

Photosynthetic Traits of Upper Three Leaves in the Vietnamese F₁ Hybrid Rice Vietlai 45 and Its Parents during the Ripening Period

Hamaoka, Norimitsu

Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University

Araki, Takuya

Faculty of Agriculture, Ehime University

Kumagai, Etsushi

National Agricultural Research Center for Tohoku Region

Hanh, Tang Thi

Faculty of Agronomy, Hanoi University of Agriculture

他

<https://doi.org/10.5109/22044>

出版情報：九州大学大学院農学研究院紀要. 57 (1), pp.27-33, 2012-02. Faculty of Agriculture, Kyushu University

バージョン：

権利関係：

Photosynthetic Traits of Upper Three Leaves in the Vietnamese F₁ Hybrid Rice Vietlai 45 and Its Parents during the Ripening Period

Norimitsu HAMAOKA^{1*}, Takuya ARAKI², Etsushi KUMAGAI³,
Tang Thi HANH⁴, Pham Van CUONG⁴ and Osamu UENO¹

Laboratory of Plant Production Physiology, Division of Agricultural Bioresource Sciences,
Department of Bioresource Sciences, Faculty of Agriculture,
Kyushu University, Hakozaki, Fukuoka 812–8581, Japan
(Received October 14, 2011 and accepted November 9, 2011)

Vietlai 45 (VL45) is F₁ hybrid rice that has been recently introduced in Vietnam. Despite its high yield potential, the grown period is about two weeks shorter than the parents. To elucidate the factor resulting in this superior trait, we investigated photosynthetic traits in upper three leaves including the flag leaf (FL) during the ripening period in VL45 in comparison with its female and male parents, 103S and R45. At 3 days after heading (DAH), shoot dry weight was higher in VL45 than in the parent cultivars, whereas the leaf morphological traits of VL45 were similar to those of R45. The nitrogen content in leaves tended to be higher in VL45 than in R45 at 14 DAH. At 24 DAH, the FL of VL45 showed higher net photosynthetic rate (P_n) and stomatal conductance (G_s) than those of the parents, with a symptom of senescence in other leaves. Values of chlorophyll fluorescence parameters did not greatly differ among the three cultivars. P_n per unit soluble protein content (P_n/SP) in the FL was much higher in VL45 than in the parents at 24 DAH. Thus, it seems that higher P_n in the FL of VL45 is because of high G_s and P_n/SP . These data demonstrate that VL45 shows the heterosis effect on the dry matter production until heading and the maintenance of high P_n in the FL at late ripening stage, which would contribute to the superior agronomic trait of VL45.

Key words: chlorophyll fluorescence, heterosis, *Oryza sativa* L., photosynthetic rate, Vietnam

INTRODUCTION

With increasing population in Asian countries, breeding of new high yielding rice cultivars and extension of cultivating area are required to increase rice productivity. Recently, F₁ hybrid rice, which exhibits high productivity due to hybrid vigor (heterosis), has been paid much attention in Asia. These cultivars generally show higher dry matter production and grain yield than their parent lines and standard selfed lines (Hirao *et al.*, 1995; Pham *et al.*, 2004; Peng *et al.*, 2008). The cultivation of hybrid rice flourishes remarkably in China and accounts for more than 50% of the rice-cultivated area (International Rice Research Institute, 2007). In China, super hybrid rice cultivars of heavy panicle type have been recently released and achieved a grain yield of more than 12 t ha⁻¹ (Cheng *et al.*, 2007; Peng *et al.*, 2008). Under subtropical conditions, their grain yield is about 12% higher than those of Chinese high yielding inbred lines and other hybrid lines (Zhang *et al.*, 2009). Such raising yield potential in rice by using heterosis leads to an increase in demand of hybrid rice in the Asian countries. However, Zhang *et al.* (2009) have reported that Chinese super

hybrid rice cultivars show lower ratios of ripened grains than inbred cultivars. Their observation suggests that in these hybrid cultivars, the source ability is relatively lower than the sink capacity. Therefore, the improvement of photosynthetic ability at the ripening period is needed for further increase in grain yield of hybrid cultivars.

Previous studies on rice plants showed that photosynthetic ability during the ripening period is closely correlated with yield potential. It is considered that 60–100% of carbon accumulated in grains come from leaf photosynthesis after heading (Yoshida, 1981). In this term, a major source organ is upper three leaves including the flag leaf (FL), and the FL is the most contributor for grain production (Zhang and Kokubun, 2004). However, it is pointed out that the degree of contribution of the upper two leaves excluding the FL to grain production differs depending on cultivars and cultivation conditions (Murata and Matsushima, 1975). Therefore, it is important to evaluate photosynthetic traits of the upper two leaves as well as the FL during the ripening period.

