conditions in historical breeding programs.

Wild *Oryza* species are widely distributed around the world including the tropical and subtropical regions and exhibit wide genotypic variation (Lu et al., 2010). The genus *Oryza* consists of several genome groups, and its AA genome group includes 6 wild species, *O. rufipogon*, *O. nivara*, *O. barthii*, *O. longistaminata*, *O. meridionalis*, and *O. glumaepatula*, together with 2 cultivated species, *O. sativa* and *O. glaberrima* (Khush, 1997; Vaughan et al., 2003). Although the wild *Oryza* species are generally lower in yielding ability than *O. sativa* and have several impertinent agronomic traits such as shattering habit and strong dormancy, they are expected to have unknown useful

genes. In fact, some genes concerning resistance to biotic (Fujita et al., 2004; Prasad and Eizenga, 2008) and abiotic stresses (Brar and Khush, 1997; Scafaro et al., 2010) have been identified in wild *Oryza* species. Therefore, they are important as genetic resources for future rice breeding (Doi et al., 2008).

With regard to wild *Oryza* species, there have been few reports about the dry matter production and growth response to N (Cook and Evans, 1983). There is a possibility that the wild species may have traits adaptable to the growth under low N conditions and show N responses different from those of the cultivated *Oryza* species. Therefore, it is also necessary to evaluate the N response in the wild species as well as the cultivated species (Futakuchi et al., 2012).

In this study, we evaluated dry matter productivity, N uptake traits, NUE, and root-related traits at the vegetative stage in the AA genome *Oryza* species under different N conditions. The main objective of this study is to clarify the genotypic variations of the growth response and NUE under N-deficient condition.

## Materials and Methods

### 1. Plant materials and cultivation

Six cultivars and 4 strains from 6 *Oryza* species (2 cultivated species and 4 wild species) were used in this study (Table 1). These species belong to the AA genome group of *Oryza* and are diploid. Of the 4 cultivars of *O. sativa*, Akenohoshi (AKN) and Nipponbare (NPB) are *japonica* type and IR24 and Kasalath (KSL) are *indica* type. Seeds of the cultivars and strain of *O. glaberrima* and wild *Oryza* species were provided from the Plant Breeding Laboratory, Faculty of Agriculture, Kyushu University, Fukuoka, Japan.

Seeds were treated with fungicide (Benlate) and incubated in water at 30°C for 48 hr for germination. Seeds of the wild *Oryza* species were dehusked to break dormancy before the fungicide treatment. Then, germinated seeds were sown in nursery boxes in a glasshouse at the

beginning of June 2011. At 4 wk after sowing, 4 seedlings per cultivar or strain were set in 4 pores of a polystyrene table ( $45 \times 31 \text{ cm}^2$ ) sealed with sponges and floated on a hydroponic culture bath filled with 32 L of nutrient solution. Except for plants, the upper surface of the bath was completely covered with vinyl film to avoid water loss by evaporation. The plants were grown in the culture solution recommended by Yoshida et al. (1972) but differing in the N levels. The standard-N (SN) and low-N (LN) culture solutions were contained 2.86 mM and 0.95 mM  $\text{NH}_4\text{NO}_3$ , respectively. These culture solutions also contained the following nutrients; 0.51 mM  $\text{K}_2\text{SO}_4$ , 1.00 mM  $\text{CaCl}_2$ , 1.67 mM  $\text{MgSO}_4$ , 0.32 mM  $\text{NaH}_2\text{PO}_4$ , 0.04 mM  $\text{FeCl}_2$ , 9.09 mM  $\text{MnCl}_2$ , 0.08 mM  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ , 18.2 mM  $\text{H}_3\text{BO}_3$ , 0.15 mM  $\text{ZnSO}_4$ , 0.16 mM  $\text{CuSO}_4$ , and 3.57 mM  $\text{Na}_2\text{SiO}_3$ . The seedlings were grown in the glasshouse under natural sunlight, wherein photosynthetic photon flux density at midday exceeded  $1,500 \mu\text{mol m}^{-2} \text{ s}^{-1}$  and the mean air temperature and relative humidity were  $30.6^\circ\text{C}$  and 66.3%, respectively. The solution was adjusted every day to pH 5.0 – 5.5 using HCl and NaOH. During the cultivation period, each solution was renewed every week and aeration was not provided.

## 2. Outline of experiments

During 4 wk after transplanting, the culture solution was renewed every week, and the amounts of water,  $\text{NH}_4\text{-N}$ , and  $\text{NO}_3\text{-N}$  uptakes of *Oryza* plants were estimated before renewing the solution from decreases in water weight and  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  concentrations. After 4 wk, stems with leaves were cut, and bleeding sap rate (BR) was measured from before daybreak to early morning. The stems and leaves were used for measurement of dry weight. After the measurement of BR, roots were sampled for measurements of root surface area and dry weight.

