Photosynthetic characteristics in *Oryza* species

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Abstract

Photosynthetic rate (P_N), SPAD value, specific leaf area (SLA), flag leaf area (FLA), and nitrogen content (LN) of genus *Oryza* were investigated and their correlation was analyzed to assess some of the main photosynthetic traits among different species in the genus *Oryza*. The results revealed wide variation in these traits. The species *O. rufipogon* and *O. australiensis* exhibited maximum photosynthetic rate. Comparison of different types of genomes (diploid: 2n=2x=24; tetraploid: 2n=4x=48) and growth habit (shade- or sun-grown) showed the species of diploid (with genome symbol EE; 2n=2x=24) genomes, with perennial and sun-grown species, had high apparent photosynthesis compared to others. The species with BB/BBCC, shade-grown and the tetraploids showed high SPAD value, and the flag leaf in sun-grown species and diploids were thicker (low SLA) compared with others. However, no significant difference could be noticed among the different types of genomes. Higher leaf area was noticed among the species of CC/CCDD genome, perennial shade-grown species and tetraploids than in others. The variety IR 36 exhibited highest leaf nitrogen concentration. Correlation analysis showed a strong relationship between P_N and leaf nitrogen concentration while no marked relationships were observed among other characteristics. It implies that the species with thick and small leaves with high nitrogen concentration and high photosynthesis evolved better than others. *O. rufipogon*, with the same genome as *O. sativa*, could be one of the wild rice resources for elite crop improvement.

Additional key words: flag leaf area; leaf N concentration; net photosynthetic rate; SPAD meter reading; specific leaf area.

Introduction

In order to break the yield plateau in rice, an increase in photosynthetic rate is an essential option (Peng 2000). Apart from C_4 transgenic rice, the other way is the use of high-photosynthesis genetic resources in the genus *Oryza*. Our previous research efforts showed the feasibility to select very high-photosynthetic-rate lines from interspecies-cross of cultivated and wild rice which were close to those of maize (Zhao *et al.* 2003).

It is known that wild rice is a rich source of agronomic traits, insect and disease resistance and increased biomass accumulation rate (Vaughan 1989, Sitch 1990). At the molecular level, isozymes for example, have clearly shown that cultivated rice was less diverse than their annual relatives, which in turn were found less diverse than their perennial relatives (Oka *et al.* 1988, Second 1985). Yeo *et al.* (1994) reported that unimproved wild germplasm of a number of species had high light-saturated assimilation rate in ambient atmosphere, more than the cultivars and elite breeding material of *O. sativa*. Quite a large range of phospho*enol*pyruvate carboxylase activity was reported in a number of species having several times higher than in many of the C_3 species (Yeo *et al.* 1994). These results indicated a wide diversity of photosynthetic characteristics in genus *Oryza* that could be exploited for further improvement of modern varieties with a potential to break the existing yield plateau in rice.

The wild species of the genus *Oryza* have been extensively evaluated in terms of photosynthetic capacity, photosynthetic rate, phospho*enol*pyruvate carboxylase, photorespiration, and absolute quantum yield (Yeo *et al.* 1994). In this paper, the authors have attempted to assess several *Oryza* species for some of the main photosynthetic traits such as P_N, SPAD value, SLA, FLA, and LN.

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Abbreviations: Chl – chlorophyll; FLA – flag leaf area; LN – nitrogen concentration in flag leaves; P_N – net photosynthetic rate; PAR – photosynthetically active radiation; SLA – specific leaf area.

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Materials and methods

Plants: Twenty-five plants, derived from 15 species, were used in the present study. The seeds were taken from Gene Bank of International Rice Research Institute (IRRI), Manila, the Philippines. The genome types and their growth characteristics are presented in Table 1. The seed material was incubated for 98 h at 50°C to break dormancy; then the seeds were dehusked, treated with fungicide (Benlate), and germinated on wet filter paper in Petri dishes at 27°C. Ten days after germination, the seedlings were transplanted into 75-mm pots filled with loam soil in a greenhouse (NG-01 in IRRI). After 4 weeks, the seedlings were transplanted into large greenhouse tanks (3.5 × 7.8 × 2.0 m width, length, and depth, respectively) filled with loam (field loam fine soil:sand, 2:1) in October 2000. Each line composed of 10 seedlings. The plants were grown under optimized conditions. The mean length of the day was about 11.5 h, with the maximal PAR of about 1,500 μmol m^{-2} s^{-1} and mean temperature 25–27°C.