In rice leaves, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) accounts for about 25% of total nitrogen (N) content and 50% of total soluble protein content, but becomes a limiting factor of photosynthesis because of low maximum specific activity (Makino *et al.*, 1984; Makino *et al.*, 1985). The photochemical activity is also one of indicators to evaluate photosynthetic ability. It is well known that photosynthetic ability is reduced by photoinhibition (Osmond *et al.*, 1997). Wang *et al.* (2005) reported that Chinese super-rice hybrid cultivars had higher tolerance for photoinhibition than a traditional hybrid rice cultivar. Hanh *et al.* (2008a) found that the FL of a Vietnamese F₁ hybrid rice showed higher

¹ Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, Hakozaki, Higashi-ku, Fukuoka 812–8581, Japan

² Faculty of Agriculture, Ehime University, Tarumi, Matsuyama, Ehime 790–8566, Japan

³ National Agricultural Research Center for Tohoku Region, Akahira, Shimokuriyagawa, Iwate 020–0198, Japan

⁴ Faculty of Agronomy, Hanoi University of Agriculture, Hanoi, Vietnam

* Correspondence author (E-mail: hama-noorii@hotmail.co.jp)

photochemical efficiency than that of the male parent during the ripening period. These data suggest that heterosis effects in photosynthetic ability may appear in the photochemical system as well. Therefore, analyses of both the photochemical and carboxylation reactions are required for the evaluation of photosynthetic ability in hybrid rice.

The cultivation system with F_1 hybrid rice has been recently introduced in Vietnam. The hybrid rice cultivar, Vietlai 20 (VL20), was registered as the first national cultivar of hybrid rice in Vietnam and has been widely cultivated in the northern provinces (Hanh *et al.*, 2008a). Vietnamese F_1 hybrid cultivars including VL20 are characterized by about two weeks shorter growth duration than the parent strains. Nevertheless, they show high yield potential, which is comparable to that in the male parents known as high yield cultivars (Hanh *et al.*, 2008a). The shorter growth duration of the F_1 hybrid rice allows farmers to cultivate three crops during a year, namely, cultivations of rice in spring and autumn with that of some winter crop (Hanh *et al.*, 2008a). This would result in an increase in income of farmers. In such background, Vietlai 45 (VL45) was produced as a candidate of next national cultivar in Vietnam. It has a trait of somewhat panicle-weight type and larger FL than previous Vietnamese hybrid rice cultivars. However, the physiological basis of high yield potential in VL45 has not yet been investigated. Until the heading stage, VL20 produces a greater amount of dry matter than the parents. During the ripening period, VL20 shows higher photosynthetic rate and photosynthetic nitrogen use-efficiency in the FL than the parents (Hanh *et al.*, 2008a, 2008b). Previous studies on photosynthetic traits of hybrid rice during the ripening period examined only the FL (Chen *et al.*, 2004; Zhang *et al.*, 2007). However, we should investigate the upper three leaves including the FL for better understanding of the heterosis effect on photosynthetic ability.

In this study, we evaluated the heterosis effect on the source ability in VL45 during the ripening period on the basis of the photosynthetic and morphological traits of the upper three leaves in comparison with its parents.

MATERIALS AND METHODS

F_1 hybrid and parent cultivars

The F_1 hybrid rice VL45 and its parental cultivars 103S and R45, which were the indica type, were made in Vietnam. The female parent 103S is a thermo-sensitive genic male sterile (TGMS) line, while the male parent R45 is a restorer line with high yield production. The growth duration of VL45 is shorter about 14 days than those of the parents under similar growth conditions.

Plant cultivation

VL45 and its parents were sown on 21 May, 2008. Seeds of them were incubated at 30°C for 48 hours for germination, and then the germinated seeds were sown in nursery boxes in a glasshouse at the beginning of June. At three weeks after sowing, the seedlings were trans-

planted into 5-liter pots (one seeding per pot) filled with sandy loam soil. As basal dressing, 0.96 g N, 1.6 g P and 1.6 g K per pot were applied in from of $(\text{NH}_4)_2\text{S}$, P_2O_5 and K_2O , respectively. In addition, top-dressed N was applied at the 40 days after transplanting at the rate of 0.64 g pot^{-1} . Afterwards, plants were grown outdoors with sufficient water, and their growth was periodically surveyed. From 40 days after transplanting, the daylength was controlled 10 h of light period/14 h of dark period in a photoperiod-controlling apparatus set outdoors. In this study, upper three leaves, the FL, a leaf below the FL, namely, the second leaf from the top (2L) and a leaf below the 2L, namely, the third leaf from the top (3L), of a main stem in a plant were examined. Three or four plants per cultivar were used for measurements of photosynthetic gas exchange, chlorophyll (Chl) fluorescence, and contents of soluble protein (SP) and Chl. The measurements were conducted on 3, 14 and 24 days after heading (DAH), beginning on 1 September at the heading stage and ending on 26 September at the late-ripening stage.

