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Changes in Photosynthetic Characteristics during Grain Filling of a Functional Stay-Green Rice SNU-SG1 and its F_1 Hybrids

Jin-Dong Fu[1], Byun-Woo Lee[1*]

[1]Department of Plant Science, College of Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Korea

Abstract

Functional stay-green is a beneficial trait that may increase grain yield through the sustained photosynthetic competence during monocarpic senescence in cereal crops. The temporal changes of photosynthesis and related characteristics throughout the grain filling period of a stay-green *japonica* rice "SNU-SG1" was compared in growth chamber conditions with three high-yielding cultivars (HYVs) and their F_1 hybrids with SNU-SG1. SNU-SG1 exhibited a typical characteristic of functional stay-green in terms of chlorophyll degradation and photosynthetic competence during grain filling. According to the photosynthesis-light response curve measured at 10 and 35 d after heading for the flag leaf, SNU-SG1 exhibited higher initial light conversion efficiency and thus higher gross photosynthetic rate at light saturation compared to HYVs. Light saturation point was not different among genotypes, ranging from 1000 to 1500 µmol photon $m^{-2}s^{-1}$. Net photosynthetic rate at light saturation (P_{max}) of the upper four leaves in SNU-SG1 was much higher and sustained longer throughout grain-filling than HYVs and F_1 hybrids. The sustained high photosynthetic competence of SNU-SG1 during grain filling was ascribed to the longer maintenance of high mesophyll conductance that resulted from not only high chlorophyll content and its delayed degradation but also the slow degeneration of photosystem II (PS II) as judged by chlorophyll fluorescence (F_v/F_m) of flag leaves. F_1 hybrids showed slow degeneration of photosystem II similar to the male parent SNU-SG1 while chlorophyll degradation pattern close to female parents, thus exhibiting a little higher P_{max} than female parents. These results suggest that SNU-SG1 has a typical functional stay-green trait that can be utilized for increasing rice yield potential through the improved dry matter production during grain filling.

Key words: Chlorophyll, photosynthesis, chlorophyll fluorescence, functional stay-green, F_1 hybrid, rice

Introduction

In Korea, with the development of a semi-dwarf rice variety "Tongilbyeo" that was bred by *indica* x *japonica* crosses, rice yield potential has been improved substantially since the early 1970s. However, *tongil*-type varieties are no longer grown in farmers' fields because of their poor palatability. Nowadays only *japonica* rice cultivars are cropped. *Japonica* varieties have been bred by incorporating semi-dwarf genes since the early 1980s and their rice yield in farmers' fields increased substantially up to 5t/ha in the early 1990s. Since then, yield of *japonica* rice varieties has stagnated at that level. Therefore, it is needed to break the yield barrier and increase the yield potential of *japonica* rice varieties.

* **To whom correspondence should be addressed**
Byun-Woo Lee
E-mail: leebw@snu.ac.kr
Tel: +82-2-880-4544 / Fax: +82-2-873-2056

The incorporation of semi-dwarf genes has brought about rice yield increases through an increased harvest index. However, the harvest index of the current varieties has almost reached a maximum, with little room remaining for further improvement (Horton 2000; Mann 1999; Murchie et al. 1999; Ying et al. 1999). For the increase of yield potential, the traits conferring higher biomass production should be identified and introduced in rice breeding programs (Cassman 1994; Peng et al. 2000; Ying et al. 1999). One of the candidate traits is functional stay-green that gives longer duration of active green leaf areas, continues photosynthesis for longer time than normal varieties, and might therefore be expected to result in higher biomass and grain yield. The functional stay-green trait can be divided into two types (A and B) on the basis of its behavior during leaf senescence (Thomas and Howarth 2000; Thomas and Smart 1993). Type A shows delayed induction of senescence, but the rate of chlorophyll degradation is the same as in the wild-type

