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# Characteristics of Gas Exchange and Chlorophyll Fluorescence during Senescence of Flag Leaf in Different Rice (*Oryza sativa* L.) Cultivars Grown under Nitrogen-Deficient Condition

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**Abstract** : Effects of nitrogen (N) deficiency on photosynthetic gas exchange and photosystem II (PSII) photochemistry of flag leaves during grain-filling stage were investigated in six rice cultivars, Kasalath (a conventional *indica*), IR36 (an improved *indica*), Shirobeniya (a conventional *japonica*), Nipponbare (an improved *japonica*), Akenohoshi (an improved *japonica-indica* intermediate type) and BSI429 (an improved tropical *japonica*, a new plant type line) grown hydroponically in N-sufficient (NS) and N-deficient (ND) solution. From 3 to 24 days after heading (DAH), net photosynthetic rate ( $P_N$ ), maximum quantum yield of photosystem II (PSII) ( $F_v/F_m$ ), quantum yield of PSII electron transport ( $\Phi_{PSII}$ ), and contents of chlorophyll (Chl) and ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) in the flag leaves decreased, particularly under the ND condition in all six cultivars. A substantial difference was observed among the ND plants for the sustainability index (SI, the ratio of the value at 24 DAH to that at 3 DAH) of  $P_N$ ,  $F_v/F_m$ ,  $\Phi_{PSII}$ , Chl content and Rubisco content; SIs of those parameters of Akenohoshi, BSI429, Nipponbare and Shirobeniya were higher than those of IR36 and Kasalath. The SI of  $P_N$  showed significant positive correlations with those of  $F_v/F_m$ ,  $\Phi_{PSII}$ , and the contents of Chl and Rubisco under the ND condition. It was concluded that the sustainability of photosynthesis in the flag leaves was mainly due to those of PSII photochemistry and electron transport, which was associated with the maintenance of Chl and Rubisco under the ND condition.

**Key words** : Chlorophyll fluorescence, Flag leaf, Leaf senescence, Nitrogen deficiency, Photoinhibition, Photosynthesis, Rice (*Oryza sativa* L.).

Rice (*Oryza sativa* L.) yield must be dramatically increased in order to sustain the growing world population (Mitchell and Sheehy, 2006). Over the last 40 years, rice production in Asia has kept pace with the increasing population. The system of managing rice production, involving high-yielding cultivars and high inputs of nitrogen (N) fertilizers and agrochemicals, contributed to an increase in the rice yield of developed and developing countries in the past few decades (Evans, 1993). However, N and agrochemicals discharged from the agricultural system lead to greater surface and underground water pollution (Jarvis, 1996). The restriction of N supply is considered an effective countermeasure to minimize environmental perturbation; however, maintaining a high yield with low N input is technically difficult. In order to solve this problem, we need to improve the N use efficiency for rice cultivars that exhibit stable yield with low N input.

In rice, 60–100% of the carbon content of grains originates from CO<sub>2</sub> assimilation during the grain-filling period (Yoshida, 1981). Among the leaves of a rice plant, flag leaves are the primary contributors to the accumulation of dry matter in grains (Murata and Matsuyama, 1975 ; Black et al., 1995). On the other hand, 70–90% of the N in rice panicles is translocated from vegetative organs. Leaf blades are the major source of remobilized N and account for approximately 60% of the mobilized N (Mae, 1997). Most of the N in leaf blades is accumulated in the chloroplasts, and the grain-filling period coincides with the onset of leaf senescence.

Leaf senescence generally induces alterations in the structure and function of the chloroplasts, which results in reduced photosynthetic activity (Woolhouse, 1984). A key event leading to this reduction during senescence is associated with the breakdown of ribulose-1,5-bisphosphate carboxylase/oxygenase

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**Abbreviations** : C<sub>a</sub>, atmospheric CO<sub>2</sub> concentration; Chl, chlorophyll; C<sub>i</sub>, intercellular CO<sub>2</sub> concentration; DAH, days after heading; F<sub>o</sub>, initial Chl fluorescence of a dark-adapted leaf; F<sub>m</sub>, the maximum Chl fluorescence of a dark-adapted leaf; F<sub>v</sub>, variable Chl fluorescence; g<sub>s</sub>, stomatal conductance; N, nitrogen; ND, N-deficient; NPQ, non-photochemical quenching; NS, N-sufficient; P<sub>N</sub>, net photosynthetic rate;  $\Phi_{PSII}$ , quantum yield of photosystem II electron transport ; PPFD, photosynthetic photon flux density; PSII, photosystem II; ROS, reactive oxygen species; Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase; SI, sustainability index; SNC, N concentration in the solution.

(Rubisco), and the amount of Rubisco and the extent of CO<sub>2</sub> assimilation thereby decline rapidly (Makino et al., 1984 ; Crafts-Brander et al., 1998). In contrast, the amount of chlorophyll (Chl), the light absorbing capacity and the photochemical activity of photosystem II (PSII) reduce slightly (Humbeck et al., 1994).

