Physiological efficiency under different nitrogen nutrition conditions and relationship with photosynthetic N-use efficiency in Myanmar local rice varieties (Oryza sativa L.)

Win, Khin Thanda
Laboratory of Plant Breeding, Division of Bioresource and Bioenvironmental Sciences, Graduate School, Kyushu University

Mochizuki, Toshihiro
Laboratory of Agricultural Ecology, Division of Agricultural Ecology, Department of Plant Resources, Faculty of Agriculture, Kyushu University

Physiological efficiency under different nitrogen nutrition conditions and relationship with photosynthetic N–use efficiency in Myanmar local rice varieties (*Oryza sativa* L.)

Khin Thanda WIN[1] and Toshihiro MOCHIZUKI*

Laboratory of Agricultural Ecology, Division of Agricultural Ecology,
Department of Plant Resources, Faculty of Agriculture,
Kyushu University, Fukuoka 811–2307, Japan
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Rice genotypes with improved N–use efficiency are becoming a prerequisite for optimizing grain yield, reducing production cost, and developing environmentally friendly cropping systems. The physiological efficiency of grain (PE–grain) and biomass (PE–biomass), photosynthetic N–use efficiency (PNUE), and in vivo carboxylation efficiency of Rubisco (Pn/Rubisco) under varying N nutrition conditions were investigated in two Myanmar varieties (Bawgyi and Sawbwa), an *indica* variety (IR24), and a *japonica* control variety (Nipponbare). Nipponbare and IR24 had significantly higher grain yield and PE–grain at both soil N and standard N supply, Bawgyi exhibited moderately high grain yield and PE–grain, and Sawbwa had a low grain yield and PE–grain at both N levels. Nipponbare and IR24 had significantly higher PE–biomass at all N levels; while Bawgyi had lower PE–biomass than Nipponbare and IR24 at all N levels, its PE–biomass was significantly higher than that of Sawbwa at the low N level. Net photosynthesis (Pn) of Bawgyi was not significantly different from that of Nipponbare and IR24, but was higher than that of Sawbwa, even with no significant difference in leaf N content. Nipponbare, IR24, and Bawgyi showed no significant difference in PNUE, but had significantly higher PNUE than Sawbwa, especially at low and standard N levels. There was no significant difference in Pn/Rubisco among Bawgyi, Nipponbare, and IR24, but Pn/Rubisco was significantly higher in these varieties than in Sawbwa at low and standard N levels. PE–biomass corresponded well to PE–grain and showed significant positive correlations with PNUE and Pn/Rubisco, indicating that rice genotypes with high physiological efficiency have high Pn with low leaf N and low Rubisco content with high Rubisco efficiency. These results suggest that, of the two Myanmar rice varieties, Bawgyi is the more desirable genotype, with considerably higher physiological efficiency and high photosynthetic activity through the highly efficient use of Rubisco.

INTRODUCTION

Nitrogen (N) is usually the limiting nutrient in rice growth. Therefore, the development and identification of germplasm with high N–use and –uptake efficiency would minimize the use of ecologically and economically expensive chemical fertilizers and encourage sustainable agriculture. Field experiments have shown that genetic variability in N–use efficiency exists in rice (Broadbent *et al.*, 1987; De Datta and Broadbent, 1993); thus, it should be possible to improve N–use efficiency in rice through genotype selection. In Myanmar, N fertilizer is the most expensive component of rice production, accounting for approximately 35–45% of the total variable costs. However, individual farmers have little capital for expensive N fertilizers; thus, it is difficult for them to increase yield potentials. As a consequence, concerned research institutions should identify and develop N–efficient genotypes from the existing germplasm to form an alternative gene pool for genetic improvements in N–use efficiency.