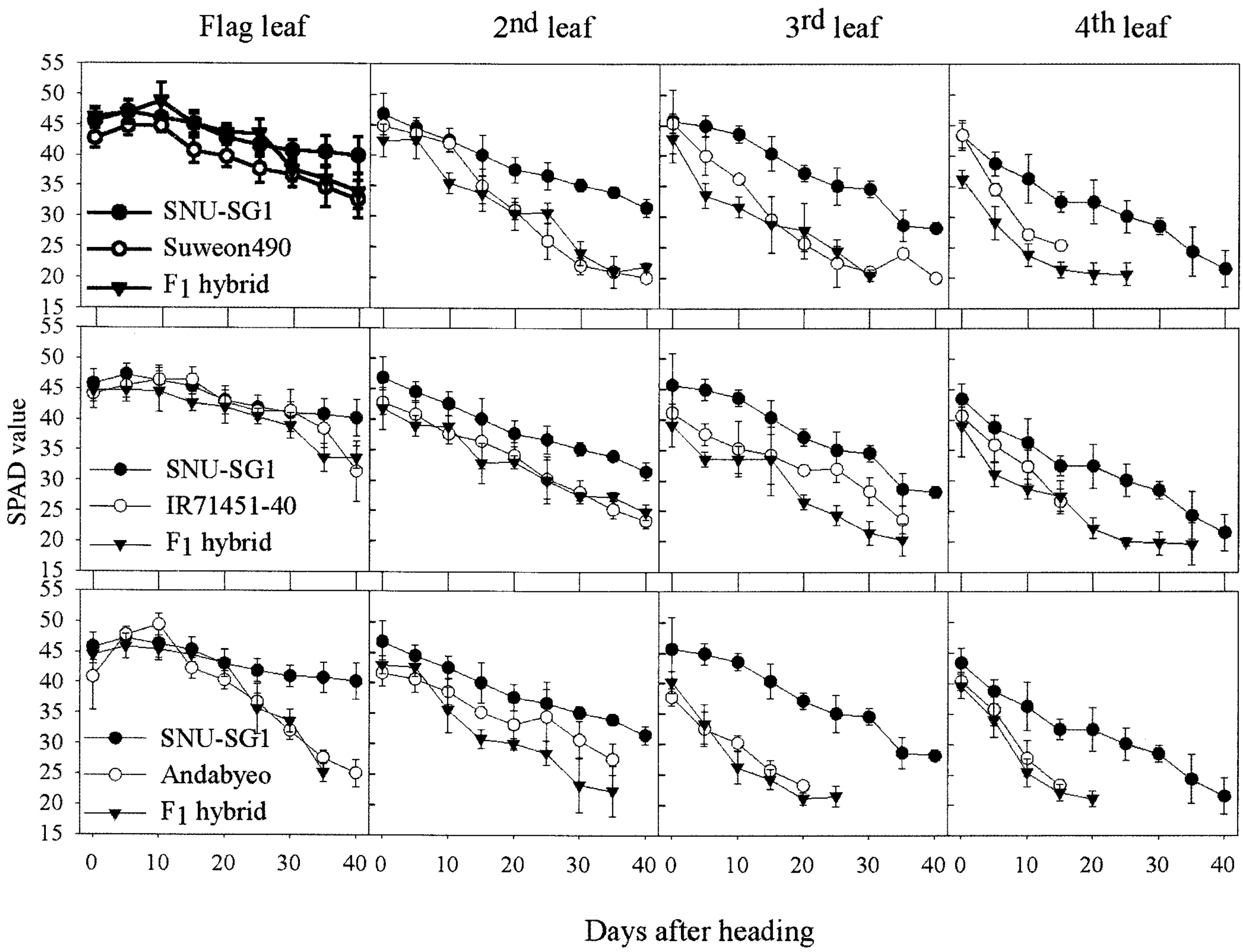

Fig. 1. Changes in SPAD value of upper four leaves during grain filling in three F_1 hybrids: SNU/Suweon490, SNU/IR71451-40, and SNU/Andabyeo, and their male parent: SNU-SG1 and female parents: Suweon490, IR71451-40, and Andabyeo.

after senescence induction. Type B initiates senescence at the same time, but the decrease of chlorophyll content and photosynthetic activity is much slower. Another stay-green phenotype is non-functional stay-green that is defined as a trait maintaining leaf greenness but lacking photosynthetic competence. Due to the possible contribution of the stay-green trait to crop production, it has been intensively studied in many crops such as sorghum (Walulu 1994), soybean (Pierce et al. 1984), maize (Gentinetta et al. 1986), kidney bean (Fang et al. 1998), durum wheat (Spano et al. 2003), and potato (Schittenhelm et al. 2004). In particular, Spano et al. (2003) reported that functional stay-green genotypes in durum wheat maintaining longer photosynthetic activity have higher seed weights and more grain yield per plant than the parental genotype. In rice, Cha et al. (2002) mapped a single recessive mutant gene, *sgr*, for non-functional stay-green in rice. Thereafter, several researchers analyzed the genetic basis of the stay-green and detected main-effect QTLs for stay green trait in rice (Abdelkhalik et al. 2005; Jiang et al. 2004; Yue et al. 2006). Park and Lee (2003) found a stay-green variant from the *japonica* rice collections, designated 'SNU-SG1', and classified it as the functional Type B stay-green *japonica* rice, which initiates leaf senescence on schedule but thereafter photosynthetic rate and chlorophyll content are higher and decrease much more slowly than several high-yielding regular *japonica* cultivars during grain filling. Yoo et al. (2007) analyzed and detected a few main-effect QTLs for functional stay-green in SNU-SG1. However, SNU-SG1 is not fully understood for the physiological basis of its functional stay-green characteristics.