P_N: Leaf photosynthesis was measured on flag leaves from 9:00 to 11:00 h on clear days using the *Licor-6400* (*Licor Inc*., Lincoln, USA) photosynthesis measuring system during the flowering stage (within five days of heading, because of genotypic difference at flowering time, measurements were time-relative to phenology of each line). The photosynthetically active radiation (PAR), leaf temperature, and CO_2 concentration were about 1,200 μmol m^{-2} s^{-1}, 30°C, and 350 ppm, respectively. P_N was measured and replicated thrice.

SPAD, FLA, and SLA: SPAD values were taken along with SLA and FLA of the above mentioned species of the genus *Oryza* after photosynthesis measurements. Three flag leaves were used to study these parameters. SPAD value was measured with SPAD meter (*SPAD-502, Konica Minolta Sensing, Inc.*, Osaka, Japan) with 10 measurements per flag leaf. FLA was determined with *Li-Cor* leaf area meter *LI-3000C* (*LI-COR Inc*., Lincoln, USA). Flag-leaf dry mass was assessed after at least 10 h in oven kept at 80°C. SLA was calculated as FLA per unit dry mass.

Table 1. Genome type and growth habits of the *Oryza* species. Note: where A/P was annual or perennial, D/T was diploid or tetraploid, S/Sh was sun or shade-grown habit.

Species	Genome type	T/D	Growth habit A/P	S/Sh
O. alta	CCDD	T(2n=48)	P	Sh
O. australiensis	EE	D(2n=24)	P	S
O. barthii	AA	D(2n=24)	A	S
O. eichingeri	CC	D(2n=24)	A	S
O. glaberrima	AA	D(2n=24)	A	S
O. glumaepatula	AA	D(2n=24)	P	S
O. grandiglumis	CCDD	T(2n=48)	P	Sh
O. latifolia	CCDD	T(2n=48)	P	Sh
O. longistaminata	AA	D(2n=24)	P	S
O. meridionalis	AA	D(2n=24)	A/P	S
O. minuta	BBCC	T(2n=48)	P	S
O. officinalis	CC	D(2n=24)	P	Sh
O. punctata	BB	D(2n=24)	A	Sh
O. rufipogon	AA	D(2n=24)	P	S
O. sativa	AA	D(2n=24)	A	S

LN: The three leaves used for measuring photosynthesis were analyzed for LN following Scheiner (1976).

Statistical methods: Data were analyzed with *MS Excel for Windows*. Multiple comparisons of the parameters between lines were conducted with LSD method.

Results

***P_N* and SPAD**: Wide variability was noticed in photosynthetic rate (P_N) and SPAD value (Fig. 1). The range of P_N varied from 14.3 μmol m^{-2} s^{-1} in *O. glumaepatula* (Acc. No. 100971) to 37.6 μmol m^{-2} s^{-1} in *O. rufipogon* (Acc. No. 105697), that was about 2.5 times more than in the former during flowing stage under saturating light (PAR 1,200 μmol m^{-2} s^{-1}) conditions. Both *O. rufipogon* and *O. australiensis* exhibited high P_N compared with *O. sativa* and others. The range of SPAD value varied from 27.4 (*O. alta,* Acc. No.1056875) to 47.4 (*O. longistaminata,* Acc. No.101741). These results showed that some wild species like *O. rufipogon* and *O. australiensis* could be donors of high photosynthesis for improving of the modern rice.

SLA and FLA: There were also wide ranges in SLA and FLA among different species (Fig. 1). The range of SLA was from 10.1 cm^2 g^{-1} (*O. sativa,* Azucena) to 29.1 cm^2 g^{-1} (*O. meridionalis,* Acc no. 101148) and FLA was from 17.1 cm^2 (*O. rufipogon,* Rampur 6) to 125.1 cm^2 (*O. grandiglumis***,** Acc. no. 105671). The maximum value was about 3 or 8 times higher than the minimum one in SLA and FLA, respectively. Both *O. rufipogon* and *O. australiensis* with high-photosynthesis resource had middle or low SLA and FLA among all species tested in this research.