Measurements of morphological traits and leaf N content

After measurements of gas exchange rate and Chl fluorescence at each stage, three or four plants per cultivar were sampled. At 3 DAH, the tillering number, total leaf area and shoot dry weight were measured. The leaf area was measured by use of an automatic area meter (AAM-8, Hayashi-denko, Japan). In addition, the upper three leaves were detached to measure leaf area, specific leaf area (SLA), leaf length and leaf width. All the samples were oven-dried at 80°C for 3 days for measurement of dry weight and determination of SLA. The upper three leaves collected at 3 and 14DAH were powdered, and N content per leaf area was determined according to the semi-micro Kjeldahl procedure.

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_2 concentration, 380±13 $\mu\text{L L}^{-1}$; relative humidity, 60±2.6%; PPFD, 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The leaf area used in the measurements was 5.9 cm^2 , and the rate of airflow into the assimilation chamber was 706 $\mu\text{mol s}^{-1}$. The CO_2 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 per unit

leaf area (P_n) and stomatal conductance (G_s) were calculated according to the method of Long and Hallgren (1985). Using a leaf that was dark-adapted for 30 min, the initial fluorescence (F_0) under non-photosynthetic conditions was determined under a measuring beam ($3.2 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPF); thereafter, the maximal fluorescence (F_m) 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 $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPF. After the steady-state fluorescence (F_s) was recorded, a 0.8-s saturation pulse was applied to determine the maximum fluorescence in the light adapted state (F_m'). The following parameters were calculated: maximum quantum yield of PSII, $F_v/F_m = (F_m - F_0)/F_m$ (van Kooten and Snel, 1990); quantum yield of PSII electron transport, $\Phi_{\text{PSII}} = (F_m' - F_s)/F_m'$ (Genty *et al.*, 1989); and non-photochemical quenching, $\text{NPQ} = (F_m - F_m')/F_m'$ (Bilger and Björkman, 1990).

Contents of Chl and SP

After the measurements of gas exchange and Chl fluorescence, leaf discs of 5 mm diameter were sampled and frozen in liquid N, and stocked at -80°C . For the measurement of Chl content, three leaf discs were soaked in 96% ethyl-alcohol in the dark for two days until the leaf colour was lost. After this extraction procedure, Chl content in the ethyl-alcohol solution was estimated from the absorbances at 649 and 665 nm using a spectrophotometer (UV-1200, Shimadzu, Japan), according to the method described by Wintermans and de Mots (1965). For measurement of SP content, six leaf discs were used. These discs were powdered in liquid N with a mortar and pestle; then a chilled extraction buffer that contains 100 mM $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ (pH 7.0), 1 mM phenylmethanesulfonyl fluoride, 1% (w/v) insoluble polyvinylpyrrolidone, and 1% (v/v) 2-mercaptoethanol was added to it, and the powder was further ground. The obtained solution sample was transferred into Eppen tubes and applied to a centrifugation ($12000 \times g$, 5 min, 4°C). Then, Bradford reagent (Bio-Rad, USA) was added to the supernatant, and the SP content in the sample was determined by measurement of absorbance at 595 nm with the spectrophotometer, according to the method of Bradford (1976).

Statistic analysis

Data were statistically analyzed using one-way ANOVA with Tukey's test (Sigmastat 3.1 for Windows, Systat Software, Inc., Richmond, USA). Significant difference was tested at $P < 0.05$.

RESULTS

Morphological traits and shoot dry weight at 3 DAH

The tillering number of VL45 at 3 DAH was intermediate between those of the parents, R45 and 103S (Table 1). There was no significant difference in the total leaf area at 3 DAH among VL45, R45 and 103S (Table 1), but the total leaf area of VL45 approached to that of R45. The shoot dry weight at 3 DAH was significantly higher

Table 1. Tillering number, total leaf area and shoot dry weight of three cultivars at 3 days after heading (3 DAH). Mean values ($n=4$) within each column followed by the same letter are not significantly different ($P < 0.05$) by the Tukey's test