The objective of this study was to characterize the physiological basis of the functional stay-green rice 'SNU-SG1'. We examined the changes in chlorophyll content (SPAD value), photosynthetic rate, mesophyll conductance, efficiency of photosystem II (PSII) in the upper four leaves or flag leaf in SNU-SG1, three regular varieties, and three F_1 hybrids crossed between SNU-SG1 and the regular varieties grown under growth chamber conditions.

Materials and Methods

Plant materials

The functional stay green rice 'SNU-SG1' that was found from the *japonica* rice collections (Park and Lee 2003), three high-yielding rice varieties; "Suweon490 (*japonica*)", "IR71451-40 (NPT)" and "Andabyeo (*tongil* type)", and three F_1 hybrids; "SNU-SG1/Suweon490", "SNU-SG1/IR71451-40", and "SNU-SG1/Andabyeo" were seeded on April 27 and transplanted on May 24, 2004 to 1/5000a Wagner pot. Pots were placed in the greenhouse until 7 d before heading and moved to growth chamber (temperature: 25 °C, humidity: 50%, lighting: 500 μmol PPFD $m^{-2}s^{-1}$).

Measurement of SPAD value, photosynthesis, and its related characteristics

SPAD value and net photosynthetic rate at light saturation (P_{max}) were measured nine times for upper four leaves at an interval of 5 days from heading. The SPAD value was taken with a chlorophyll meter (SPAD-502, Minolta, Japan) and recorded as a mean of six measurements for each individual leaf between 11:00 and 14:00. P_{max} was measured between 11:00 and 14:00 with a Li-Cor 6400 portable photosynthesis measurement system (Li-Cor, Lincoln, NE, USA) at CO_2 concentration of 350 ppmv, flow rate of 500 μmol s^{-1} and PPFD of 1500 μmol m^{-2} s^{-1}. Block temperature was set to 25 °C and ambient relative humidity was maintained at approximately 60%. Leaf temperature was slightly below that of block temperature. Irradiance was provided by a red LED array (Li-Cor).

Mesophyll conductance (C_m) was calculated by equation ① and ②, where F_n is net photosynthesis rate, C_i is internal CO_2 concentration, τ is CO_2 compensation point.

$$\tau = 40 \cdot Q_{10}^{(T-20)/10} \quad [\text{ppmv}] \qquad ①$$

$$C_m = \frac{F_n}{C_i - \tau} \quad [\mu\text{mol } CO_2 m^{-2} s^{-1}] \qquad ②$$

Photosynthesis-photosynthetic photon flux density (PPFD) response curves were constructed for flag leaf at 10 and 35 d after heading by measuring photosynthesis at PPFD of 2000 to 0 μmol $m^{-2}s^{-1}$ (2000, 1750, 1500, 1400, 1250, 1200, 1000, 900, 700, 400, 200, 100, 50, and 0 μmol $m^{-2}s^{-1}$).

Chlorophyll fluorescence of flag leaf was measured at an interval of 5 d during grain filling with a portable PAM2000 chlorophyll fluorometer (Heinz Walz, Germany) as described by Fukushima et al. (2001). Minimum fluorescence (F_o) and maximum fluorescence (F_m) were measured in the dark-adapted leaves, using a 2-second light pulse (3000 μmol photons $m^{-2}s^{-1}$ in the range of 350 to 700 nm) to saturate all photosystem II (PSII) reaction centers. The photochemical efficiency of PSII was calculated as the ratio of variable fluorescence ($F_v = F_m - F_o$) to maximum fluorescence (F_m) to determine the potential activity of PSII (F_v/F_m) as previously described by Genty et al. (1989) and Kooten and Snel (1990).