The correlation of *P_N*, SPAD, SLA, and FLA: There were no close correlations between P_N, SPAD, SLA, and FLA. It was shown that the photosynthetic rate did not depend on the chlorophyll (Chl) concentration, thickness, and size of leaves. These items of leaves were independent; therefore, they formed the diversity of photosynthetic characteristics in genus *Oryza*. In other words,

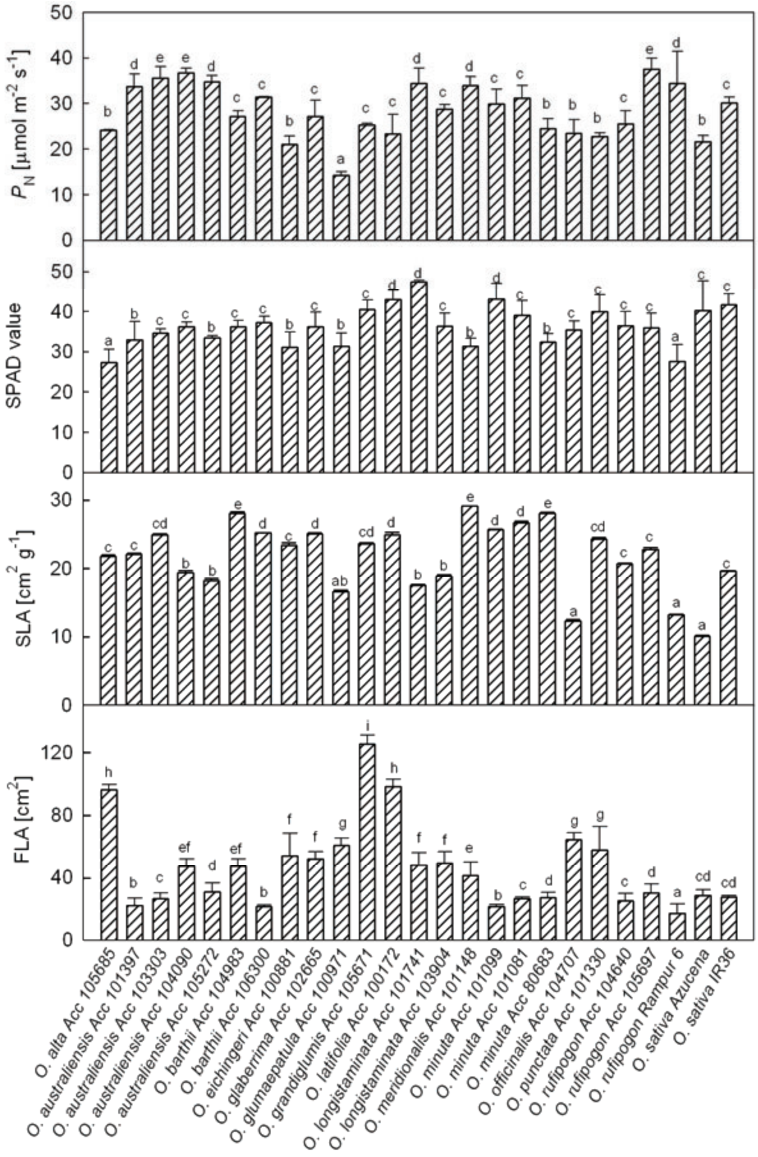

Fig. 1. Photosynthetic rate (P_N), SPAD values, specific leaf area (SLA) and flag leaf area (FLA) in genus *Oryza.* The column means the average of the parameters in each line, and the error bars show the standard deviation of three replicates. The letters on the top of the column means the multiple comparisons by SLD method. *Error bars* are +SE.

good resources of high photosynthesis were not certainly high Chl concentration and thick leaves. It also implies that the Chl concentration and thickness might not be the main factors related to photosynthesis and they could not be used to select high-photosynthesis in genus *Oryza*.