N supply affects absorption and utilization of excitation energy in plant leaves (Chen and Cheng, 2003). Leaf Chl content decreases in response to N deficiency, leading to a decrease in light absorption (Chen and Cheng, 2003). N deficiency also decreased the light saturated photosynthetic rate, which is associated with the decrease in Rubisco content (Evans and Terashima, 1987). However, the decrease in Rubisco content is not proportional to the decrease in Chl content (Chen et al., 2003). Thus, the marked decline in CO<sub>2</sub> assimilation accompanying the slight decrease in the light harvesting capacity and PSII photochemical activity was induced by N deficiency, which results in the accumulation of light excess excitation energy. If the excess energy is not dissipated efficiently, reactive oxygen species (ROS) are generated, which phenomenon reflects the severe damage to the photosynthetic apparatus (photodamage) and accelerate the progress of leaf senescence. Huang et al. (2004) reported that N-deficient (ND) leaves of rice plants showed more rapid reduction in the maximum quantum yield of PSII ( $F_v/F_m$ ) during its senescence, indicating that the decreased CO<sub>2</sub> assimilation induced by N deficiency causes photodamage to chloroplasts and accelerates the progress of leaf senescence.

Several researchers reported that many rice cultivars bred in Japan over a hundred year period showed improvements in both grain yield and photosynthetic capacity of flag leaves in the two to three weeks after heading (Sasaki and Ishii, 1992 ; Zhang and Kokubun, 2004). So far many studies have been conducted on the response of leaf photosynthesis to N applications using different rice cultivars (Hayami, 1982 ; Sasaki and Ishii, 1992 ; Sarker et al., 2002). In a number of studies, rice photosynthesis has been discussed mainly from the viewpoint of gas exchange rate; however, we consider that in order to deepen the understanding of leaf photosynthesis, it should be examined and discussed on the basis of the mutual relationship between gas exchange and PSII photochemical functions. For the breeding and selection of a cultivar that has stable yield under the conditions of low N input, it is important to gain better information of the cultivar variation in the duration of photosynthesis in relation to gas exchange and PSII photochemical functions of flag leaves under the ND condition. However, the difference among cultivars in terms of the duration of gas exchange and PSII photochemical functions of flag leaves under the ND condition has received little attention thus far.

The objective of this study was to clarify the differ-

ence of the response of photosynthesis in the flag leaves to N deficiency among various rice cultivars. For this purpose, we selected six cultivars with genotypic diversity and investigated the effects of N deficiency on gas exchange, Chl fluorescence, and contents of Chl and Rubisco in flag leaves of these cultivars during the grain-filling stage.

## Materials and Methods

### 1. Plant materials and N treatments

Kasalath (a conventional *indica*), IR36 (an improved *indica*), Shirobeniya (a conventional *japonica*), Nipponbare (an improved *japonica*), Akenohoshi (an improved *japonica-indica* intermediate type) and BSI429 (an improved tropical *japonica*, a new plant type line) were used in this experiment. Water-soaked seeds of these six cultivars were sown in nursery boxes in a glasshouse on 8 June 2006. After three weeks, two young seedlings of each cultivar were transplanted in a bottomless polyvinyl chloride cylinder (8 cm in diameter and 7 cm in height) filled with sponges, which was floated in N-sufficient (NS) and ND nutrient solutions in four water-baths with a 500 L capacity. Two water-baths were prepared for each N condition. Thirty-six cylinders (12 cylinders per cultivar) were placed in a water-bath. The water bathes contained the following solution recommended by Yoshida et al. (1972): 1.43 mM NH<sub>4</sub>NO<sub>3</sub>, 0.51 mM K<sub>2</sub>SO<sub>4</sub>, 1.00 mM CaCl<sub>2</sub>, 1.67 mM MgSO<sub>4</sub>, 0.32 mM NaH<sub>2</sub>PO<sub>4</sub>, 0.04 mM FeCl<sub>2</sub>, 9.09 μM MnCl<sub>2</sub>, 0.08 μM (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>, 18.2 μM H<sub>3</sub>BO<sub>3</sub>, 0.15 μM ZnSO<sub>4</sub>, 0.16 μM CuSO<sub>4</sub>, and 3.57 mM Na<sub>2</sub>SiO<sub>3</sub>. The N concentration of the solution (SNC) was set at two levels: NS and ND, which was 2.86 and 1.43 mM, respectively. De-ionized water was used to make up a solution. The seedlings were grown in the glasshouse under natural sunlight. During the cultivation period, the SNC was monitored once a week by using RQ flex plus (Merck, Germany), and the pH of the solution was adjusted every day to between 5.0 and 5.5 using HCl and NaOH. The solution renewal method was as described by Kumagai et al. (2007) with slight modification. Each solution was renewed at a two-week interval. The N concentration in each renewed solution was adjusted to the same SNC level that was measured in each bath at every renewal time except at four and six weeks after heading. The concentrations of nutrients other than N were adjusted to the standard level. Under the NS condition, SNC was detectable (approximately 0.3 mM) at the heading stage, but under the ND condition, it was eventually reduced completely at the heading stage, and the six cultivars thus grown suffered from severe N deficiency during the grain-filling period (data not shown).