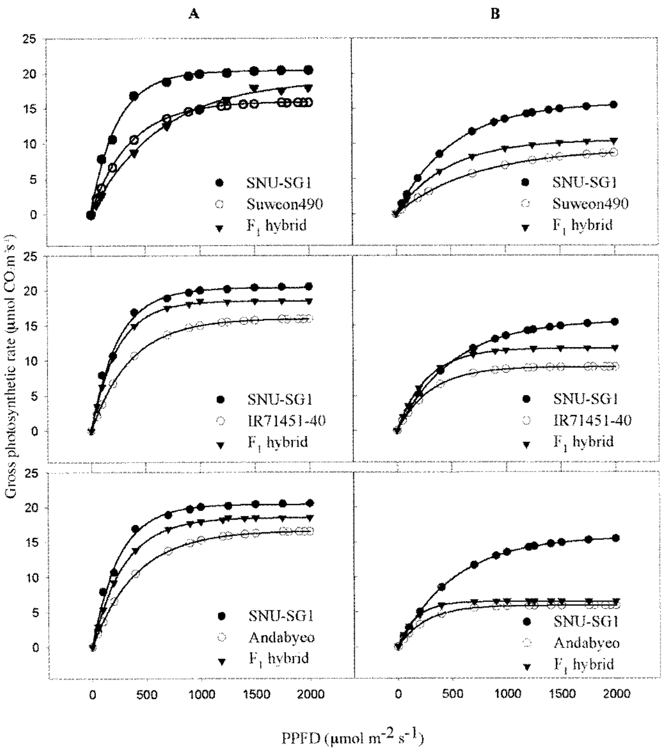

Fig. 2. Responses of gross photosynthetic rate to photosynthetic photon flux density (PPFD) in the flag leaves at 10 (A) and 35 d after heading (B) in three F_1 hybrids: SNU-SG1/Suweon490, SNU-SG1/IR71451-40, and SNU-SG1/Andabyeo, and their male parent: SNU-SG1 and female parents: Suweon490, IR71451-40, and Andabyeo.

Data analysis

Analysis of variance and Tukey's test were applied to experimental results to determine the significance of differences between varieties by SAS 9.1 version (SAS Institute 2003). Sigmaplot 9.0 (Systat Software 2004) was used for all the figures and regression analysis.

Results

Chlorophyll content (SPAD value)

The changing pattern of SPAD value during grain filling was significantly different among the tested genotypes (Fig. 1). For the flag leaf, SPAD value reached the maximum around 5 to 10 d after heading (DAH) and thereafter began to decrease in all genotypes tested. However, its decreasing rate was very different according to genotypes, being the lowest in SNU-SG1 and the highest in Andabyeo. SNU-SG1 not only had a higher SPAD value but also sustained it longer throughout grain filling, showing a SPAD value of above 40 even at 40 d after heading. SPAD value of leaf 2 to 4 decreased from heading and the decreasing rate was slower in the order of leaf 2, 3, and 4 and slowest in SNU-SG1 among genotypes. SNU-SG1 also had higher SPAD value of 2 to 4 leaf than the regular cultivars and F_1 hybrids that