Among the various agronomic indices used to assess N–use efficiency, physiological efficiency (PE) for grain or biomass production per unit N uptake is considered a key parameter in rice (De Datta and Broadbent, 1988; Cassman *et al.*, 1998). Furthermore, since PE integrates both N–uptake and –use efficiency, it may be the most comprehensive index of rice N–use efficiency. Moreover, Makino and Osmond (1991) found a highly positive correlation between photosynthetic capacity and N content in leaves, because the amount of N invested in chloroplasts accounts for 70–80% of leaf N content. In addition, Evans and Seemann (1989) and Makino and Osmond (1991) defined photosynthetic N–use efficiency (PNUE) as the rate of CO_2 assimilation per unit leaf N. Therefore, PNUE is an important expression for describing how efficiently plants use N to sustain growth and photosynthesis.

Ribulose–1, 5–bisphospate carboxylase/oxygenase (Rubisco, EC 4.11.39) is the key enzyme in photosynthetic fixation of CO_2 in green plants and is the dominant leaf protein (Ray *et al.*, 1983). In rice plants, this protein accounts for more than 50% of total soluble protein and over 25% of total leaf N (Makino *et al.*, 1984). Rubisco is also a rate–limiting factor for potential photosynthesis under present atmospheric conditions, and the specific activity of Rubisco controls the rate of carbon fixation (Makino *et al.*, 1992). Therefore, genetic modification to improve Rubisco efficiency in crops to enhance photosynthetic N–use efficiency would be of great agronomic importance.

The present study aimed to identify N–efficient

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[1] Laboratory of Plant Breeding, Division of Bioresource and Bioenvironmental Sciences, Graduate School, Kyushu University, Fukuoka 812–8581, Japan
* Corresponding author (E–mail: mochizuki@farm.kyushu–u.ac.jp)

Myanmar rice varieties for further genetic improvement in N–use efficiency and to clarify N–use efficiency at the whole–plant and the photosynthetic levels.

MATERIALS AND METHODS

Plant materials and growth conditions

To evaluate physiological efficiency for grain (PE–grain), a local Myanmar rice variety (Bawgyi) that is widely cultivated in major rice production areas, an *indica* variety (IR24) that is popular as an early maturing variety in irrigated summer rice areas in Myanmar, and a *japonica* control variety (Nipponbare) were grown individually in pots (Wagner's pots, 2×10^{-6} ha, Tokyo, Japan) filled with wetland paddy soil containing 2.2 g N kg^{-1} soil during the 2005 summer season at the University Farm, Kyushu University, Fukuoka, Japan. The plants were grown in a plastic greenhouse under natural sunlight. Two N treatments were implemented, 0N (soil N, 0 kg N ha^{-1}) and SN (standard N, 0.18 g N pot^{-1}), with four equal splits (basal, mid–tillering, 5–7 days before panicle initiation, and heading), and five replications for each N treatment. In addition to the basal N (NH_4NO_3), 0.21 g P_2O_5 pot^{-1} and 0.36 g K_2O pot^{-1} were applied as basal fertilization.

For another Myanmar rice variety, Sawbwa, 21–day–old seedlings were individually transplanted to a field at the Experimental Farm, Okinawa Prefectural Agricultural Experimental Station, Ishigaki Island, Okinawa, Japan, with a spacing of 30 cm$\times$15 cm in a 2.4×1.8 m^2 subplot (96 hills per subplot) during the 2005 summer season. N treatments, times of application, and basal application of P_2O_5 and K_2O were the same as in the pot experiment.

To assess physiological efficiency for biomass (PE–biomass) and N–use efficiency for potential photosynthesis, the above varieties were grown in a temperature–controlled greenhouse with a day/night temperature of 25/20 °C under natural sunlight during the 2005 winter season. Seven–day–old seedlings of four plants (one from each genotype) were transplanted to each container ($48\times38\times22$ cm) filled with 5 kg nutrient–free gravel as planting medium and 5 l Yoshida's nutrient solution (Yoshida *et al.*, 1976). The three N treatments were LN (low N, 1.4 mM NH_4NO_3), SN (standard N, 2.8 mM NH_4NO_3), and HN (high N, 5.6 mM NH_4NO_3) in Yoshida's standard nutrient solution with five replications. Nutrient solution was renewed weekly and pH was adjusted to 5.0–5.5 regularly during the culture period.