P_N, SPAD, SLA, and FLA of different genomes: There were large differences in P_N, SPAD, SLA, and FLA among genomes (Fig. 2). Generally speaking, P_N of EE genome was high (35.2±1.2 µmol m^{-2} s^{-1}), SPAD of BB or BBCC was high (40.7±1.7), and FLA of CC or CCDD was high (87.6±28.7 cm^2) compared with other genomes. However, there were no significant differences in SLA among genomes. *O. rufipogon* with high photosynthesis (with the average of 32.5±6.2 µmol m^{-2} s^{-1} and the maximum of 37.6±2.3 µmol m^{-2} s^{-1}) belonged to AA genome, which is the same with *O. sativa.* We suggested that AA genome wild resources with good photosynthetic characteristics might be most benefiting for improving modern rice variety photosynthesis to break the yield barrier.

Comparison of P_N, SPAD, SLA, and FLA in species with different growth characteristics and chromosome number: Differences in P_N, SPAD, SLA, and FLA among the annual and perennial species, sun- and shade-grown plants and between diploid and tetraploid species were noticed (Fig. 3). The photosynthetic rates of perennial, sun-growing and diploid species were higher than those in annual, shade-grown and tetraploid species, respectively. There was no significant difference in SPAD among these types and by their chromosome number. FLA of perennials, shade-grown plants, and tetraploids was larger than annual, sun-grown and diploid species. These results imply that photosynthetic characteristics also were affected by original growth habitat and chromosome number of genome in *Oryza*.

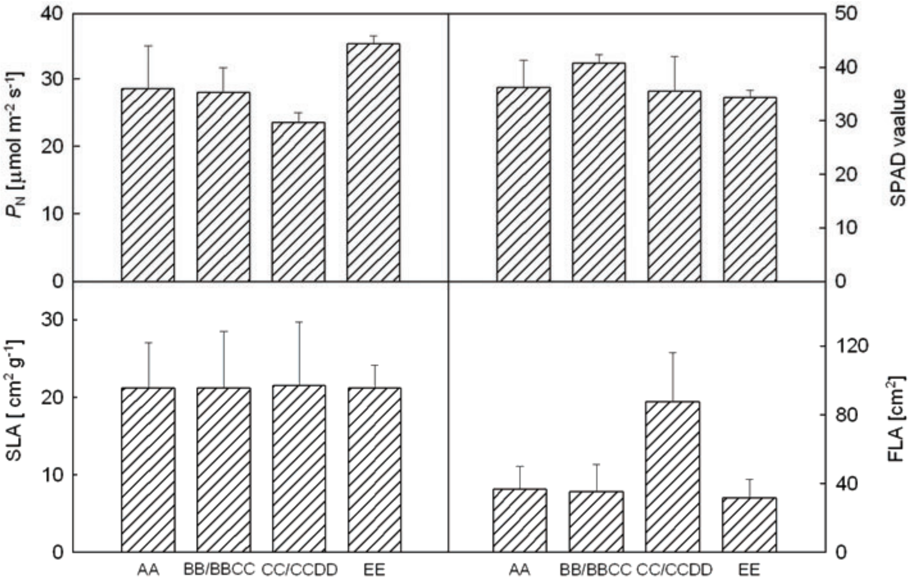

Fig. 2. The comparison of P_N, SPAD, SLA, and FLA among 4 types of genomes. The data were average values of AA (n = 12), BB/BBCC (n = 4), CC/CCDD (n = 5), and EE (n = 4), respectively in three repetitions. *Error bars* are +SE.

Leaf nitrogen concentration: There were wide changes in leaf nitrogen concentration among species tested (Fig. 4). The highest nitrogen concentration was 1.36 g m^{-2} [*O. sativa* (IR36)], was about 7 times of the lowest one, which was 0.2 g m^{-2} (*O. latifolia*). The species of relative high nitrogen *were O. meridionalis, O. gradiglumis, O. australiensis, and O. logistaminata*. The analysis of correlation (Fig. 4) revealed a tendency, which photosynthetic rate seems to have a positive correlation with leaf nitrogen. The leaf nitrogen had also negative correlation with SLA and FLA.