Plant growth was periodically surveyed, and the panicles that emerged on one day after the onset of heading were selected and tagged. Only tagged main stems were used for photosynthesis measurements

and sample collections. Four tagged stems per cultivar for each N condition were used for measurements of photosynthetic CO<sub>2</sub> assimilation, Chl fluorescence, and the contents of soluble protein, Chl, and Rubisco. The measurements were conducted on 3 DAH and 24 DAH, beginning on 24 August at the heading stage and ending on 5 October at the late-ripening stage. The day of the onset of heading was earlier in the ND plants than in the NS plants, and the measurements were staggered: under the NS condition, IR36, Nipponbare and Akenohoshi headed on 27, 28 and 29 August, respectively. Both Shirobeniya and Kasalath headed on 6 September, and BSI429 headed on 10 September. Under the ND condition, IR36, Nipponbare and Akenohoshi headed on 24, 26 and 28 August, respectively. Both Shirobeniya and Kasalath headed on 1 September, and BSI429 headed on 6 September.

## 2. Measurements of photosynthetic gas exchange and Chl fluorescence

Photosynthetic gas exchange and Chl fluorescence were measured simultaneously by using a system that combined an open gas exchange system and a portable fluorometer (PAM-2000, Waltz, Germany). The temperature-controlled chamber of the open system was modified as follows: the fiberoptic of the PAM-2000 was attached onto the side of the chamber at a 60° angle without significantly interfering with photosynthetic photon flux density (PPFD) distribution at the leaf surface, yet it allows for delivery of saturation pulse and measuring beam and the detection of measured signals.

Gas exchange was measured under the following condition: leaf temperature, 30 ± 0.4°C; CO<sub>2</sub> concentration, 380 ± 13 μL L<sup>-1</sup>; relative humidity, 60 ± 2.6%; and PPFD, 1000 μmol m<sup>-2</sup> s<sup>-1</sup>. The leaf area used in the measurements was 5.9 cm<sup>2</sup>, and the rate of airflow into the assimilation chamber was 706 μmol s<sup>-1</sup>. The CO<sub>2</sub> concentration and water vapour pressure in the reference and sample air were monitored with an infrared gas analyzer (Li-6262, LI-COR, USA). The net photosynthetic rate (P<sub>N</sub>), stomatal conductance (g<sub>s</sub>), and intercellular CO<sub>2</sub> concentration (C<sub>i</sub>) were calculated from the measurements according to the method of Long and Hallgren (1985).

Using a leaf that was dark-adapted for 30 min, the initial fluorescence (F<sub>0</sub>) in non-photosynthetic conditions was determined with a low intensity measuring beam; thereafter, the maximal fluorescence (F<sub>m</sub>) was measured by applying a 0.8-s saturation pulse onto the leaf in order to reduce all the PSII centres. Then, the leaf was continuously irradiated by actinic light of 1000 μmol m<sup>-2</sup> s<sup>-1</sup> PPFD. After the steady-state fluorescence (F<sub>s</sub>) was recorded, a 0.8-s saturation pulse was applied to determine the maximum fluorescence in the light-adapted state (F<sub>m</sub>'). The following parameters were calculated: maximum quantum yield of PSII, F<sub>v</sub>/F<sub>m</sub> = (F<sub>m</sub> -

F<sub>0</sub>)/F<sub>m</sub> (van Kooten and Snel, 1990); quantum yield of PSII electron transport, Φ<sub>PSII</sub> = (F<sub>m</sub>' - F<sub>s</sub>)/F<sub>m</sub>' (Genty et al., 1989); and non-photochemical quenching, NPQ = (F<sub>m</sub> - F<sub>m</sub>')/F<sub>m</sub>' (Bilger and Björkman, 1990).

## 3. Determination of soluble protein, Chl, and Rubisco contents

The contents of soluble protein, Chl, and Rubisco in the flag leaves were measured according to Kumagai et al. (2007). After the gas exchange and Chl fluorescence were measured, leaf discs of 5-mm diameter were sampled, frozen in liquid N<sub>2</sub>, and stored at -80°C. To measure the Chl content, three of these discs were soaked in 96% ethyl-alcohol in the dark for two days until the leaf colour was bleached. After this extraction procedure, the Chl content in the alcohol solution was estimated from the absorbance at 649 and 665 nm by using a spectrophotometer (UV-1200, Shimadzu, Japan) according to the method described by Wintermans and de Mots (1965).