Determination of grain yield, total biomass, N uptake, and physiological efficiency for grain (PE–grain) and biomass (PE–biomass)

To evaluate grain yield and PE–grain, plants were harvested at physiological maturity and grain dry weight was determined at 14% moisture content. For the field experiment, six plants for each N treatment were individually harvested in six replications. Dry weight of leaves, and stem plus panicle rachis were determined separately after oven drying to constant weight at 70 °C for 3 days. For determination of total N content, first, com-posite samples of leaf, stem plus panicle rachis, and grain (caryopsis plus hull) were separately ground to a fine powder and then digested using the general Digesdahl digestion method with H_2SO_4–H_2O_2 at 440 °C. The digested samples were used for determination of total N content in a spectrophotometer (DR/2000, HACH, USA), following the total Kjeldahl (Nessler) method. Total N uptake was determined on the basis of total dry weight and total N content. PE–grain was determined from the following equation:

PE–grain = Grain yield / Total N uptake

To evaluate total biomass and PE–biomass, plants were sampled 43 days after transplanting and separated into leaves, stems (including sheaths), and roots. Total biomass was determined as the dry weight of leaves, stem, and roots after oven drying to a constant weight at 70 °C for 3 days. Total N content of these dried samples was determined as described above. Total N uptake was determined on the basis of total biomass and total N content. PE–biomass was determined using the following equation:

PE–biomass = Total biomass / Total N uptake

Measurement of net photosynthesis (Pn) and photosynthetic N–use efficiency (PNUE)

Single leaf photosynthesis was determined 1 week before sampling, on the uppermost fully expanded leaf, using a portable, open photosynthesis system with CO_2 control (CIRAS–1, PP Systems, Hitchin, Herts, UK) equipped with an automatic rice leaf cuvette. Measurements were made at a photosynthetic photon flux density (PPFD) of 1000 μmol quanta m^{-2} s^{-1}, a leaf temperature of 26$\pm$1 °C, and ambient CO_2 concentration of 350$\pm$2 μmol mol^{-1} with three replications for each genotype. Leaf N content was determined from the leaves used for gas exchange measurement as described above. PNUE was determined from the following equation:

PNUE = Pn / Leaf N content

Determination of Rubisco content (Rubisco) and in vivo carboxylation efficiency (Pn/Rubisco)

For determination of Rubisco, soluble protein was first determined from frozen leaf samples punched from leaves after gas exchange measurement, by spectrophotometry (UV–1200, Shimadzu, Japan) at 595 nm, using BSA (bovine serum albumin) as standard (Bradford, 1976). Rubisco subunits were separated from the soluble protein by SDS–PAGE (sodium dodecyl sulphate–polyacrylamide gel electrophoresis). After electrophoresis, the bands of Rubisco subunit polypeptides were treated according to Makino *et al.* (1985). The intensity of the band corresponding to the large subunit of Rubisco was determined with an image analyzer (Image J, version 1.36). The indirect estimate of in vivo carboxylation efficiency of Rubisco was evaluated using the following equation (Debabrata *et al.*, 2003):

In vivo Rubisco efficiency = Pn / Rubisco

Statistical analyses

Data were analyzed using the SPSS version 13.0 com-

puter package. N treatment effect (N) and interaction effect of genotype and N treatment (genotype × N) were calculated by two–way ANOVA (repeated measures design) using a GLM, while genotype effect was analyzed by one–way ANOVA. Mean comparisons were done with a post–hoc Tukey's HSD test.

RESULTS AND DISCUSSION

Grain yield, N uptake, and physiological efficiency for grain (PE–grain)

Grain yield and plant N uptake increased significantly with N supply in all genotypes (Table 1). Nipponbare showed significantly higher grain yield than the other genotypes at soil N level, but no significant difference from IR24 at standard N supply. Bawgyi showed no significant difference in grain yield from IR24 at soil N level and significantly higher yield than Sawbwa at both N levels. Nipponbare and Sawbwa showed significantly higher N uptake at soil N, and IR24 exhibited significantly higher N uptake at standard N supply, while Bawgyi had low N uptake at both N levels. Since grain yield is the integrated result of various factors related to the plant and

its environment, an increase in N accumulation might not always result in a proportional increase in grain yield. Thus, low grain yield with high N uptake in Sawbwa was apparently not limited by N availability and might be attributed to the inherent yield potential of this genotype.