## 3. Quantification of water, $\text{NH}_4\text{-N}$ , and $\text{NO}_3\text{-N}$ uptakes

Every week before renewing the culture solution, the weight of the old culture solution in which plants had been cultured for one week was measured to estimate the amount of water uptake (amount of culture solution absorbed by plants) in a week. The amounts of  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  in the solution were also measured to estimate their uptake by plants. On the other hand, the culture solution (32000 g) without plants was used as a control to determine the water loss by evaporation during the same period. The amount of water uptake by plants was determined from the difference in the amount of decrease in culture solution with and without plants (Cont). The amounts of  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  in the culture solution were determined by using RQ flex plus (Merck, Germany).

The ratio of  $\text{NO}_3\text{-N}$  uptake to total N ( $\text{NH}_4\text{-N}$  plus  $\text{NO}_3\text{-N}$ ) uptake ( $\text{NO}_3\text{-N}/\text{total N}$  ratio) was calculated from the data of the first 2 wk after transplanting, because no

exhaustion of N occurred in all cultivars and strains during this period.

## 4. Measurement of bleeding sap rate (BR)

Four wk after transplanting, BR was measured for each plant (4 plants per cultivar or strain). The measurement of BR was performed during 0300 to 0800. Shoots were cut with a sharp razor blade at a height of approximately 10 cm from solution surface, and the cut ends were covered with pre-weighed cotton pads, which were sealed with plastic film and rubber bands to protect against water loss. The sap released from cut ends during 180 min was collected, and then cotton pads were weighed to determine BR. BR was calculated on the basis of root surface area per plant (RSA).

## 5. Measurements of dry matter production and morphological traits

Plants were sampled at 4 wk and divided into roots and the remaining organs (leaves and stems). The RSA was measured using a root analysis instrument (WinRHIZO; Regent Instruments Inc., Quebec, ON, Canada). Subsequently, the samples were oven-dried at  $80^\circ\text{C}$  for 3 d to determine dry weight. From dry weight of the aboveground part and roots, top-root ratio (T-R ratio) was calculated.

## 6. Calculation of NUE and water use efficiency (WUE)

NUE and WUE were calculated according to the following formulas:  $\text{NUE} = [\text{Total dry matter weight (g plant}^{-1})] \text{ at 4 wk} / [\text{Apparent total N uptake (g plant}^{-1})]$ , where apparent total N uptake was total amount of  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  absorbed during 4 wk.  $\text{WUE} = [\text{Total dry matter weight (g plant}^{-1})] / [\text{Apparent total water uptake (L plant}^{-1})]$ , where apparent total water uptake was total amounts of water absorbed during 4 wk. In this study, dry weight of seedling at the start of experiment was not subtracted from total dry matter weight, because it was less than 2 to 3% of the dry weight at 4 wk after transplanting.

## 7. Statistic analysis

The data were statistically evaluated by analysis of variance (ANOVA), and Tukey's test at  $P < 0.05$  was used for the comparison of means.

## Results

### 1. Dry matter production and T-R ratio

Fig. 1 shows total dry weight, root dry weight, and top-root ratio (T-R ratio) in the AA genome *Oryza* species. In general, similar patterns of cultivar and specific difference were recognized in total and root dry weight under the SN and LN conditions (Fig.1A-D). The total dry weight was lighter in plants grown under LN condition than under the SN condition; the ratios of the values under LN to SN