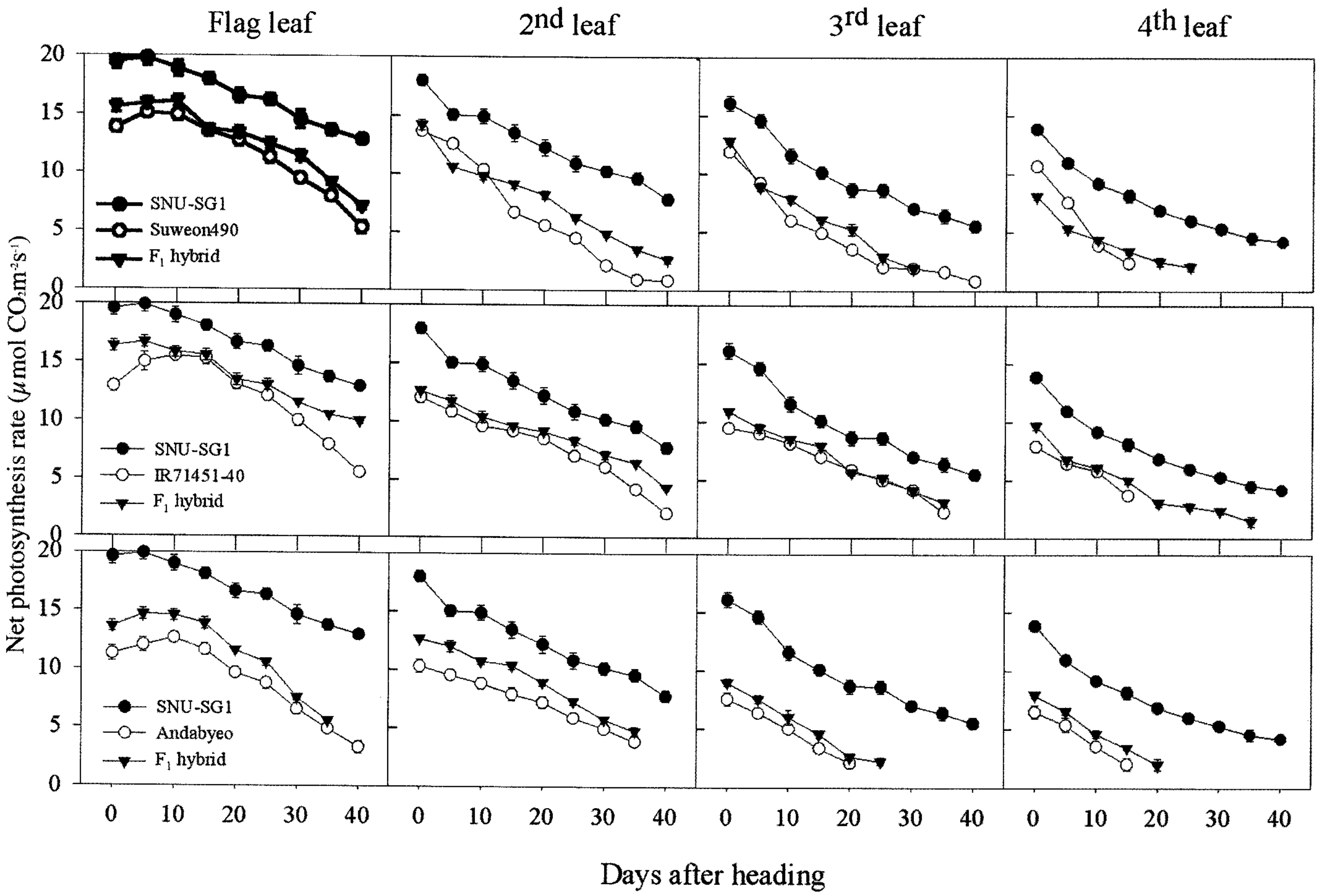

Fig. 3. Changes in light-saturated net photosynthesis (Pmax) of upper four leaves during grain-filling in three F_1 hybrids: SNU/Suweon490, SNU/IR71451-40, and SNU/Andabyeo, and their male parent: SNU-SG1 and female parents: Suweon490, IR71451-40, and Andabyeo.

Table 1. Parameters of photosynthesis-light response curve in flag leaf at 10 and 35 d after heading DAH) in three F_1 hybrids: SNU-SG1/Suweon490, SNU-SG1/IR71451-40, and SNU-SG1/Andabyeo, and their male parent: SNU-SG1 and female parents: Suweon490, IR71451-40 'and Andabyeo.

Varieties and F_1 hybrids		10DAH			35DAH		
		F_{gmax} [a]	α [b]	R_d [c]	F_{gmax}	α	R_d
Parents	SNU-SG1	20.76	0.0502	1.56	15.82	0.0304	0.82
	Suweon490	16.02	0.0442	1.52	9.29	0.0128	0.49
	IR71451-40	16.65	0.0417	1.45	9.13	0.0294	0.43
	Andabyeo	15.03	0.0328	1.63	5.92	0.0236	0.52
F_1 hybrids	SNU-SG1/Suweon490	18.88	0.0300	1.78	10.54	0.0223	0.44
	SNU-SG1/IR71451-40	18.52	0.0766	1.82	11.76	0.0422	0.36
	SNU-SG1/Andabyeo	16.98	0.0638	1.88	6.51	0.0399	0.41
	$LSD_{0.05}$	3.1	0.0142	0.34	3.8	0.0126	0.31

$$F_g = F_n + R_d = F_{gmax} \times [1 - \exp\left(-\alpha \frac{I}{F_{gmax}}\right)]$$

[a] F_{gmax} : μmol CO_2 $m^{-2}s^{-1}$ (Maximum gross photosynthetic rate)
[b] α: μmol CO_2 μE^{-1} PAR (Initial light conversion factor)
[c] R_d: μmol CO_2 $m^{-2}s^{-1}$(Respiration rate)

showed faster senescence and thus not measurable SPAD value at later grain filling. F_1 hybrids had similar SPAD values of flag leaf to male parent SNU-SG1 during early to mid grain filling, while they had similar SPAD values to their female parents at late grain filling (40 DAH). For leaf 2 to 4, they showed SPAD value between the male and female parents.