PE–grain gave a contrary response to N supply, *i.e.*, high PE–grain was observed at low N supply (Table 1). We also detected genotypic variation in PE–grain among the tested varieties. Nipponbare had significantly higher PE–grain at both N levels; Bawgyi showed no significant difference in PE–grain at the standard N level, but was significantly lower at soil N level than IR24 and was significantly higher than Sawbwa at the soil N level. These results suggest that, among the Myanmar rice varieties, Bawgyi is a more efficient genotype for N uptake, with a moderately high grain yield and PE–grain, especially at soil N level.

Total biomass, N uptake, and physiological efficiency for biomass (PE–biomass)

Total biomass accumulation in all varieties increased with increasing N supply, although the incremental rate

Table 1. Grain yield and physiological efficiency (PE–grain) of rice varieties under different N nutrition

Genotype	Grain yield (g plant^{-1})		N uptake (mg plant^{-1})		PE–grain (g grain g^{-1} N uptake)	
	0N	SN	0N	SN	0N	SN
Nipponbare	8.7a	11.9a	103.8a	176.7ab	78a	61a
IR24	6.1ab	10.8a	76.5b	220.0a	79a	44b
Bawgyi	6.3ab	7.4ab	88.0b	164.7b	64ab	40b
Sawbwa	4.2b	5.8b	124.2a	188.8ab	41b	38b
P (genotype)	**	**	**	**	**	**
P (N)	***		***		***	
P (genotype×N)	**		***		***	

Mean values within each column followed by the same letter are not significantly different at the 5% level by Tukey's HSD test. Significance levels are *, *P*<0.05; **, *P*<0.01; ***, *P*<0.001; ns, not significant (*P*>0.05). 0N, soil N; SN, standard N. The dotted line represents that the data for Sawbwa come from the different experiment.

Table 2. Total biomass accumulation, N uptake, and physiological efficiency (PE–biomass) of rice varieties under different N nutrition

Genotype	Total biomass (g plant^{-1})			N uptake (mg plant^{-1})			PE–biomass (g biomass g^{-1} N uptake)		
	LN	SN	HN	LN	SN	HN	LN	SN	HN
Nipponbare	3.38a	8.95b	13.68a	35.3a	95.4b	178.0a	96a	94b	77a
IR24	2.47a	8.48b	9.42b	25.3a	85.9b	125.7b	97a	99a	75ab
Bawgyi	2.71a	6.50b	11.70ab	30.8a	77.3b	157.8b	88b	84c	74b
Sawbwa	3.47a	11.92a	14.88a	42.0a	141.8a	200.0a	83c	84c	74b
P (genotype)	ns	***	**	ns	***	*	**	***	**
P (N)	***			***			***		
P (genotype×N)	*			*			**		

Mean values and significance levels are as described in the legend of Table 1. LN, low N; SN, standard N; HN, high N.

was considerably slower from SN to HN relative to that from LN to SN (Table 2). Sawbwa had the highest biomass accumulation at all rates of N supply, while Bawgyi was not significantly different in biomass accumulation from Nipponbare and IR24.

Plant N uptake in all genotypes showed similar responsiveness to N supply in terms of total biomass (Table 2). Genotypic variation in N uptake was only observed in SN and HN, but not in LN. Sawbwa exhibited the highest N uptake at all N levels, although N concentration was not significantly different from those of the other varieties (data not shown). This might be attributed to Sawbwa's considerably higher biomass accumulation. There was no significant difference in N uptake between Bawgyi and Nipponbare and IR24. In fact, less than 50% of the applied fertilizer N was taken up by the plants (Craswell and Godwin, 1984; Strong, 1995), and the remainder was lost to the off–farm environment, contributing to another pernicious global environmental challenge. Therefore, high N uptake efficiency in Sawbwa would be a desirable trait from an ecological standpoint to minimize leakage of applied N into the environment. Nipponbare and IR24 had significantly higher PE–biomass at all N levels, while Bawgyi had relatively lower PE–biomass than Nipponbare and IR24 at all N levels, but was significantly higher than Sawbwa only at the low N level.