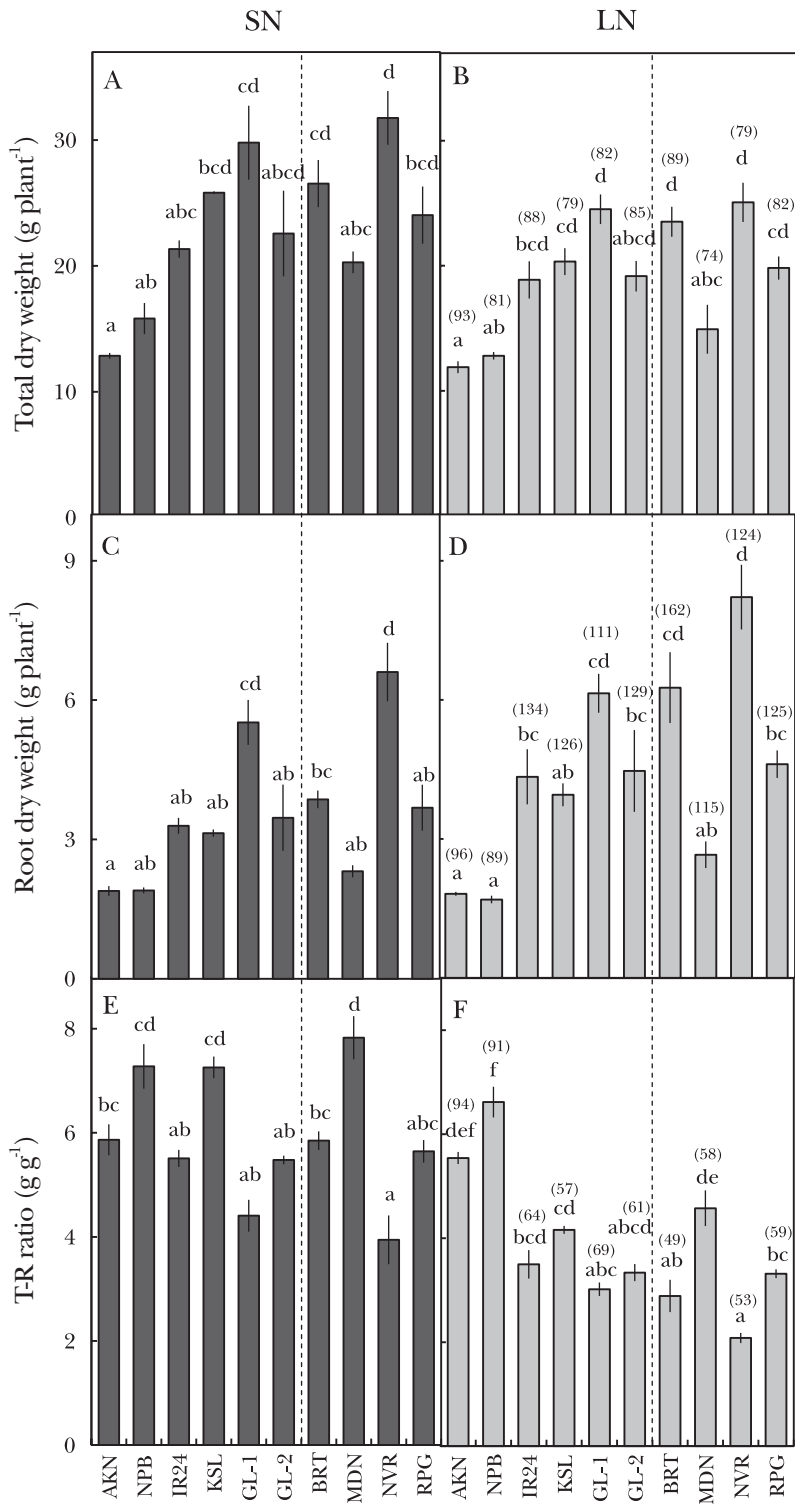


Fig. 1. Total dry weight, root dry weight and top-root ratio (T-R ratio) in cultivars and strains of the AA genome *Oryza* species grown hydroponically under the standard-N (SN; A, C and E) and the low-N (LN; B, D and F) conditions, measured at 4 wk after transplanting. Values are the means  $\pm$  S.E of 4 replications derived from different plants. Bars followed by the same letter represent no significant difference by the Tukey's test at 5% level. Values in parentheses indicate percentage of LN values to SN values.

condition from 74% (MDN) to 93% (AKN) (Fig. 1A, B). With regard to root dry weight in two *japonica* cultivars of *O. sativa*, AKN was almost unchanged under both N conditions, and NPB decreased under the LN condition (Fig. 1C, D). In other *Oryza* plants, however, root dry weight increased under the LN condition; the ratios of the values in LN to SN ranged from 111% (GL-1) to 162% (BRT). Total and root dry weights were heavier in the

*indica* cultivars, IR24 and KSL, than in the *japonica* cultivars under both N conditions. In all cultivars of *O. sativa* and *O. glaberrima*, GL-1 showed the greatest total and root dry weight.

In the wild *Oryza* species, there were also considerable variations in total and root dry weight (Fig. 1A-D). With regard to total dry weight (Fig. 1A, B), BRT, NVR, and RPG showed high values under both N conditions, which were