Cultivar	Tillering number (plant ⁻¹)	Total leaf area (m ² plant ⁻¹)	Shoot dry weight (g plant ⁻¹)
VL45	28.2 ab	0.55 a	79.8 a
R45	23.2 b	0.57 a	65.2 b
103S	35.4 a	0.49 a	59.9 b

Table 2. Leaf area, specific leaf area (SLA), leaf length and leaf width in upper three leaves (FL, flag leaf; 2L, leaf below FL; 3L, leaf below 2L) of three cultivars at 3 days after heading (3 DAH). Mean values ($n=4$) within each column followed by the same letter are not significantly different ($P < 0.05$) by the Tukey's test

Leaf	Cultivar	Leaf area (cm ² leaf ⁻¹)	SLA (cm ² g ⁻¹)	Leaf length (cm)	Leaf width (cm)
FL	VL45	55.3 a	311 a	33.9 a	2.51 a
	R45	59.6 a	312 a	33.7 a	2.60 a
	103S	32.7 b	253 b	25.2 b	1.87 b
2L	VL45	61.1 b	300 a	50.5 a	1.72 b
	R45	75.0 a	316 a	44.8 b	2.48 a
	103S	43.8 c	219 b	38.4 c	1.54 b
3L	VL45	58.6 b	288 a	50.4 a	1.51 b
	R45	74.5 a	309 a	49.9 a	1.82 a
	103S	44.6 c	251 b	45.2 a	1.45 b

in VL45 than in the parents (Table 1). Table 2 showed leaf area, SLA, leaf length and leaf width in the upper three leaves of the three cultivars at 3 DAH. The leaf areas of the upper three leaves were significantly higher in VL45 than in 103S. The leaf areas of 2L and 3L were lower in the VL45 than in R45, although there was no significant difference in that of FL between VL45 and R45. The SLAs in the upper three leaves of VL45 were similar to those of R45 but higher than those of 103S (Table 2). Thus, the upper three leaves of VL45 were greater and thinner than those of the female parent 103S and showed morphological traits rather similar to the male parent R45. This trend was also recognized in the data on leaf length and leaf width of these leaves (Table 2).

N contents of the upper three leaves at 3 and 14 DAH

Table 3 shows N contents per leaf area in the upper three leaves at 3 and 14 DAH. There was no significant difference in the N contents of leaves among the three cultivars at 3 DAH. At 14 DAH, however, the N content in the 2L of VL45 was intermediate between those of the parents. Since the female parent 103S is a TGMS line, it seems that 103S shows delayed senescence and main-

Table 3. Leaf N content in upper three leaves of three cultivars at 3 and 14 days after heading (3 and 14 DAH). Mean values (n=3–4) within each column followed by the same letter are not significantly different ($P \leq 0.05$) by the Tukey's test

Cultivar	N content (g m^{-2})					
	3DAH			14DAH		
	FL	2L	3L	FL	2L	3L
VL45	1.12 a	1.10 a	0.93 a	1.12 a	1.06 ab	0.95 a
R45	1.02 a	1.10 a	0.96 a	0.88 a	0.78 b	0.72 a
103S	1.18 a	1.32 a	1.15 a	1.21 a	1.33 a	1.09 a

tains higher N contents in leaves than VL45 and R45. Therefore, when N content was compared only between VL45 and R45 at 14 DAH, values of the upper three leaves were significantly higher in VL45 than in R45 ($P < 0.05$; data not shown).

P_n and G_s in the upper three leaves during 3 to 24 DAH

Figure 1 shows P_n in the upper three leaves of the three cultivars at 3, 14 and 24 DAH. P_n in the three leaves were not significantly different among the three cultivars at 3 DAH (Fig. 1A). At 14 DAH, P_n in the FL and 2L of 103S increased compared to those at 3 DAH (Fig. 1B). This is due probably to delayed leaf senescence in 103S. When P_n at 14 DAH was compared between VL45 and R45, there was no significant difference in P_n of the three leaves. However, a tendency was found that P_n in 2L and 3L of VL45 was somewhat lower than those of R45 (Fig. 1B). At 24 DAH, the FL of VL45 maintained higher P_n than that of R45 (Fig. 1C). P_n in the 3L of VL45 was not measured because of distinct senescence. The ratio of P_n at 24 DAH to P_n at 3 DAH, namely, the maintenance ratio of P_n from 3 to 24 DAH, in the FL was highest in VL45 (76%; Fig. 1C). However, the ratio in the 2L of VL45 (23%) was lower than that of 103S (64%) and almost the same with that of R45 (27%). The patterns