Nipponbare and IR24 consistently showed higher PE for both biomass and grain yield, while Bawgyi had significantly higher PE for both biomass and grain than Sawbwa at the low N level. High biomass accumulation by Sawbwa at standard and high N levels might contribute to reasonable PE–biomass at these N levels. From these results, PE–biomass corresponded well to PE–grain and, among the two Myanmar varieties, Bawgyi was found to be a N–efficient genotype with a considerably higher PE for both biomass and grain yield, especially at the low N level.

Net photosynthesis (Pn) and photosynthetic N–use efficiency (PNUE)

A strong correlation has been observed between the light–saturated rate of photosynthesis of a single leaf and its N content (Field and Mooney, 1986; Reich *et al.*, 1994). The same result was observed in this study: Pn of young, fully expanded leaves increased linearly with leaf N content in all genotypes (Table 3). Bawgyi did not differ significantly in Pn from Nipponbare and IR24 at all N levels; whereas Sawbwa had the lowest Pn at all N levels, even though there was no significant difference in leaf N content among the genotypes. These results confirm that a high rate of photosynthesis with low leaf N content is a desirable physiological trait for improving N–use efficiency of rice.

PNUE varied with genotype and with N nutrition (Table 3), decreasing with increased N supply and leaf N

Table 3. Genotypic variability in leaf N content, net photosynthesis (Pn) and photosynthetic N–use efficiency (PNUE) of rice varieties under different N nutrition

Genotype	Leaf N ($g\ m^{-2}$)			Pn ($\mu mol\ m^{-2}\ s^{-1}$)			PNUE ($\mu mol\ CO_2\ g^{-1}\ N\ s^{-1}$)		
	LN	SN	HN	LN	SN	HN	LN	SN	HN
Nipponbare	0.68a	0.92a	1.15a	11.6a	12.1a	14.4a	16.9a	13.1a	12.5a
IR24	0.74a	0.96a	1.26a	11.3a	11.9a	13.5ab	15.3a	12.3a	10.7b
Bawgyi	0.72a	0.96a	1.18a	10.6a	11.2a	12.8ab	14.7a	11.8a	10.9b
Sawbwa	0.85a	1.01a	1.10a	9.0a	9.5b	11.9b	10.6b	9.4b	10.8b
P (genotype)	ns	ns	ns	ns	*	*	**	*	*
P (N)	***			***			***		
P (genotype×N)	*			**			**		

Mean values, significance levels, and abbreviations are as described in the legends of Tables 1 and 2.

Table 4. Genotypic variability in soluble protein content, Rubisco content and in vivo carboxylation efficiency of rice varieties under different N nutrition

Genotype	Soluble protein ($g\ m^{-2}$)			Rubisco ($g\ m^{-2}$)			Pn/Rubisco ($\mu mol\ CO_2\ g^{-1}\ Rubisco\ s^{-1}$)		
	LN	SN	HN	LN	SN	HN	LN	SN	HN
Nipponbare	4.74a	5.48a	5.81a	1.60b	2.60b	3.08b	7.2a	4.6a	4.7a
IR24	3.17b	5.35a	6.02a	1.74b	3.16a	3.85a	6.5a	3.8a	3.5b
Bawgyi	4.03a	4.73a	6.23a	1.85ab	2.70b	4.05a	5.7a	4.2a	3.2b
Sawbwa	4.67a	5.26a	6.15a	2.12a	3.08a	3.90a	4.3b	3.1b	3.0b
P (genotype)	**	ns	ns	*	*	*	*	*	*
P (N)	***			***			**		
P (genotype×N)	ns			*			*		

Mean values, significance levels, and abbreviations are as described in the legends of Tables 1 and 2.

content in all genotypes, except in Sawbwa at the high N level. This last response to N supply was also observed in rice by Ligeng *et al.* (2005). There was no significant difference between PNUE of Bawgyi and Nipponbare at low and standard N levels or IR24 at all N levels, but the PNUE of Bawgyi was significantly higher than that of Sawbwa at low and standard N levels, although there was no significant difference in leaf N content. From these results, Bawgyi appears to be the desirable genotype, as its N–use efficiency at the photosynthetic level is as high as Nipponbare and IR24.