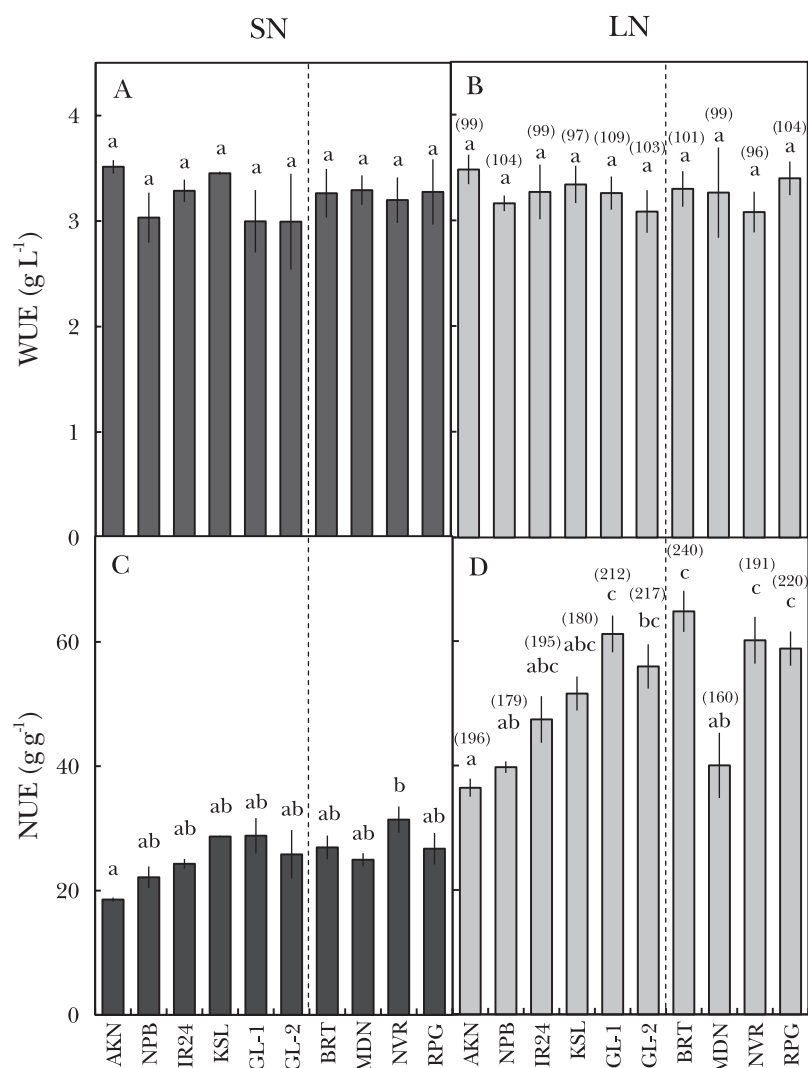


Fig. 2. Water use efficiency (WUE) and nitrogen use efficiency (NUE) in cultivars and strains of the AA genome *Oryza* species grown hydroponically under the standard-N (SN; A and C) and the low-N (LN; B and D) conditions measured at 4 wk after transplanting. Values are the means  $\pm$  S.E. of 4 replications derived from different plants. Bars followed by the same letter represent no significant difference by the Tukey's test at 5% level. Values in parentheses indicate percentage of LN values to SN values.

comparable to the cultivars of *O. sativa* and *O. glaberrima* with high values, but MDN showed lower values than other wild species. Under the SN condition, NVR showed heavier root dry weight than other wild *Oryza* species, which showed a somewhat heavier value than GL-1 (Fig. 1C). Under the LN condition also, the pattern of specific difference was similar to that found under the SN condition (Fig. 1D). NVR had also markedly high root dry weight. The root dry weight in NVR was about 3.5 and 4.8 times heavier than that in the two *japonica* cultivars under the SN and LN conditions, respectively.

The T-R ratio was lower in the plants grown under the LN condition than under the SN condition, and the percentage of the ratio of LN to SN condition ranged from 49% in BRT to 94% in AKN (Fig. 1E, F). Under the SN condition, NPB and KSL of *O. sativa*, and MDN showed higher T-R ratio than other cultivars and strains (Fig. 1E). Under the LN condition, the *japonica* cultivars, AKN and NPB, showed higher T-R ratio than others (Fig. 1F). As a result, T-R ratios in AKN and NPB were only slightly reduced under the LN condition (Fig. 1F). Under both SN

and LN conditions, NVR showed the lowest T-R ratios among all cultivars and strains (3.9 and 2.1; Fig. 1E, F).

## 2. WUE and NUE

WUE and NUE were determined by dividing the amounts of water and N absorbed by plants during 4 wk by the amount of culture solution and the total dry weight of plants, respectively, at 4 wk after transplanting (Fig. 2). The WUE ranged from 3.00 to 3.51 g L<sup>-1</sup> under the SN condition and from 3.08 to 3.48 g L<sup>-1</sup> under the LN condition (Fig. 2A, B). There was no significant difference in WUE among the cultivars and strains under each N condition (Fig. 2A, B). In addition, there was almost no difference in WUE between the two N conditions.