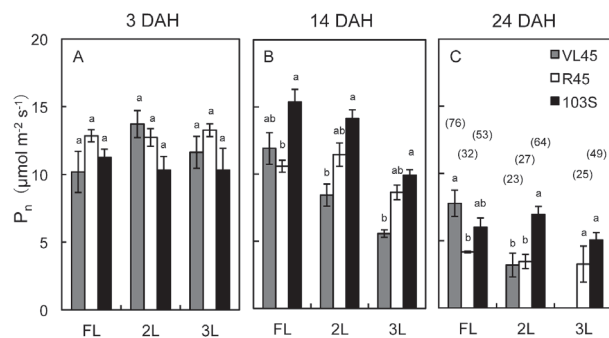


Fig. 1. Change in net photosynthetic rate (P_n) in upper three leaves (FL, flag leaf; 2L, leaf below FL; 3L, leaf below 2L) of three cultivars at 3, 14 and 24 days after heading (3, 14 and 24DAH). Values are given as the mean \pm SE (n=3–4). Bars followed by the same letters are not significantly different as determined by the Tukey's test at $P < 0.05$. Values in parentheses indicate ratios of P_n at 24 DAH to P_n at 3 DAH (%).

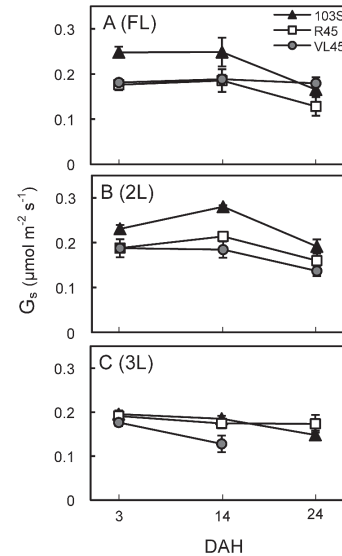


Fig. 2. Change in stomatal conductance (G_s) in upper three leaves of three cultivars at 3, 14 and 24 days after heading (3, 14 and 24 DAH). Values are given as the mean \pm SE (n=3–4).

of change in G_s of the upper three leaves in VL45 from 3 to 24 DAH was approximately similar to those in P_n (Fig. 2). G_s in the FL of VL45 maintained a constant value even at 24 DAH, contrasting to G_s in the FLs of parents (Fig. 2A). With respect to the 2L and 3L (Fig. 2B, C), the extents of decrease in G_s of VL45 were larger than those of R45. G_s in the FL and 2L of 103S tended to be higher than those of VL45 and R45 (Fig. 2A, B).

Chl fluorescence parameters in the upper three leaves at 24 DAH

At 24 DAH, F_v/F_m , Φ_{PSII} and NPQ in the FL and 2L were compared among the three cultivars (Table 4). Except for NPQ in the FL, all the Chl fluorescence param-

Table 4. Maximal quantum yield of PSII (F_v/F_m), quantum yield of PSII electron transport (Φ_{PSII}) and non-photochemical quenching (NPQ) in upper three leaves of three cultivars at 24 days after heading (24 DAH). Mean values (n=3–4) within each column followed by the same letter are not significantly different ($P \leq 0.05$) by the Tukey's test

Leaf	Cultivar	F_v/F_m	Φ_{PSII}	NPQ
FL	VL45	0.750 a	0.153 a	2.50 b
	R45	0.735 a	0.155 a	2.75 a
	103S	0.741 a	0.173 a	2.66 ab
2L	VL45	0.686 a	0.111 a	2.79 a
	R45	0.686 a	0.113 a	2.28 a
	103S	0.725 a	0.153 a	2.40 a
3L	VL45	–	–	–
	R45	0.682 b	0.119 b	1.80 a
	103S	0.725 a	0.165 a	2.67 a

eters in the FL and 2L were not significantly different among them. Therefore, heterosis effect was not found in activity of the photochemical system at 24 DAH. The Chl fluorescence parameters in 3L of VL45 were not measured because of distinct senescence. In the 3L, F_v/F_m and Φ_{PSII} of R45 were lower than those of 103S. At 3 and 14 DAH also, F_v/F_m , Φ_{PSII} and NPQ in the FL were not significantly different among the three cultivars (data not shown).

Chl and SP contents and P_n per unit Chl and SP contents in the upper three leaves at 24 DAH

The Chl contents in the FL at 24 DAH were not significantly different among the three cultivars, but Chl content in the 2L of VL45 was significantly lower than that of 103S (Fig. 3A). On the other hand, Chl contents in the upper three leaves of the parent cultivars were not significantly different from each other. The SP contents in the upper three leaves of 103S were higher than those of VL45 and R45 (Fig. 3B). The SP contents in the FL and 2L of VL45 were almost the same to those of R45. P_n per unit Chl content (P_n/Chl) in the upper three leaves were not significantly different among the three cultivars, although P_n/Chl in the FL of VL45 tended to be higher than that of R45 (Fig. 3C). In contrast, P_n per unit SP content (P_n/SP) in the FL of VL45 was much higher than those of the parents (Fig. 3D). In the 2L and 3L, there was no cultivar difference in P_n/SP .