Rubisco content (Rubisco) and in vivo carboxylation efficiency (Pn/Rubisco)

Because a large fraction of leaf N is invested in the photosynthetic apparatus, and this fraction is generally constant (Evans and Seemann, 1989), leaf N reflects the amount of photosynthetic proteins. Both soluble protein and Rubisco content increased with leaf N content because of the increasing N supply (Table 4). Genotypic variation in soluble protein content was only observed at the low N level. Sawbwa had the highest Rubisco content at all N levels, while Nipponbare had the lowest; there was no significant difference in soluble protein content. This might be due to genotypic differences in investment of total soluble protein in Rubisco protein. Rubisco content per unit leaf area is a rate–limiting factor for photosynthesis under adequate light conditions (Makino *et al.*, 1983). Evans (1989) and Makino *et al.* (1992) reported that not only Rubisco content, but also its specific activity, controls the rate of carbon fixation. Therefore, a high Rubisco content will not necessarily result high Pn. Nipponbare showed a higher Pn than the other genotypes, but a lower Rubisco content, while Sawbwa had a significantly lower Pn and a higher Rubisco content.

In this study, we did not measure specific activity of fully activated Rubisco; only the Pn to Rubisco content (Pn/Rubisco) was calculated as an indirect estimate of in vivo carboxylation efficiency (Debabrata *et al.*, 2003). Nipponbare had consistently higher Pn/Rubisco at all N

levels with a lower Rubisco content. In contrast, Pn/ Rubisco of IR24 and Bawgyi did not differ significantly from that of Nipponbare, but was significantly higher than that of Sawbwa at low and standard N levels, even though Bawgyi had a lower Rubisco content than Sawbwa. Genotypes having high carboxylation efficiency with low Rubisco content should have high productivity under low N input conditions.

A significant inverse relationship was observed between Rubisco content and its in vivo carboxylation efficiency (data not shown). This suggests that genotypes with higher Rubisco efficiency could economize on allocation of leaf N to Rubisco without an appreciable reduction in Pn. This savings in N may have a large effect on N partitioning into other components that limit photosynthesis and could therefore substantially increase N–use efficiency.

Relationship between PE–biomass, PNUE, and Pn/ Rubisco

Physiological efficiency (PE) is a measure of the ability of a plant to produce grain and biomass with N acquired at the whole–plant level. Similarly, Pn and PNUE indicate the ability and efficiency, respectively, of plant biomass production relative to N at the single leaf level. Wu and Tao (1995) stated that PE in rice genotypes reflects an association with photosynthetic features: rice genotypes with a higher PE have higher photosynthetic N–use efficiency. In the present study, a significant ($P<0.01$) positive correlation between PE–biomass and PNUE was observed (Fig. 1A). However, PE–biomass was not related to Pn of young, fully expanded leaves.