The NUE ranged from 18.6 to 31.4 g g<sup>-1</sup> under the SN condition and from 36.4 to 64.8 g g<sup>-1</sup> under the LN condition (Fig. 2C, D). In each cultivar and strain, the ratio of NUE in LN to that in SN ranged from 160% (MDN) to 240% (BRT). Under the SN condition, NVR showed a significantly higher NUE than AKN, and other cultivars and strains showed intermediate NUE (Fig. 2C). Under

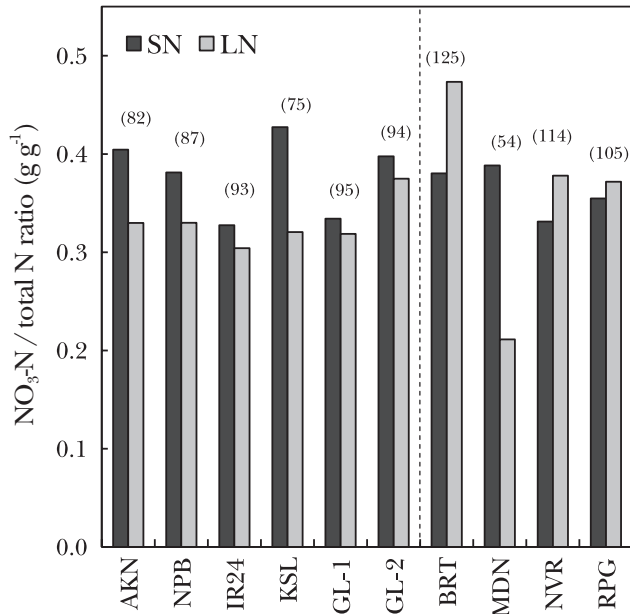


Fig. 3. The NO<sub>3</sub>-N to total N uptake ratio in cultivars and strains of the AA genome *Oryza* species grown hydroponically under the standard-N (SN) and the low-N (LN) conditions during 2 wk after transplanting. Values in parentheses indicate percentage of LN values to SN values.

the LN condition, GL-1 and the wild *Oryza* strains, BRT, NVR, and RPG, showed significantly higher NUE than the *japonica* cultivars and MDN, whereas other three cultivars, IR24, KSL, and GL-2, showed intermediate NUE (Fig. 2D).

### 3. NO<sub>3</sub>-N / total N uptake ratio

Fig. 3 shows NO<sub>3</sub>-N / total N uptake ratios in plants grown under the SN and LN conditions during 2 wk after transplanting. Under the SN condition, NO<sub>3</sub>-N / total N uptake ratios ranged from 0.33 to 0.43, showing some difference among the cultivars and strains. Under the LN condition, the ratios in AKN, NPB, and KSL of *O. sativa* and MDN decreased, whereas those in BRT and NVR increased. In other cultivars and strains, the ratios were almost the same under the two N conditions.

### 4. BR and RSA

BR in AKN, NPB, IR24, and RPG was not greatly influenced by the N conditions, whereas BR in other cultivars and strains decreased under the LN condition (Table 2). BR in NVR was significantly higher than that in AKN, NPB, IR24, and RPG under the SN condition and than that in AKN and IR24 under the LN condition. RSA in IR24, BRT, and NVR increased under the LN condition,

Table 2. Bleeding sap rate (BR), root surface area (RSA), BR per unit RSA in cultivars and strains of the AA genome *Oryza* species grown hydroponically under the standard-N (SN) and the low-N (LN) conditions, measured 4 wk after transplanting.

N condition	Cultivar and Strain	BR (g h <sup>-1</sup> plant <sup>-1</sup> )	RSA (cm <sup>2</sup> plant <sup>-1</sup> )	BR per unit RSA (g h <sup>-1</sup> m <sup>-2</sup> )
SN	AKN	0.79 ± 0.04 <sup>a</sup>	242 ± 10 <sup>a</sup>	32.8 ± 1.0 <sup>ab</sup>
	NPB	1.58 ± 0.43 <sup>ab</sup>	263 ± 10 <sup>a</sup>	40.6 ± 1.6 <sup>ab</sup>
	IR24	0.93 ± 0.08 <sup>ab</sup>	390 ± 33 <sup>ab</sup>	26.5 ± 3.5 <sup>a</sup>
	KSL	1.64 ± 0.24 <sup>abc</sup>	460 ± 38 <sup>ab</sup>	35.6 ± 4.4 <sup>ab</sup>
	GL-1	2.57 ± 0.34 <sup>bc</sup>	587 ± 42 <sup>bc</sup>	43.6 ± 5.1 <sup>ab</sup>
	GL-2	2.32 ± 0.44 <sup>abc</sup>	476 ± 65 <sup>abc</sup>	48.5 ± 5.0 <sup>ab</sup>
	BRT	2.57 ± 0.39 <sup>bc</sup>	420 ± 30 <sup>ab</sup>	62.9 ± 12.2 <sup>b</sup>
	MDN	1.55 ± 0.25 <sup>abc</sup>	302 ± 19 <sup>a</sup>	51.1 ± 7.1 <sup>ab</sup>
	NVR	2.93 ± 0.53 <sup>c</sup>	689 ± 72 <sup>c</sup>	43.5 ± 7.0 <sup>ab</sup>
	RPG	1.12 ± 0.32 <sup>ab</sup>	418 ± 49 <sup>ab</sup>	25.0 ± 3.9 <sup>a</sup>
LN	AKN	0.61 ± 0.02 <sup>a</sup>	205 ± 07 <sup>a</sup>	29.7 ± 0.3 <sup>bc</sup>
	NPB	0.83 ± 0.07 <sup>abc</sup>	213 ± 13 <sup>a</sup>	39.0 ± 3.1 <sup>c</sup>
	IR24	0.68 ± 0.08 <sup>ab</sup>	505 ± 46 <sup>bc</sup>	13.5 ± 1.6 <sup>a</sup>
	KSL	0.88 ± 0.18 <sup>abc</sup>	445 ± 36 <sup>bc</sup>	19.7 ± 3.3 <sup>ab</sup>
	GL-1	1.38 ± 0.19 <sup>bc</sup>	580 ± 28 <sup>c</sup>	23.5 ± 2.2 <sup>ab</sup>
	GL-2	0.95 ± 0.16 <sup>abc</sup>	492 ± 37 <sup>bc</sup>	19.1 ± 2.6 <sup>ab</sup>
	BRT	1.39 ± 0.11 <sup>bc</sup>	601 ± 53 <sup>c</sup>	23.3 ± 1.7 <sup>ab</sup>
	MDN	0.94 ± 0.12 <sup>abc</sup>	352 ± 34 <sup>ab</sup>	29.5 ± 2.9 <sup>bc</sup>
	NVR	1.64 ± 0.24 <sup>c</sup>	846 ± 52 <sup>d</sup>	18.3 ± 4.4 <sup>ab</sup>
	RPG	1.02 ± 0.08 <sup>abc</sup>	440 ± 23 <sup>bc</sup>	23.0 ± 0.8 <sup>ab</sup>