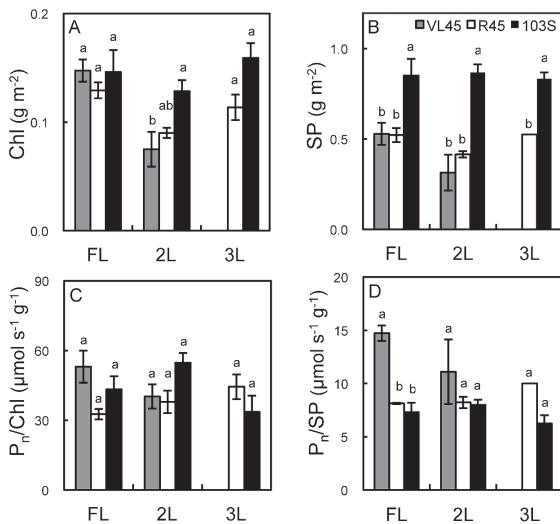


Fig. 3. Chlorophyll (Chl) content, soluble protein (SP) content, net photosynthetic rate per unit Chl content (P_n/Chl) and net photosynthetic rate per unit SP content (P_n/SP) in upper three leaves of three cultivars at 24 days after heading (24 DAH). Values are given as the mean \pm SE ($n=3-4$). Bars followed by the same letters are not significantly different as determined by the Tukey's test at $P<0.05$.

DISCUSSION

In this study, we investigated photosynthetic and morphological traits in the upper three leaves including the FL during the ripening period to evaluate the heterosis effect on source ability in VL45. The shoot dry weight

of VL45 at the heading stage (3 DAH) was the highest in the three cultivars (Table 1), indicating that the dry matter production of VL45 until heading stage was higher than those of parental cultivars. Similar traits have been found in other Vietnamese F₁ hybrid rice (Hanh *et al.*, 2008a). The morphological traits of the upper three leaves in VL45 were intermediate between those in the parents or somewhat similar to those of the male parent R45. Especially, the FL of VL45 showed similar features to the FL of R45 (Table 2). Pham *et al.* (2004) examined the heterosis effect on growth traits of some F₁ hybrid rice cultivars and their parents and reported that the heterosis effect was found mainly in increased tillering number and expanded leaf area at the vegetative growth stage. In our study, VL45 showed significantly higher dry matter production than R45 but did not differ in leaf area from R45 at the heading stage (Table 1). The rate of leaf emergence in VL45 was faster than that in R45 at the vegetative growth stage, and dying-off in leaves below the 3L in VL45 were also faster than that in R45 at the heading stage (data not shown). Accordingly, if these dead leaves were added, total leaf area formed until the heading stage in VL45 would be greater than that in R45. This agrees with the data of Pham *et al.* (2004). In addition, the plant type of VL45 was more spread than that of R45 (data not shown). Therefore, such better light-intercepting feature in VL45 may result in higher dry matter production.

P_n of the upper three leaves were not significantly different among the three cultivars at 3 DAH (Fig. 1A). At 14 DAH, P_n in the FL of VL45 was intermediate between those of the parents, whereas P_n in the 2L and 3L were lower than those of the parents (Fig. 1B). Therefore, it appears that leaf senescence in the 2L and 3L of VL45 began more early than those of the parents. In addition, a tendency was found that P_n in the upper three leaves of 103S were higher than those of VL45 and R45 at 14 DAH (Fig. 1B). It is well known that TGMS rice lines show higher P_n values than ordinary rice cultivars at the ripening stage (Kato *et al.*, 2004; Zhou *et al.*, 2006). Thus, it is thought that the photosynthetic trait of 103S reflects that of the TGMS lines. At 24 DAH, P_n in the FL of VL45 was significantly higher than that of R45 (Fig. 1C). Furthermore, the ratio of P_n at 24 DAH to P_n at 3 DAH in the FL was remarkably higher in VL45 than in R45 (VL45, 76%; R45, 32%). Thus, it is suggested that the heterosis effect on photosynthetic traits in VL45 occurred in the FL at late ripening stage. With respect to the 2L and 3L of VL45, senescence gradually progressed from 14 to 24 DAH. These data suggest that photosynthetic traits in the upper three leaves of VL45 during the ripening period differ depending on the leaf position; the FL maintains higher photosynthetic ability than those of the parents, whereas the 2L and 3L begin more early leaf senescence than those of the parents. Yamauchi and Yoshida (1985) reported that leaf thickness of F₁ rice cultivars were intermediate between or thinner than those of the parents. The SLA of the FL did not differ between VL45 and R45 (Table 2), but P_n of the FL was higher in VL45 than in R45 at late ripening stage. These

facts indicate that such photosynthetic traits in VL45 are caused by physiological factors rather than morphological factors.