To measure the soluble protein content, we powdered six leaf discs in liquid N<sub>2</sub> in a mortar; then, added a chilled extraction buffer, 100 mM potassium buffer (pH 7.0) containing 1 mM phenylmethanesulfonyl fluoride and 1% (v/v) 2-mercaptoethanol, and 1% (w/v) insoluble polyvinyl polypyrrolidone, and the powder was further ground. The obtained sample solution was transferred into Eppendorf tubes and centrifuged (12 000 g, 4°C, 5 min). Bradford reagent (Bio-Rad, USA) was then added to the supernatant according to the method of Bradford (1976), and the amount of soluble protein in the sample was determined based on the absorbance at 595 nm determined using a spectrophotometer. The amount of Rubisco in the soluble protein obtained as above was quantified by SDS-polyacrylamide gel electrophoresis, according to the method described by Makino et al. (1985).

## Results

### 1. Photosynthetic gas exchange parameters in flag leaves

Under both the NS and the ND conditions, P<sub>N</sub> of the six cultivars decreased significantly from 3 DAH to 24 DAH (Fig. 1A, B). After heading, the ND plants of the six cultivars had lower P<sub>N</sub> than the NS plants, and the effects of restricted N supply became more significant at 24 DAH. Moreover, P<sub>N</sub> differed significantly among the cultivars under the ND condition at 24 DAH; it was highest in Akenohoshi followed by BSI429, Nipponbare, Shirobeniya, IR36, and Kasalath in this order. Under the NS condition, g<sub>s</sub> of the five cultivars except Nipponbare did not change from 3 DAH to 24 DAH (Fig. 1C, D). The effect of N deficiency on g<sub>s</sub> was small at 3 DAH. However, g<sub>s</sub> decreased greatly with senescence in the ND plants, particularly in Kasalath. The ratio of C<sub>i</sub> to the atmospheric CO<sub>2</sub> concentration (C<sub>i</sub>/C<sub>a</sub>) increased

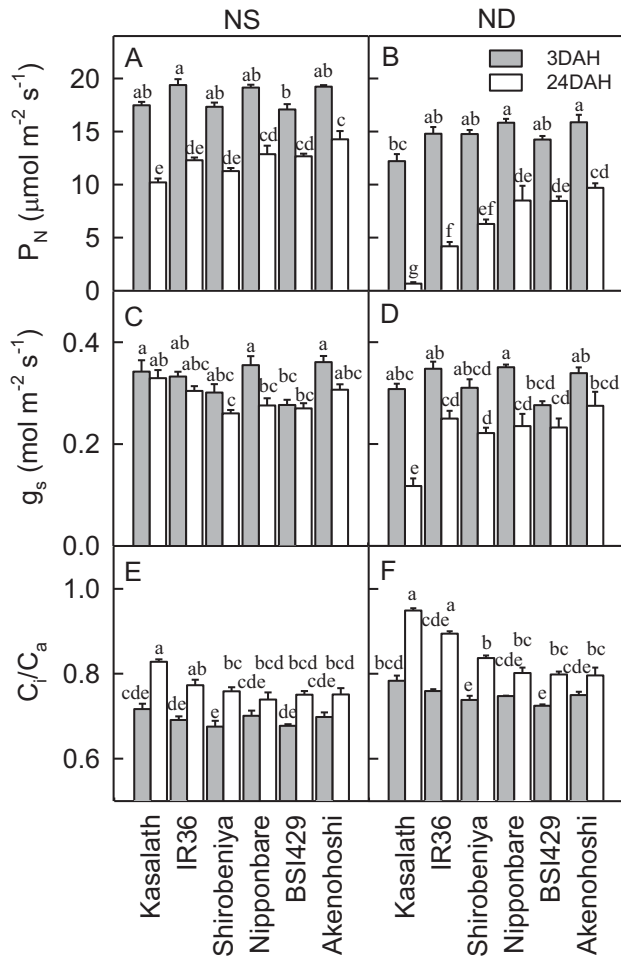


Fig. 1. Net photosynthetic rate ( $P_N$ ), stomatal conductance ( $g_s$ ) and ratio of intercellular to atmospheric  $\text{CO}_2$  concentration ( $C_i/C_a$ ) of the flag leaves in the six rice cultivars grown hydroponically in the N-sufficient (NS: A, C and E) and the N-deficient (ND: B, D and F) conditions measured at 3 and 24 days after heading (DAH). Values are the means  $\pm$  S.E. of four replications derived from different plants. Bars followed by the same letter represent no significant difference by the Tukey's test at 5% level.

with leaf senescence under both N conditions in all six cultivars (Fig. 1E, F). At both 3 DAH and 24 DAH,  $C_i/C_a$  was higher in the NS plants than in the ND plants, particularly in Kasalath and IR36.