Values are the means ± S.E of 4 replications derived from different plants.

Values followed by the same letter represent no significant difference by the Tukey's test at 5% level. SN, standard-N; LN, low-N.

whereas RSA in other plants were almost unchanged (Table 2). RSA was also the largest in NVR under both N conditions, showing significantly larger value than in most cultivars and strains.

BR per unit RSA was calculated to evaluate physiological activity of roots (Table 2). Although BR per unit RSA in most cultivars and strains decreased under the LN condition, those in AKN, NPB, and RPG were unchanged. BRT showed a significantly higher value than IR24 and RPG under the SN condition. Under the LN condition, AKN, NPB, and MDN maintained relatively high values.

## Discussion

### 1. Dry matter production and NUE under different N conditions

Total and root dry weights of *O. sativa* were heavier in the *indica* type than in the *japonica* type under both N conditions (Fig. 1A-D). The cultivars of *O. glaberrima* showed similar or somewhat higher dry matter productivity than the *indica* type of *O. sativa*. As a result, the dry matter productivity in the cultivated species is in the following order: *japonica* type < *indica* type  $\leq$  *O. glaberrima*. The difference in root dry weight of the cultivated *Oryza* species was larger under the LN condition. Currently, there are many reports on the physiological difference between the subspecies of *O. sativa*. Namai et al. (2009) reported that the *indica* type has higher N sensitivity in the dry matter productivity than the *japonica* type. In addition, the *indica* type develops roots more extensively than the *japonica* type under the LN condition (Namai et al., 2009). Kubota et al. (1992) also showed that the dry matter productivity and N sensitivity at the vegetable stage were higher in *O. glaberrima* than in *O. sativa*. These traits of the cultivated *Oryza* species were also observed in this study (Fig. 1A-D).

In the AA genome *Oryza* species including wild species, BRT and NVR had a higher dry matter productivity and lower T-R ratio than other cultivars and strains (Fig. 1). Note that NVR had the highest dry matter productivity under both SN and LN conditions (Fig. 1A-D); NVR had 2.2 and 2.0 times heavier total dry weight than the *japonica* cultivars under the SN and LN conditions, respectively, whereas NVR grown under the LN condition had 4.8 and 1.8 times heavier root dry weight than the *japonica* and *indica* cultivars, respectively. The difference in root dry weight was reflected in that in RSA (Table 2). Under N-deficient condition, the expansion of root system is an important morphological trait for N acquisition from soil (Shi et al., 2010). Therefore, it seems that cultivars capable of high root development under LN conditions are favorable for growth under N deficiency. We carried out a similar experiment under the same N conditions in summer, 2010 and obtained results similar to those of the present study (data not shown). Thus, our data demonstrate that the response of dry matter productivity to N deficiency largely differs with the species or strain of AA genome *Oryza* species.