Photosynthesis, mesophyll conductance, and photochemical efficiency

The responses of gross photosynthetic rate (F_g) to photosynthetic photon flux density (PPFD) in the flag leaf at 10 DAH and 35 DAH were presented in Fig. 2 A and Fig. 2 B, respectively and the parameter values of the response curve in Table 1. Light saturation point (LSP) of F_g was estimated at PPFD of 1000 to 1500 μmol photon $m^{-2}s^{-1}$ in all genotypes and measuring dates except in Andabyeo and its F_1 hybrid with SNU-SG1 with very low LSP of about 500 μmol photon $m^{-2}s^{-1}$ at 35 DAH measurement. SNU-SG1 had significantly greater light-saturated F_g (F_{gmax}) at 10 and 35 DAH than the female parents and F_1 hybrids. F_1 hybrids showed F_{gmax} similar to the average of their parents. And the decreasing rate of F_{gmax} with leaf senescence was slower in SNU-SG1 than F_1 hybrids and their female parents. SNU-SG1 exhibited higher initial light conversion efficiency (ILCE) than in HYVs. ILCE in F_1 hybrids was similar to or less than SNU-SG1 in intra-subspecific hybrid (SNU-SG1 x Suwon490) while

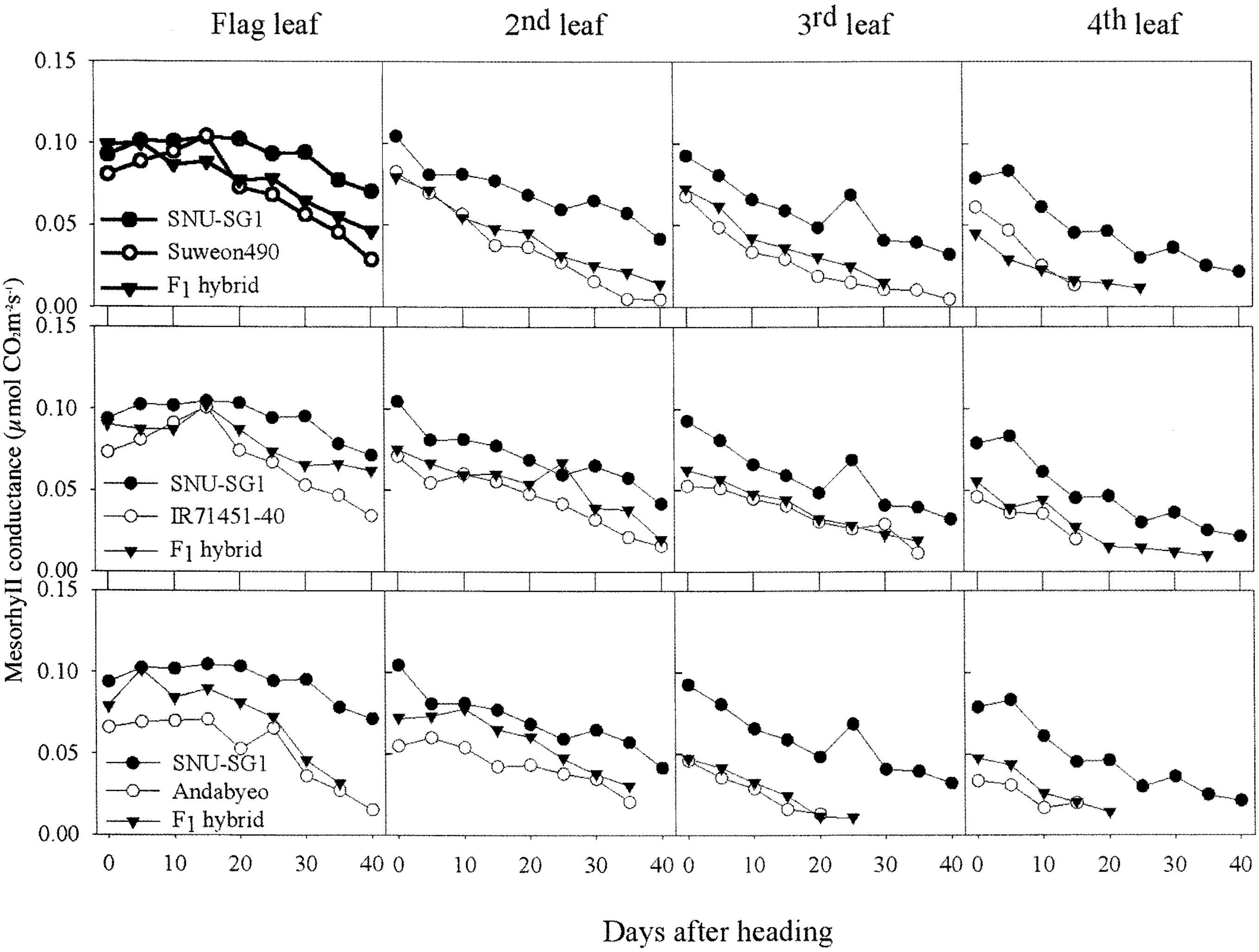

Fig. 4. Changes in mesophyll conductance of upper four leaves during grain filling in three F_1 hybrids: SNU/Suweon490, SNU/IR71451-40, and SNU/Andabyeo, and their male parent: SNU-SG1 and female parents: Suweon490, IR71451-40, and Andabyeo.