## 2. Chl fluorescence parameters in flag leaves

$F_v/F_m$  of the five cultivars except Kasalath did not change during the entire experimental period in the NS plants (Fig. 2A). In contrast,  $F_v/F_m$  decreased clearly during the senescence in the ND plants (Fig. 2B). Under the ND condition,  $F_v/F_m$  differed among the cultivars at 24 DAH; it was highest in Akenohoshi followed by BSI429, Nipponbare, Shirobeniya, IR36, and Kasalath in this order. With the progress of leaf senescence,  $\Phi_{\text{PSII}}$  decreased more markedly in the ND plants than in the NS plants (Fig. 2C, D). Moreover, there was a substantial difference among the cultivars

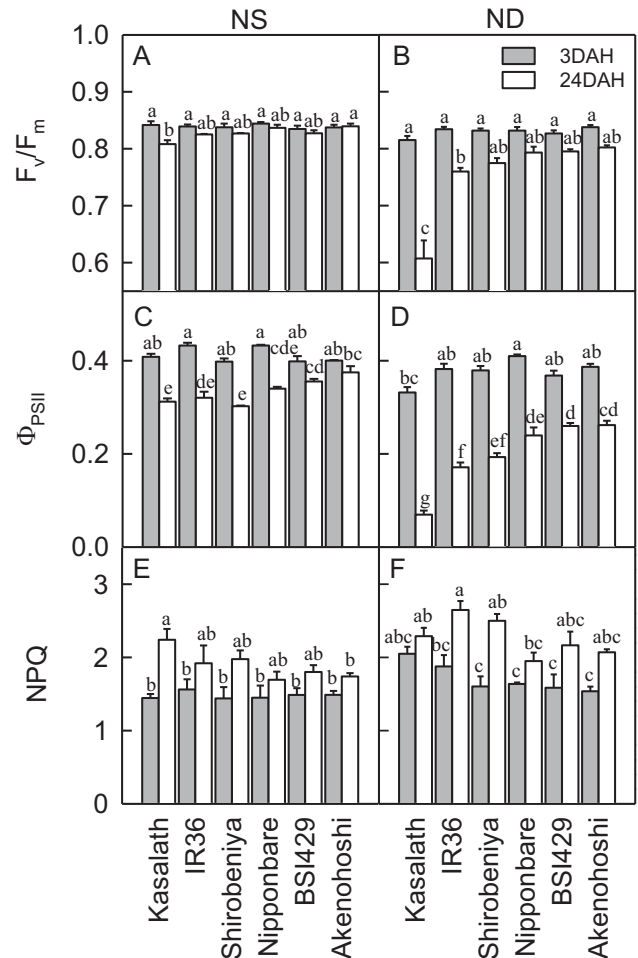


Fig. 2. Maximum quantum yield of PSII ( $F_v/F_m$ ), quantum yield of PSII electron transport ( $\Phi_{\text{PSII}}$ ) and non-photochemical quenching (NPQ) of the flag leaves in the six rice cultivars grown hydroponically in the N-sufficient (NS: A, C and E) and the N-deficient (ND: B, D and F) conditions measured at 3 and 24 days after heading (DAH). Values are the means  $\pm$  S.E. of four replications derived from different plants. Bars followed by the same letter represent no significant difference by the Tukey's test at 5% level.

in  $\Phi_{\text{PSII}}$  in the ND plants at 24 DAH; the order of  $\Phi_{\text{PSII}}$  was in agreement with that of  $P_N$  and  $F_v/F_m$ . NPQ of the six cultivars tended to increase during leaf senescence under both N conditions (Fig. 2E, F). NPQ of IR36 and Shirobeniya increased significantly with leaf senescence under the ND condition whereas NPQ of Kasalath increased under the NS condition.

## 3. The contents of Chl and Rubisco in flag leaves

The reduction in Chl content with leaf senescence was observed more markedly in the ND plants than in the NS plants (Fig. 3A, B). At 24 DAH, Chl content of Akenohoshi, BSI429, Nipponbare and Shirobeniya was higher than that in IR36 and Kasalath under the ND condition (Fig. 3B). Rubisco content decreased significantly as leaf senescence progressed, particularly under the ND condition (Fig. 3C, D). Under the ND condition,