There was no significant difference in WUE among the AA genome *Oryza* species, and WUE was almost constant, regardless of the difference in N level (Fig. 2A, B). On the other hand, NUE increased under the LN condition as compared with the SN condition (Fig. 2C, D). Under the LN condition, NUE was higher in the *indica* cultivars and *O. glaberrima* than in the *japonica* cultivars, and the wild *Oryza* strains such as BRT, NVR and RPG showed much higher NUE than *O. sativa* cultivars. The difference in NUE among the cultivars and wild strains was positively correlated with those in root dry weight (Fig. 4A) and RSA (Fig. 4B) under the SN and LN conditions. This suggests

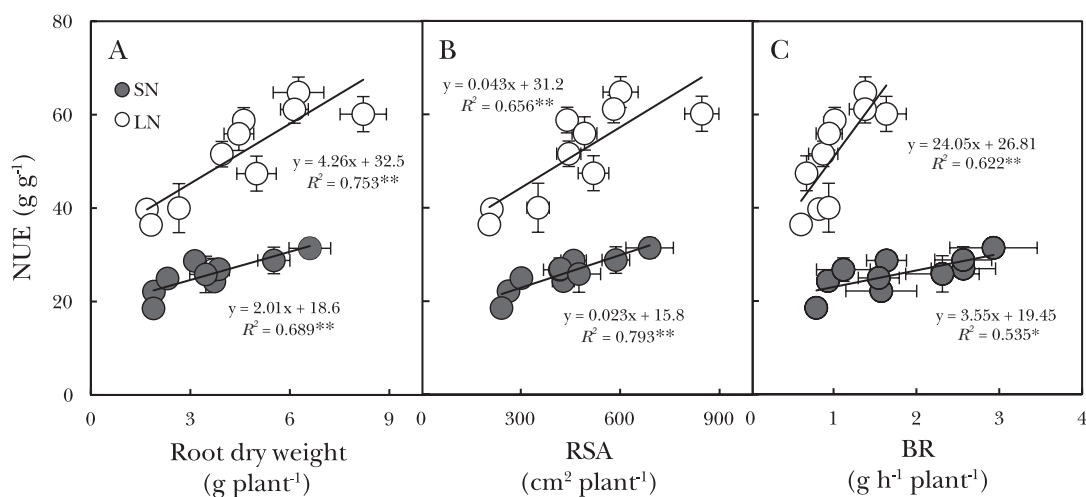


Fig. 4. Relationships between nitrogen use efficiency (NUE) and (A) root dry weight, (B) root surface area (RSA), and (C) bleeding sap rate (BR) in cultivars and strains of the AA genome *Oryza* species grown hydroponically under the standard-N (SN) and low-N (LN) conditions. \* and \*\* indicate a significant correlation at 5% and 1% level, respectively. Bars indicate standard error (S.E).

that the degree of root development and some physiological ability of N uptake, which is not directly related to water uptake traits, is concerned with the cultivar and specific difference found in NUE. It has been reported that a part of N is emitted as  $\text{NH}_3$  from leaves in some plants including rice (e.g., Kumagai et al., 2011). In this study, the  $\text{NH}_3$  emission was not included in the estimation of NUE. It will be necessary for more strict estimation of NUE to consider the effect of  $\text{NH}_3$  emission.

## 2. Change in $\text{NO}_3\text{-N}$ /total N uptake ratio under N-deficient condition

Song et al. (2011) reported that, when both  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  were applied, rice cultivars with high NUE developed more lateral roots and increased growth and N accumulation. They suggested that it was caused by enhanced synthesis of auxin induced by  $\text{NO}_3^-$ . Li et al. (2008) found that cultivars with high NUE showed higher activity of nitrification in rhizosphere than those with low NUE. Thus, it has been considered that the absorption and assimilation of  $\text{NO}_3\text{-N}$  as well as  $\text{NH}_4\text{-N}$  play an important role in the growth of rice plants.

In our study, some cultivars and strains exhausted N in culture solution at the end of the third and later weeks under the LN condition, because of their active absorption (data not shown). Thus, we calculated the  $\text{NO}_3\text{-N}$ /total N uptake ratio, an index of usability of  $\text{NO}_3\text{-N}$ , from the amounts of N uptake during 2 wk after transplanting. BRT and NVR having high NUE under the LN condition tended to increase  $\text{NO}_3\text{-N}$ /total N uptake ratio with the decrease in N level (Fig. 3). In contrast, AKN, NPB, KSL, and MDN having low NUE under the LN condition showed the opposite trend. These data suggest that they may change the usability of inorganic N source depending on the level of N application. However,  $\text{NO}_3\text{-N}$ /total N uptake ratios in GL-1 and RPG, which also showed high NUE under the LN condition, was almost the same under the SN and LN conditions. Therefore, it appears that plants with a high NUE under the LN condition increase or maintain the usability of  $\text{NO}_3\text{-N}$  under a low N environment. This needs to be investigated in relation to expression of nitrate transporter and N assimilatory enzymes (Shi et al., 2010; Hakeem et al., 2011).