Discussion

There is a rich source for improving of agronomic traits including insect- and disease resistance to biotic stresses, and increased biomass (Vaughan 1989, Sitch 1990) in the genus *Oryza*. Therefore, there are good resources to improve the rice photosynthesis. Our research showed that it can help select the high-photosynthesis plants from the offspring of the cultivated and wild rice species similar to those of maize (Zhao *et al*. 2001). It may be most important to identify donor species for improving the modern varieties to break the yield barrier in rice. The comparison of photosynthetic rate in 23 species showed that *O. australiensis* unimproved wild germplasm had maximum light-saturated assimilation rates (19.8 µmol m^{-2} s^{-1}) and PEPC [3.26 µmol min^{-1} mg^{-1} (Chl)] in ambient air (Yeo *et al*. 1994). Among 17 species studied, *O. rufipogon* and *O. australiensis* exhibited highest photosynthesis, and *O. rufipogon* was the best wild resource species for improving rice photosynthesis among all species tested because it had maximum P_N (37.4 µmol m^{-2} s^{-1}), same AA genome and presents a successful example for enhanced photosynthesis in offspring crossed with *O. sativa*. In addition, Yeo *et al*. (1994) also reported that a range of *O. rufipogon* accessions had photorespiration rate significantly (P=0.01) lower than in the *O. sativa* genotypes tested. On the basis of these results, we suggested that *O. rufipogon* is the most important wild species for improvement of rice photosynthesis and yield potential.

The range of P_N within the genus was about 2.5 fold in this research and other studies (Yeo *et al.* 1994), both results were similar. However, the maximum P_N was 37.6 µmol m^{-2} s^{-1} (*O. rufipogon,* Acc. no. 105697) and 19.8 µmol m^{-2} s^{-1} (*O. australiensis*, Acc. no. 105273), and the minimum photosynthetic rate was 14.3 µmol m^{-2}s^{-1} (*O. glumaepatula,* Acc. no. 100971) and 7.96 µmol m^{-2} s^{-1} (*O. granulata*, Acc. no. 104503) in this research and Yeo *et al.* (1994) reported respectively under saturating light. There might be a lot of factors that result in the differences in photosynthetic rate found by different researchers. Another reason may be that photosynthesis of rice in tropic area is higher than that in other area. Yoshida (1981) also reported that maximum rates of leaf photosynthesis ranged from 25 to 32 µmol m^{-2} s^{-1} for C_3 rice in IRRI, Philippines. Comparison of range of 12 modern rice varieties with very high yield potential also showed photosynthetic rate to be from 27.2 to 32.5 µmol m^{-2} s^{-1} during flowering under saturation light (data supplied by Hu 2000, personal communcations) in IRRI. Ohno (1976) reported that certain Indica cultivars grown in the dry season of the tropical region under high solar