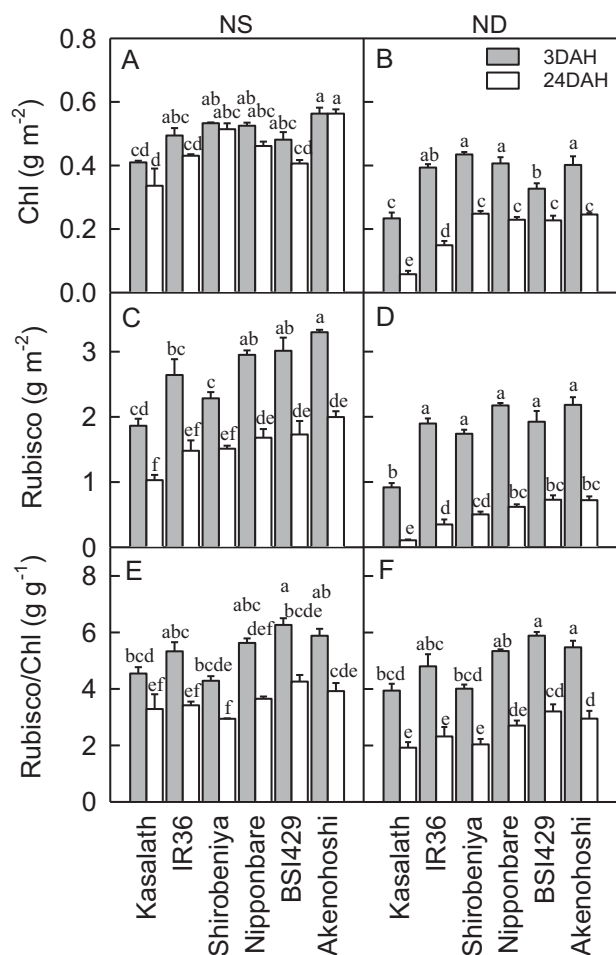


Fig. 3. Chl content, Rubisco content and Rubisco/Chl ratio of the flag leaves in the six rice cultivars grown hydroponically in the N-sufficient (NS: A, C and E) and the N-deficient (ND: B, D and F) conditions measured at 3 and 24 days after heading (DAH). Values are the means  $\pm$  S.E of four replications derived from different plants. Bars followed by the same letter represent no significant difference by the Tukey's test at 5% level.

the order of Rubisco content was in agreement with those of  $P_N$ ,  $F_v/F_m$  and  $\Phi_{PSII}$  at 24 DAH: Rubisco content was highest in Akenohoshi followed by BSI429, Nipponbare, Shirobeniya, IR36, and Kasalath. With the progress of leaf senescence, Rubisco/Chl ratio of the six cultivars also declined more significantly under the ND condition than under the NS condition (Fig. 4E, F). The cultivar difference in the ratio was observed evidently at 24 DAH under the ND condition; The ratio in Kasalath, IR36 and Shirobeniya tended to be lower than in the other three cultivars.

#### 4. Sustainability index (SI) of photosynthetic parameters

Table 1 shows the sustainability index (SI) of parameters related to gas exchange and PSII photochemical functions in the flag leaves of the rice cultivars grown under the NS and the ND conditions. SI is calculated as (the values at 24 DAH)/(the values

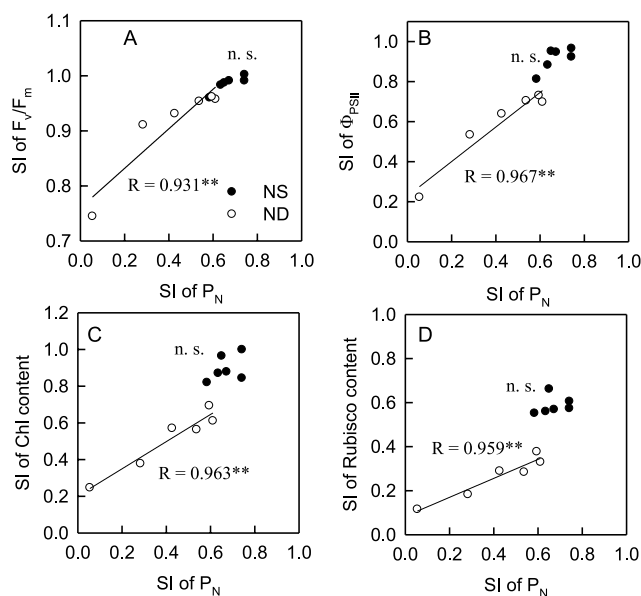


Fig. 4. Correlation of the sustainability index (SI) of  $P_N$  and SI of  $F_v/F_m$  (A),  $\Phi_{PSII}$  (B), Chl content (C) and Rubisco content (D) under N-sufficient (NS) and the N-deficient (ND) conditions.  $^{**}$  and n.s. indicates a significant correlation at 1% level and no significance, respectively.

at 3 DAH) and indicates the degree of maintenance of parameters during the grain-filling stage. SIs of  $P_N$ ,  $F_v/F_m$ ,  $\Phi_{PSII}$ , and the contents of Chl and Rubisco were lower in the ND plants. A large difference among the cultivars was observed in SIs of all parameters of the ND plants; SIs of those parameters of Akenohoshi, BSI429, Nipponbare and Shirobeniya were higher than those of IR36 and Kasalath. SI of  $P_N$  in the NS plants did not correlate significantly with that of  $F_v/F_m$ ,  $\Phi_{PSII}$ , Chl content, and Rubisco content (Fig. 4). In contrast, there was a significant correlation between SI of  $P_N$  and that of  $F_v/F_m$ ,  $\Phi_{PSII}$ , Chl content, and Rubisco content under the ND condition ( $R = 0.931$ ,  $0.967$ ,  $0.963$ , and  $0.959$ , respectively).