N contents of the upper three leaves in VL45 did not significantly differ from those in R45 at 3 DAH but were somewhat higher than them at 14 DAH (Table 3). At 14 DAH, there were positive correlations between N contents and P_n in the FL of three cultivars. In the 2L and 3L, however, P_n tended to be lower in VL45 than in R45, despite that N content tended to be higher in VL45 than in R45. It seems that factors other than N content regulates P_n . Zhang *et al.* (2003) investigated senescence of upper three leaves of rice at the ripening stage under field conditions, and found that the degree of senescence was not necessarily correlated with leaf age because Rubisco content in the FL was more early decreased than that in the 3L. VL45 showed delayed senescence in the FL and distinct senescence in the 3L during the ripening period. In the upper three leaves of VL45, therefore, the degree of senescence corresponded with the leaf age. However, leaf senescence in R45 occurred almost simultaneously in the upper three leaves. These data show that the progress of senescence in the upper three leaves differs between VL45 and the male parent. They also suggest that in VL45, N was translocated from the 3L to the FL at late ripening stage.

It is interesting to discuss the heterosis effect on senescence in the FL of VL45 in relation to photosynthetic function. At 3 and 14 DAH, G_s in the FL of VL45 was similar to G_s of R45. At 24 DAH, the FL of VL45 maintained the same G_s value, despite a decrease in G_s in the FL of R45 (Fig. 2). G_s in the 2L and 3L of VL45 tended to be lower than G_s of R45 after the heading. Therefore, higher photosynthetic ability in the FL of VL45 would be due to the maintenance of high stomatal aperture. F_v/F_m and Φ_{PSII} in the FL and 2L did not significantly differ among the three cultivars at 24 DAH, although they were somewhat higher in the FL than in the 2L (Table 4). These results indicate that activities of PSII electron transport in each leaf position did not greatly differ among the three cultivars, suggesting that the photochemical parameters are not limiting factors of photosynthetic ability at late ripening stage. Kumagai *et al.* (2009) have reported that F_v/F_m and Φ_{PSII} in the FL of rice did not change during the ripening stage. In the three cultivars, we confirmed that F_v/F_m and Φ_{PSII} in the upper three leaves at 3 and 14 DAH also showed similar trends to those at 24 DAH, and values of F_v/F_m in FL and 2L maintained more than 0.8 until 14 DAH (data not shown). These data demonstrate that the heterosis effect did not occur in activities of PSII electron transport of the upper three leaves. NPQ reflects activity of the xanthophyll-cycle and is an index for dissipation of excess excitation energy (Bilger and Björkman, 1990; Adams and Demmig-Adams, 1995). At 24 DAH, NPQ in the FL of VL45 was significantly lower than that of R45 (Table 4), whereas F_v/F_m and Φ_{PSII} did not differ between VL45 and R45. These results indicate that there is no large difference in energy distribution of PSII in the upper three leaves between VL45 and R45 at late ripening stage.

At 24 DAH, Chl content in the FL did not significantly differ between VL45 and the parents. However, Chl content in the 2L of VL45 was lower than that of 103S. The 3L of VL45 showed distinct senescence (Fig. 3A). The SP contents in the upper three leaves of 103S were higher than those of VL45 and R45, probably due to that 103S lacking grains (sink) keeps SP in the leaves. There was no difference in SP contents of the FL between VL45 and R45. These facts suggest that high photosynthetic ability in the FL of VL45 is not explained from the contents of Chl and SP. Thus, P_n per unit Chl and SP contents (P_n/Chl and P_n/SP) were calculated as indicators of photosynthetic ability (Fig. 3C, D). The P_n/Chl of the FL and 2L did not significantly differ among the three cultivars. This suggests that there is no large difference in CO_2 assimilation rate relative to light harvesting rate, if it is postulated that Chl content is an indicator for the photochemical function. In contrast, P_n/SP in the FL of VL45 was much higher than those of the parents, whereas P_n/SP in the 2L did not differ among the three cultivars, indicating the heterosis effect on P_n/SP in the FL of the F_1 hybrid at 24 DAH. This may be caused by a higher ratio of Rubisco protein per unit SP content or a higher specific activity of Rubisco, although further analysis will be needed. Interestingly, Hirao *et al.* (1995) reported that photosynthetic rate per unit Rubisco content in a high yielding F_1 cultivar was higher than those of its parents.