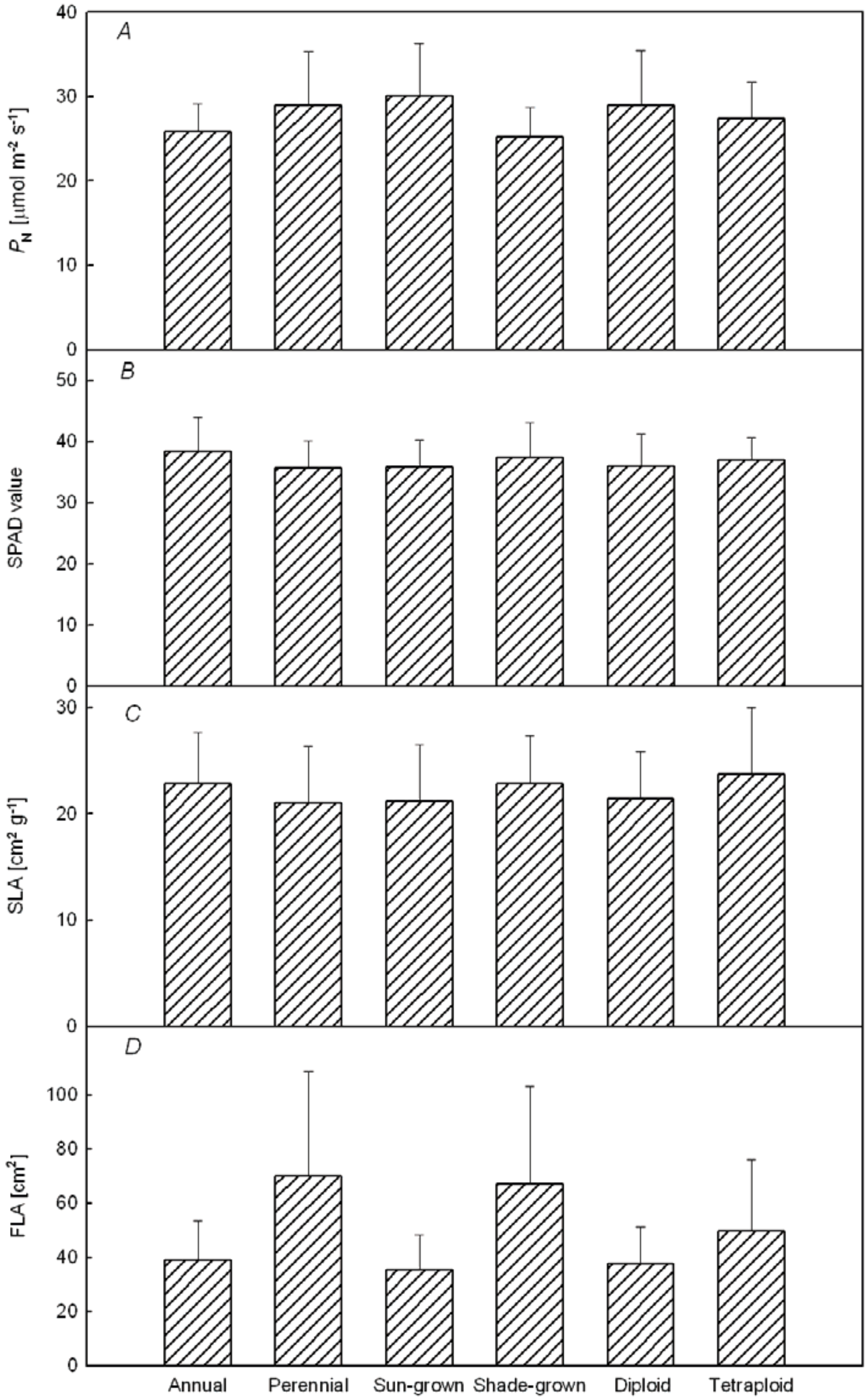

Fig. 3. P_N, SPAD, SLA, and FLA in different growth characteristics and chromosome in *Oryza* species. The data are average of 8 annual and 17 perennial species; 20 sun-grown and 5 shade-grown habit species; 19 diploid and 6 tetraploid species. *Error bars* are +SE.

radiation did not show the light saturation of photosynthesis up to about 1,500 μmol m^{-2} s^{-1} (original data 80 kLx), and exhibited a photosynthetic rate as high as more than 45 μmol m^{-2} s^{-1} [original data 60 mg(CO_2) dm^{-2} h^{-1}]. In our other research, one plant was identified with a photosynthetic rate of 46 μmol m^{-2} s^{-1} under 2,400 μmol m^{-2} s^{-1} PAR among the F_2 progeny of *O. sativa* and *O. rufipogon*. The photosynthetic rate of the selected high-photosynthesis plant was about 90% that of maize (50.5 μmol m^{-2} s^{-1}) grown under similar field conditions.