In vivo carboxylation efficiency of Rubisco (Pn/ Rubisco) was also positively ($P<0.001$) correlated with PE–biomass (Fig. 1B). This is in agreement with the results of Debabrata *et al.* (2003), who found that higher PE was associated with higher Pn at lower Rubisco content. Since both PNUE and Pn/Rubisco were significantly correlated with PE–biomass, rice genotypes with
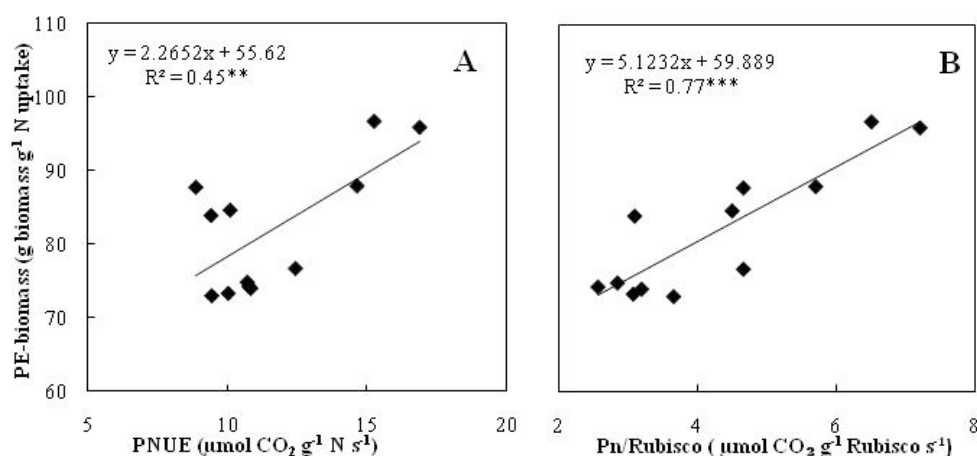

Fig. 1. Relationship between physiological efficiency and photosynthetic N–use efficiency (A), and in vivo carboxylation efficiency of Rubisco (B) in young, fully expanded leaves of rice varieties. Significance levels are **, $P<0.01$; ***, $P<0.001$.

high PE–biomass should have a high Pn with a low leaf N content and low Rubisco content with high efficiency of the enzyme.   Genotypes with higher PE–biomass (Nipponbare, IR24, and Bawgyi) achieved higher PNUE and Pn/Rubisco, especially at low and standard N levels. Therefore, among the Myanmar varieties, high PE in Bawgyi can be attributed to efficient Pn activity with high efficiency of Rubisco. In addition, PE–biomass at the vegetative stage corresponded to PE–gain. Therefore, assessment of PE–biomass could be used as a rapid screening method to evaluate N–efficient genotypes in the existing germplasm.

Generally, local Myanmar rice varieties were less responsive to N fertilizer and had lower N–use efficiency than modern improved varieties at an equivalent N uptake. However, we also found that some local varieties have high N–use efficiencies, both at the photosynthetic and whole–plant levels, that match the high–yielding varieties. Such local varieties would be appropriate not only for low N input conditions with unfavourable environments, but also for high–yield potential environments. Of the two tested Myanmar rice varieties, Bawgyi showed higher PE, PNUE, and Pn/Rubisco. Such a genotype would be ecologically and physiologically desirable for maximizing photosynthetic carbon fixation and productivity with efficient use of applied N. Because of the large variation among rice genotypes (Broadbent *et al.*, 1987; De Datta and Broadbent, 1990), germplasm improvement is commonly regarded as an ideal and cheap measure to improve PE in rice (Ligeng *et al.*, 2005). The present study is a first attempt to improve N–use efficiency of local Myanmar rice varieties and provides useful information for the development of locally adapted, N–efficient genotypes and further genetic improvement in N–use efficiency. This study also demonstrates a rapid screening method for evaluating PE at the vegetative stage.

REFERENCES

Bradford, M. M.   1976   A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Anal. Biochem.* **72**: 248–254

Broadbent, F. E., S. K. De Datta and E. V. Laureles   1987   Measurement of nitrogen utilization efficiency in rice genotypes. *Agron. J.* **79**: 786–791

Cassman, K. G., S. Peng, D. C. Olk, J. K. Ladha, W. Reichardt, A. Dobermann and U. Singh   1998   Opportunities for increased nitrogen–use efficiency from improved management in irrigated rice systems. *Field Crops Res.* **56**: 7–39

Craswell, E. T. and D. C. Godwin   1984   The efficiency of nitrogen fertilizers applied to cereals in different climates. *In* "Advances in Plant nutrition", Volume 1, ed. by P. B. Tinker and A. Lauchli, Praeger Publishers, New York, pp. 1–55