it was significantly higher in inter-subspecific hybrids (SNU-SG1 x Andabyeo and SNU-SG1 x IR71451-40)

As shown in Fig. 3, photosynthesis at light saturation (P_{max}) of upper four leaves in SNU-SG1 were much higher and sustained longer during grain filling than the regular cultivars and F_1 hybrids. For the flag leaf, P_{max} reached the maximum around 10 d after heading (DAH) and thereafter began to decrease in all the tested genotypes. However, SNU-SG1 exhibited slower decreasing rate and maintained much higher Pmax throughout grain filling than the other genotypes. SNU-SG1 showed P_{max} of above 14 μmol CO_2 $m^{-2}s^{-1}$ even at 40 d after heading. P_{max} of leaf 2 to 4 has decreased from heading and the decreasing rate was similar in all genotypes. However SNU-SG1 also sustained a higher P_{max} of leaf 2 to 4 than the regular cultivars and F_1 hybrids that showed faster senescence and thus not measurable Pmax at later grain filling stage. F_1 hybrids had P_{max} of upper four leaves a little higher than but close to female parents.

The changing pattern of mesophyll conductance (C_m) during grain filling was significantly different among the tested genotypes (Fig. 4). For the flag leaf, C_m reached the maximum between 5 to 15 d after heading (DAH) and thereafter began to decrease in all the genotypes tested. However, its decreasing rate was very different according to genotypes, being the significantly lowest in SNU-SG1 and the highest in Andabyeo. SNU-SG1 not only had higher C_m but also sustained it longer throughout grain filling. SNU-SG1 maintained C_m at the level of above 75% even at 40DAH as compared to that at heading. C_m of leaf 2 to 4 decreased steadily from heading and the decreasing rate was not different among genotypes. Nevertheless, SNU-SG1 maintained higher C_m of leaf 2 to 4 throughout grain filling as it had much higher C_m at heading. F_1 hybrids had C_m of upper four leaves between the male and female parents but close to female parents.

Temporal changes of chlorophyll fluorescence were measured for flag leaf during grain filling. SNU-SG1 showed slower decline of PSII photosynthetic competence as represented by the F_v/F_m ratio and maintained much higher level of F_v/F_m ratio during grain filling while other regular varieties exhibited very rapid decrease of F_v/F_m ratio with senescence after heading (Fig. 5). F_1 hybrids followed similar changes in F_v/F_m ratio to male parent SNU-SG1.

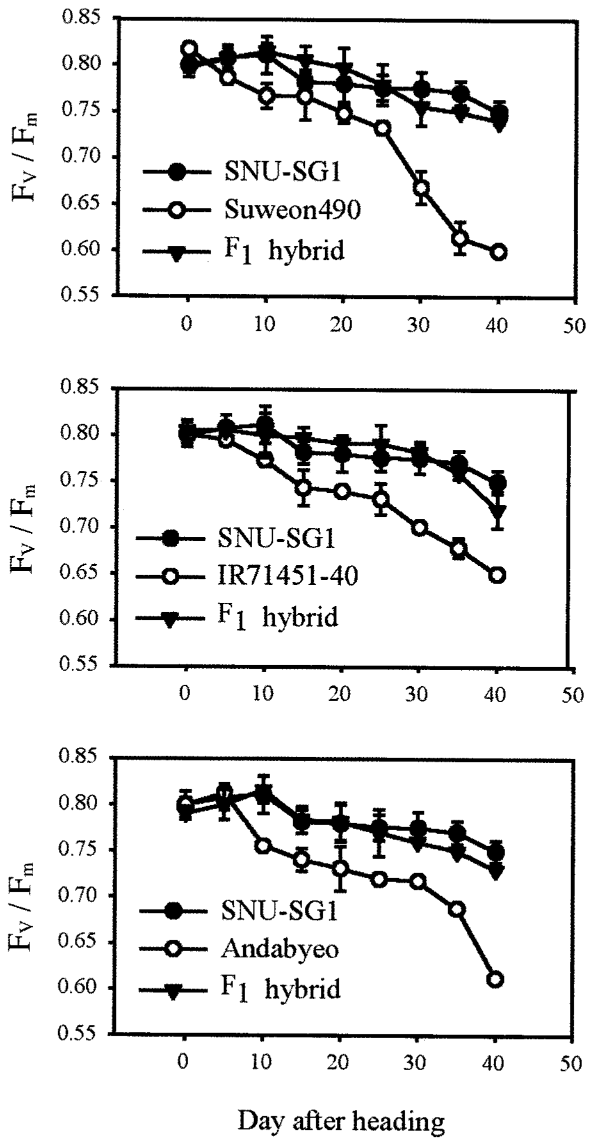

Fig. 5. Changes in the maximal efficiency of PSII photochemistry (F_v/F_m) of flag leaf during grain filling in three F_1 hybrids: SNU-SG1/Suweon490, SNU-SG1/IR71451-40, and SNU-SG1/Andabyeo, and their male parent: SNU-SG1 and female parents: Suweon490, IR71451-40, and Andabyeo.