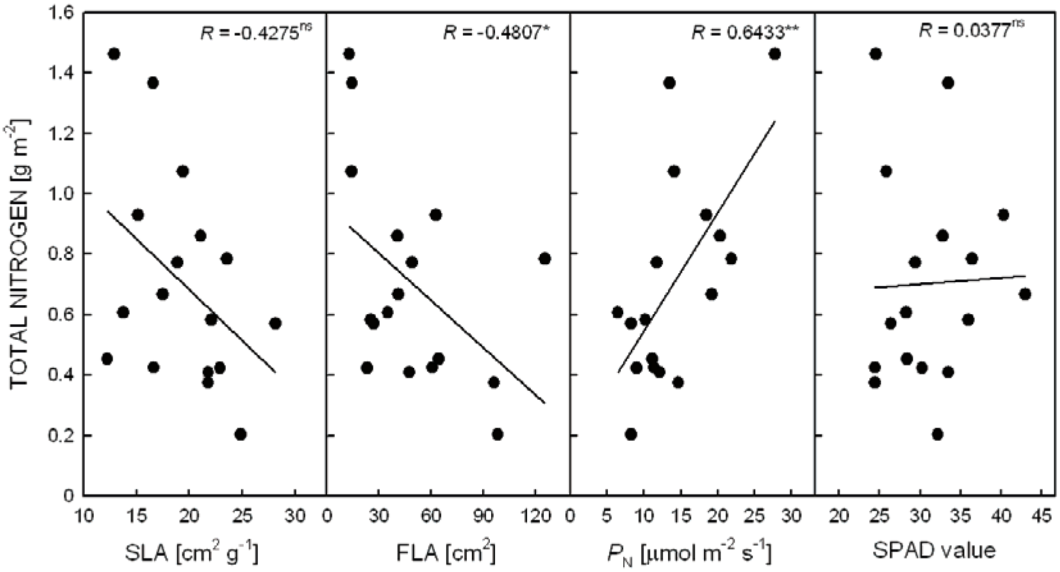

Fig. 4. Correlation of leaf nitrogen with SLA, FLA, P_N, and SPAD value. Every correlation analysis had 17 samples in total and each sample had three repetitions.

If photosynthesis of rice in tropic area is indeed higher than that in other area, a problem arises, which factors result in high photosynthesis in tropical area and requests a further research.

On the other hand, photosynthetic characteristics also depended on genomes, chromosome ploidy and growth habit in genus *Oryza*. In this research, some results showed that the species of EE genome, diploid, perennial, and sun-growing plants had higher photosynthesis compared to others. The species of BB/BBCC, shade-growing plants and tetraploid had high SPAD reading. The flag leaf in species of perennial, sun-growing and diploid were thicker (SLA was lower) compared to others, however, there was no significant difference among different types of genomes. Comparing the FLA showed that the species of CC/CCDD genome, perennial shade growing and tetraploid were larger than others, respectively. The species with the highest leaf nitrogen concentration was *O. sativa* (IR36) among all tested species. Therefore, some of these results were similar to those of others, in which diploid species had higher assimilation rate, as has been observed in the genus *Triticum* (Kaminski *et al.* 1990), and in which the sun species had a higher assimilation rate, light requirements, $V_{Rubisco}$ and PEP carboxylase activities (Yeo *et al.* 1994).

There are widely diverse photosynthetic characteristics in genus *Oryza*. At the molecular level, isozymes for example, it has been clearly shown that cultivated rice species are less diverse than their annual relatives, which in turn are less diverse than their perennial relatives (Oka *et al.* 1988, Second 1985). The correlation analysis in this paper showed that there were no markedly close relationships among P_N, SPAD, SLA, FLA, and leaf nitrogen except the relationship between P_N and nitrogen concentration. These characteristics may not be linked to each other during evolution of rice. Because of the diversity in the photosynthetic characteristics, there should be a wide possibility to select and redesign rice photosynthesis to obtain an increased yield. Several wild species are adapted to full shade and may have useful genes for low-light tolerance (Vaughan 1994). Therefore, some physiologists working on photosynthesis might be interested in shade-loving species of the *O. meyeriana* complex or *O. officinalis* complex (Sasahara *et al.* 1982). However, in fact, strong light often results in photosynthesis decrease because of photodamage or photoinhibition in rice, especially in tropic area. We suggest that the wild species with high photosynthesis and low photorespiration under strong light, such as *O. rufipogon*, should be useful for improvement rice photosynthesis.

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