#### Discussion

We showed that  $P_N$  in the flag leaves of the six cultivars decreased significantly as senescence progressed, and  $P_N$  in the ND plants was lower than that in the NS plants during the grain-filling stage (Fig. 1A, B). Moreover, SI of  $P_N$  in the ND plants was lower than that in the NS plants (Table 1). These results indicate that N deficiency induced the acceleration of flag leaf senescence in the rice cultivars. Difference in leaf senescence was identified between the rice subspecies *indica* and *japonica*, and the former showed more early senescence than the latter (Yoshida, 1981; Abdelkhalik et al., 2005). In our study, the differences in  $P_N$  at 24 DAH and SI of  $P_N$  among cultivars become evident in the ND plants; those in the *japonica* cultivars, Akenohoshi, BSI429, Nipponbare and Shirobeniya were higher than those in the *indica* cultivars, IR36

Table 1. The sustainability index (SI) of  $P_N$ ,  $F_v/F_m$ ,  $\Phi_{PSII}$ , Chl content and Rubisco content of flag leaves in the six rice cultivars grown hydroponically in the N-sufficient (NS) and the N-deficient (ND) conditions.

Treatment	Cultivar	Sustainability Index (SI)				
		$P_N$	$F_v/F_m$	$\Phi_{PSII}$	Chl	Rubisco
NS	Kasalath	0.584	0.960	0.813	0.821	0.552
	IR36	0.634	0.983	0.883	0.871	0.560
	Shirobeniya	0.650	0.987	0.952	0.965	0.662
	Nipponbare	0.672	0.991	0.948	0.878	0.569
	BSI429	0.741	0.991	0.924	0.844	0.574
	Akenohoshi	0.742	1.002	0.966	1.000	0.606
ND	Kasalath	0.055	0.745	0.223	0.247	0.116
	IR36	0.283	0.911	0.534	0.378	0.184
	Shirobeniya	0.426	0.931	0.639	0.571	0.290
	Nipponbare	0.537	0.954	0.705	0.564	0.284
	BSI429	0.594	0.962	0.731	0.694	0.378
	Akenohoshi	0.611	0.958	0.698	0.612	0.330

SI was calculated as {the values at 24 days after heading (DAH)}/(the values at 3 DAH).

and Kasalath (Fig. 1B and Table 1). In addition, we observed that greater sustainability of photosynthesis in the improved cultivar than in the conventional one in both *japonica* and *indica* groups. Ding et al. (2005) reported that previously released maize hybrids had higher grain weight than recently released maize hybrids under N deficiency, which was mainly due to the slow senescence of the flag leaves during the grain-filling period. In rice cultivars, factors that lower the photosynthetic rate in the flag leaves during the grain-filling period could limit grain yield (Murchie et al., 1999). Horton (2000) proposed that prolonging the duration of photosynthesis offers an opportunity to increase the total amount of carbon fixed by a crop plant, and that delaying leaf senescence is a prime target for crop improvement. Similarly, we consider that it is likely that the delay of flag leaf senescence is beneficial for improving rice grain yield under low N input condition.

In all the cultivars tested,  $C_i/C_a$  in the flag leaves increased continuously throughout senescence, particularly in the ND plants of Kasalath and IR36 (Fig. 1E, F), which showed the greater reduction of  $P_N$  during leaf senescence (Fig. 1B). This remarkable increase in  $C_i/C_a$  accompanied with the decrease in  $P_N$  in the ND plants suggested that the decrease in photosynthetic capacity was not due to stomatal limitation, and these results were in agreement with those reported by Huang et al. (2004). Under the conditions used in this study which were not water-limited,  $g_s$  would not be expected to be a large component of photosynthetic capacity.

$F_v/F_m$  did not change during the grain-filling period in the NS plants except for Kasalath (Fig. 2A). In contrast, it decreased greatly at the late stage in the

ND plants particularly in Kasalath (Fig. 2A), indicating that the ND plants of Kasalath suffered from chronic photoinhibition. We also observed that SI of  $F_v/F_m$  in the ND plants was lower than that in the NS plants (Table 1). The data obtained in this study show that N-dependent leaf senescence influences the degree of photoinhibition and/or photodamage of PSII in the flag leaves of rice cultivars.  $\Phi_{PSII}$  of the six cultivars decreased significantly under both N conditions, particularly under the ND condition (Fig. 2C, D). The ND plants had lower SI of  $\Phi_{PSII}$  than the NS plants (Table 1). There was a substantial cultivar difference in  $\Phi_{PSII}$  in the ND plants at the late stage, which was parallel to the trends of  $P_N$ . Additionally, we observed a significant correlation between SI of  $P_N$ , and those of  $F_v/F_m$  and  $\Phi_{PSII}$  under N deficiency (Fig. 4). It was suggested that the sustainability of photosynthesis in the flag leaves was mainly due to those of PSII photochemistry and electron transport under the ND condition.