Discussion

Leaf senescence that is the final stage of leaf development is accompanied by leaf-yellowing due to chlorophyll degradation in chloroplasts and a series of other biochemical and physiological changes (Matile 1992). However, these changes vary among varieties in terms of the degree and rate of leaf senescence. SNU-SG1 not only had higher chlorophyll content (SPAD value) but also sustained it longer throughout grain filling than the regular cultivars and F_1 hybrids (Fig. 1), showing that the process of leaf senescence was delayed in SNU-SG1. Delayed senescence, or stay-green of leaf can be generally divided into two groups, functional and non-functional (Thomas and Howarth 2000). A functional stay-green is defined as retaining both leaf greenness and photosynthetic competence much longer during leaf senescence, while non-functional stay-green is defined as maintaining only leaf greenness (Thomas and Howarth 2000; Thomas and Smart 1993). SNU-SG1 had significantly greater light-saturated gross photosynthetic rate (F_{gmax}) at early (10DAH) and late phase (35DAH) of grain filling than regular varieties and F_1 hybrids, and also exhibited higher initial light conversion efficiency (ILCE) (Fig.2, Table 1). Similarly, the SNU-SG1 had much higher net photosynthetic rate at light saturation (P_{max}) and sustained it longer in upper four leaves during grain filling than the regular cultivars and F_1 hybrids (Fig. 3). These results for SNU-SG1 answer the definition of functional stay-green that retains both chlorophyll content and photosynthetic competence much longer during leaf senescence (Thomas and Howarth 2000), suggesting that SNU-SG1 exhibited a typical characteristic of functional stay-green. Similar results were also observed for SNU-SG1 under field conditions (Park and Lee 2003).

Most previous studies have shown that the mesophyll conductance was linearly correlated with photosynthetic capacity (Evans and Loreto 2000; Park and Lee 2003; Siddique et al. 1999). SNU-SG1 had not only higher mesophyll conductance but also sustained it longer throughout grain filling, maintaining it at the level of above 75% even at 40DAH as compared to that at heading (Fig. 3). Changes in Rubisco and/or Rubisco activity and electron transport lead to changes in mesophyll conductance (Flowers et al. 2007). The ratio of Rubisco to total leaf nitrogen content is not influenced by environmental changes (Mae 1997) and also Rubisco content is reported to be controlled by the same QTL controlling soluble protein content (Ishimaru et al. 2001). Thus, it can be inferred, although not measured directly, that SNU-SG1 would sustain a higher amount of Rubisco during grain filling as it was reported to have much higher leaf nitrogen and soluble protein content throughout grain filling (Fu 2008; Park and Lee 2003). The F_v/F_m ratio representing the maximal efficiency of PSII photochemistry was measured because photosynthesis depends on the function of the light-harvesting and electron transport systems within the chloroplasts. SNU-SG1 showed slower decline of F_v/F_m ratio and maintained much higher level of F_v/F_m ratio in flag leaf during grain filling while other regular varieties exhibited very rapid decrease with senescence after heading (Fig. 5). This suggested that the sustained high photosynthetic competence of SNU-SG1 during grain filling could be ascribed to the longer maintenance of high mesophyll conductance due to the delayed degradation of Rubisco and electron transport system.

All three F_1 hybrids followed similar changes in F_v/F_m ratio of flag leaf to the male parent SNU-SG1. However, F_1 hybrids exhibited a photosynthetic rate significantly lower than SNU-SG1 and slightly higher than the female parents as they might have followed the decline in dark reactions of the Calvin cycle of the mid parents. Rate of degradation in chlorophyll-protein complexes of PSI was greater than in chlorophyll-protein complexes of PSII during flag leaf senescence of rice (Tang et al. 2005). These suggest that the degradation of Rubisco and photochemical system during leaf senescence is controlled by different genetic mechanisms (Ishimaru et al. 2001; Thomas et al. 2002).

In conclusion, as compared with regular varieties, the SNU-SG1 sustained high photosynthetic competence, mesophyll conductance, and photochemical efficiency (F_v/F_m) as well as leaf chlorophyll content throughout grain filling. These results suggest that a functional stay-green trait in SNU-SG1 can be utilized for increasing rice yield potential through the improved dry matter production during grain filling.

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