## 3. BR and physiological activity of roots

In rice plant, BR has been reported to be involved in physiological traits of roots, such as respiratory rate (Yamaguchi et al., 1995) and N uptake rate (Samejima et al., 2004). Thus, BR is used as an index of physiological activity of roots for water and nutrient uptakes (Morita and Abe, 2002; Sakaigaichi et al., 2007). Under the LN condition, BR was unchanged in the 2 *japonica* cultivars (AKN and NPB), IR24, and RPG but decreased in other plants (Table 2). The difference in BR among the cultivars

and strains was positively correlated with that in NUE (Fig. 4C). On the other hand, there were positive correlations between BR and root dry weight under the SN and LN conditions ( $R^2 = 0.516^*$  and  $0.708^{**}$ , respectively), suggesting that plants having well developed root system exhibit higher BR. Thus, it seems that BR is also concerned with NUE through the development of root system.

We examined BR per unit RSA in the cultivars and strains to evaluate more strictly the physiological activity of roots (Table 2). There were no significant correlations between this value and NUE under both N conditions. On the other hand, BR per unit RSA in AKN, NPB and RPG was unchanged under both N conditions, but decreased under the LN condition in other plants. The root dry weight was also unchanged in the *japonica* cultivars, AKN and NPB under both N conditions, but tended to increase under the LN condition in other plants. This suggests that there are different patterns in the physiological and morphological responses of roots to low N: plants that cope with well development of roots rather than the enhancement of the physiological activity, and plants that are unchanged or maintain these traits. However, the N responses of RPG differed from such patterns. Under the LN condition, the cultivars and strains with high NUE, such as GL-1, BRT and NVR, showed low physiological activities of roots together with high development of root system. This respect is suggestive for understanding of the relationship between NUE and root traits under LN condition. In the measurement of dry weight and surface area of roots, we did not take the differences in the age and form of roots into consideration. It remains unknown whether these traits of roots affect the evaluation of physiological ability of roots.

## 4. Relationship between habitat and the morphological and physiological responses to N deficiency in the AA genome *Oryza* species

The AA genome *Oryza* species generally grow in sunny wet habitats in regions characterized by rainy and dry seasons, where water levels are seasonally changed (Vaughan et al., 2003). Such change in water environments is considered to have brought about the adaptive variation in *Oryza* species (Khush, 1997). Under flooded conditions such as ponds, the major form of N source absorbable by rice plants is  $\text{NH}_4\text{-N}$ . In soil environments with low water and fluctuating water levels, however,  $\text{NO}_3\text{-N}$  also becomes N source together with  $\text{NH}_4\text{-N}$ , because nitrification is promoted in the oxidized soil layer due to an increase in oxygen supply (Kirk and Kronzucker, 2005). In fact,  $\text{NO}_3\text{-N}$  uptake accounts for 34% of the total N uptake by rice plants in paddy field (Kirk and Kronzucker, 2005), which corresponds with the results of our study (Fig. 3). In our study also, the cultivars and strains with high NUE tended to increase or maintain the usability of  $\text{NO}_3\text{-N}$  under the



LN condition (Fig. 3). BRT and NVR, which have high dry matter productivity and NUE under the LN condition, are annual plants occurring in environments where the water level decreases in the dry season (Vaughan et al., 2003). Interestingly, these plants also increased the usability of  $\text{NO}_3\text{-N}$  under the LN condition. Therefore, the usability of  $\text{NO}_3\text{-N}$  also seems to be concerned in NUE, reflecting the environmental conditions of habitats.

The N responses of the root dry weight, NUE, and BR per unit RSA of the *japonica* type plant differed considerably from those of the *indica* type of *O. sativa*. On the other hand, the responses of the *indica* type were somewhat similar to those of BRT, NVR and *O. glaberrima* (Figs. 1, 2 and Table 2). Currently it is considered that the ancestor of *indica* type of *O. sativa* is *O. nivara* (Cheng et al., 2003) whereas that of *O. glaberrima* is *O. barthii* (Vaughan et al., 2008). Therefore, the N responses of root growth and related traits may represent the genetic traits that were inherited from the ancestral species to the cultivated species.

## 5. Conclusion

This study suggests that a high NUE under the LN condition is related to the development of root system, increased BR, and probably the change in the ability of  $\text{NO}_3\text{-N}$  uptake. In addition, this study demonstrated that wild rice strains such as BRT and NVR show higher dry matter production and NUE than *O. sativa* cultivars under low N condition. These strains had the superior morphological and physiological traits for N absorption under low N conditions and might become promising genetic resource for breeding of cultivars suitable for low N-input agriculture. Further studies would be required to evaluate the N response of photosynthetic traits and to characterize the physiological mechanism of N uptake in the AA genome *Oryza* species. It also remains unknown how the concentration of N and the composition of N-forms ( $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$ ) affect the N uptake traits of the *Oryza* species. Finally, detailed analyses including the growth analysis at various stages will be needed for the *Oryza* species grown in the field to clarify the N response at the population level.

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\* In Japanese with English abstract.