De Datta, S. K. and F. E. Broadbent   1988   Methodology for evaluating nitrogen utilization efficiency by rice genotypes. *Agron. J.* **80**: 793–798

De Datta, S. K. and F. E. Broadbent   1990   Nitrogen–use efficiency of 24 rice genotypes on an N–deficient soil. *Field Crops Res.* **23**: 81–92

De Datta, S. K. and F. E. Broadbent   1993   Development changes related to nitrogen–use efficiency in rice. *Field Crop Res.* **34**: 47–56

Debabrata, R., M. S. Sheshshayee, K. Mukhopadhyay, H. Bindumadhava, T. G. Prasad and K. M. Udaya   2003   High nitrogen use efficiency in rice genotypes is associated with higher net photosynthetic rate at lower Rubisco content. *Biol. Plant* **46**: 251–256

Evans, J. R.   1989   Photosynthesis and nitrogen relationships in leaves of C_3 plants, *Oecologia* **78**: 9–19

Evans, J. R. and J. R. Seemann   1989   The allocation of protein nitrogen in the photosynthetic apparatus: costs, consequences and control. *In* "Photosynthesis", ed. by W. R. Briggs, Alan R. Liss Press, New York, pp. 281–304

Field, C. and H. A. Mooney   1986   The photosynthesis–nitrogen relationship in wild plants. *In* "On the economy of plant form and function", ed. by T. J. Givnish, Cambridge Univ. Press, Cambridge, pp. 25–55

Ligeng J., D. Dong, X. Gan and S. Wei   2005   Photosynthetic efficiency and nitrogen distribution under different nitrogen management, and relationship with physiological N–use efficiency in three rice genotypes. *Plant and Soil* **271**: 321–328

Makino, A. and B. Osmond   1991   Effects of nitrogen nutrition on nitrogen partitioning between chloroplasts and mitochondria in pea and wheat. *Plant Physiol.* **96**: 355–362

Makino, A., T. Mae and K. Ohara   1983   Photosynthesis and ribulose–1,5–bisphosphate carboxylase in rice leaves.   *Plant Physiol.* **73**: 1002–1007

Makino, A., T. Mae and K. Ohira   1985   Photosynthesis and rubulose–1,5–bisphosphate carboxylase/oxygenase in rice leaves from emergence through senescence. Quantitative analysis by carboxylation/oxygenation and regeneration of ribulose–1,5–bisphosphate. *Planta* **166**: 414–420

Makino, A., T. Mae and K. Ohiro   1984   Relation between nitrogen and ribulose–1,5–bisphoshate carboxylase in rice leaves from emergence to senescence. *Plant Cell Physiol.* **25**: 429–437

Makino, A., H. Sakashita, J. Hidema, J. Mae, K. Ojima and B. Osmond   1992   Distinctive response of ribulose–1,5–bisphosphate carboxylase and carbonic anhydrase in wheat leaves to nitrogen nutrition and their possible relationships to CO_2 transfer resistance. *Plant Physiol.* **100**: 1737–1743

Ray, S., W. A. Mondal and M. A. Choudhuri   1983   Regulation of leaf senescence, grain filling and yield of rice by kinetin and abscisic acid. *Plant Physiol.* **59**: 343–346

Reich, P. B., M. B. Walters, D. S. Ellsworth and C. Uhl   1994   Photosynthesis–nitrogen relations in Amazonian tree species. I. Patterns among species and communities. *Oecologia* **97**: 62–72

Strong, W. M.   1995   Nitrogen fertilization of upland crops. *In* "Nitrogen Fertilization in the Environment", ed. by P. E. Bacon, Marcel Dekker Press, New York, pp. 129–169

Wu, P. and Q. N. Tao   1995   Genotypic response and selection pressure on nitrogen use efficiency in rice under different nitrogen regimes. *J. Plant Nutr.* **18**: 487–500

Yoshida, S., D. A. Forna, J. A. Cock and K. A. Gomez   1976   Laboratory Manual for Physiological Studies of Rice. International Rice Research Institute Press, Manila (Philippines), p. 62