Many reports showed that the activity of the xanthophyll cycle, as measured using NPQ, is enhanced under N deficiency (Verhoeven et al., 1997 ; Chen et al., 2003 ; Chen and Cheng, 2003 ; Kumagai et al., 2007). Moreover, several reports on rice suggested the existence of a difference among rice cultivars in terms of both the susceptibility to photoinhibition and the activity of the xanthophyll cycle (Jiao and Ji, 2001 ; Jiao et al., 2003). We also observed that the NPQ in the flag leaves of the six cultivars increased with senescence, particularly in the ND plants (Fig. 2E, F). Under the ND condition, the NPQ in IR36 and Shirobeniya increased more remarkably than that of the other cultivars. It is interesting that the NPQ in Kasalath did not change with the senescence under

the ND condition, but it increased significantly under the NS condition. Dai et al. (2004) reported that the activity of the xanthophyll cycle in the flag leaves of barley plants decreased dramatically at the late stage when leaf senescence progressed severely. Our finding indicated that the susceptibility of Kasalath to photoinhibition might be associated with the lower activity of xanthophyll cycle at the late stage under the ND condition.

N deficiency decreased the amounts of Chl and Rubisco in the rice leaves (Chen et al., 2003 ; Huang et al., 2004 ; Kumagai et al., 2007). We also observed that the Chl content in the flag leaves of the NS plants did not change with senescence; however, that in the flag leaves of the ND plants decreased significantly (Fig. 3A, B). SI of Chl content of Akenohoshi, BSI429, Nipponbare and Shirobeniya was higher than that of IR36 and Kasalath under the ND condition (Table 1). The amount of Rubisco in the flag leaves of the six cultivars reduced significantly from the heading to the mature stage, particularly in the ND plants (Fig. 3C, D). The large cultivar difference in the Rubisco content at 24 DAH and SI of Rubisco content in the ND leaves was observed (Fig. 3D and Table 1); Akenohoshi, BSI429, Nipponbare and Shirobeniya showed the higher SI of Rubisco than other two cultivars.  $P_N$  at saturating irradiance and ambient  $CO_2$  levels frequently correlated with Rubisco contents in rice plants (Makino et al. 1985). We also observed significant correlation between  $P_N$  measured at  $1000 \mu mol m^{-2} s^{-1}$  PPFD and Rubisco content under this condition ( $R = 0.957$ , data not shown). It is notable that SI of  $P_N$  correlated with either SI of Chl or Rubisco in the ND plants (Fig. 4). Therefore, we concluded that the sustainability of photosynthesis was strongly associated with the maintenance of Chl and Rubisco contents under the ND condition.

The changes in the ratios of light-harvesting capacity to electron transport and  $CO_2$  assimilation activity in a leaf may be reflected in the ratio between Rubisco and Chl (Chen et al., 2003 ; Kumagai et al., 2007). During the senescence of the flag leaves in the six cultivars, the reduction in the Rubisco/Chl ratio was larger in the ND plants than in the NS plants (Fig. 3E, F), implying the occurrence of an energy imbalance between absorbed light and the availability of electron sinks in the senescing flag leaves under N deficiency. Furthermore, there was a clear cultivar difference in the Rubisco/Chl ratio at late grain-filling stage. The flag leaves of rice plants are positioned at the top of the canopy. In the summer season in tropical and temperate regions, these leaves are frequently exposed to excessively high irradiance. In such circumstances, flag leaves with a low Rubisco/Chl ratio may easily accumulate excess energy, rendering them more susceptible to photoinhibition when the Rubisco content is reduced and  $CO_2$  assimilation is limited by

N deficiency. Among all the cultivars examined in our study, Kasalath had the lowest Rubisco content and Rubisco/Chl ratio; therefore, it was more susceptible to photodamage and its flag leaves showed shorter duration of photosynthesis during the grain-filling stage under the N deficiency.

In this paper, there was a substantial cultivar difference in the response to N deficiency with regard to the sustainability of photosynthesis in the flag leaves: SIs of  $P_N$ ,  $F_v/F_m$ ,  $\Phi_{PSII}$ , and the contents of Chl and Rubisco of Akenohoshi, BSI429, Nipponbare and Shirobeniya were higher than those of IR36 and Kasalath. The sustainability of photosynthesis was strongly associated with the maintenance of Chl and Rubisco contents under N deficiency. Thus, this variation could be used for the selection of cultivars whose yield is stable even in low N input cultivation systems.

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