Katsura *et al.* (2007) have showed that Chinese super hybrid rice cultivars have high yielding ability, whereas their growth duration is longer than that of high yielding inbred rice cultivars. The growth traits of Vietnamese F_1 hybrid cultivars differ from those of F_1 hybrid cultivars in other countries, because the former was produced for the cultivation in three-crop system (Hanh *et al.*, 2008a). Our study was performed to evaluate the photosynthetic traits of the F_1 hybrid rice useful for such cropping system. Our results suggest that high P_n in the FL of VL45 at late ripening stage results from high G_s and P_n/SP . In addition, it was demonstrated that VL45 shows the heterosis effect on the dry matter production until heading and the maintenance of high P_n in the FL at late ripening stage. These properties may contribute to the ripening of grains in VL45. Further studies on the allocation patterns of photosynthate from the upper three leaves to grains will be required for the physiological characterization of VL45. In Vietnam, dense planting of hybrid rice has been recently planned to rise yield potential per area. The photosynthetic traits of VL45, which depends mainly on the FL, may be useful for such cropping system, because the FL is superior in the interception of radiation to the 2L and 3L on high density culture.

ACKNOWLEDGEMENTS

We thank Prof. Atsushi Yoshimura for the use of his photoperiod-controlling apparatus. This study is supported by Kyushu University Interdisciplinary Programs in Education and Projects in Research Development.

REFERENCES

- Adams, W. W. and B. Demmig-Adams 1995 The xanthophyll cycle and sustained thermal energy dissipation activity in *Vinca minor* and *Euonymus kiautschovicus* in winter. *Plant Cell Environ.*, **18**: 117–127
- Bilger, W. and O. Björkman 1990 Role of the xanthophyll cycle in photoprotection elucidated by measurements of light induced absorbance changes, fluorescence and photosynthesis in leaves of *Hedera canariensis*. *Photosynth. Res.*, **25**: 173–185
- Bradford, M. M. 1976 A rapid and sensitive method for the quantitation of microgram quantities of protein utility the principle of protein-dye binding. *Anal. Biochem.*, **72**: 248–254
- Chen, G. X., S. H. Liu, C. J. Zhang and C. G. Lu 2004 Effects of drought on photosynthetic characteristics of flag leaves of a newly-developed super-high-yield rice hybrid. *Photosynthetica*, **42**: 573–578
- Cheng, S. H., J. Y. Zhuang, Y. Y. Fan, J. H. Du and L. Y. Cao 2007 Progress in research and development on hybrid rice: a super-domesticated in China. *Ann. Bot.*, **100**: 959–966.
- Genty, B., J. M. Briantais and N. R. Baker 1989 The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim. Biophys. Acta*, **990**: 87–92
- Hanh, T. T., T. Araki, V. C. Pham, T. Mochizuki, A. Yoshimura and F. Kubota 2008a Characteristics of CO₂ exchange rate of flag leaves in a Vietnamese F₁ hybrid rice variety and its parents during grain filling stage. *Trop. Agr. Develop.*, **52**: 104–110
- Hanh, T. T., T. Araki, V. C. Pham, T. Mochizuki, A. Yoshimura and F. Kubota 2008b Effects of nitrogen supply restriction on photosynthetic characters and dry matter production in Vietlai 20, a Vietnamese hybrid variety, during grain filling stage. *Trop. Agr. Develop.*, **52**: 111–118
- Hirao, K., F. Kubota, W. Agata and X. F. Song 1995 Characteristics of leaf photosynthesis in Chinese F₁ hybrid rice cultivar, Shanyou 63. *Jpn. J. Crop Sci.*, **64**: 209–215 (In Japanese with English summary)
- International Rice Research Institute, 2007 Atlas of Rice (World Rice Statistics). Available online at <http://www.irri.org/science/ricestat/index.asp>.
- Kato, M., K. Kobayashi, E. Ogiso and M. Yokoo 2004 Photosynthesis and dry-matter production during ripening stage in a female sterile line of rice. *Plant Prod. Sci.*, **7**: 184–188
- Katsura, K., S. Maeda, T. Horie and T. Shiraiwa 2007 Analysis of rice attributes and crop physiological traits of Liangyoupeijiu, a hybrid rice recently bred in China. *Field Crops Res.*, **103**: 170–177
- Kumagai, E., T. Araki and F. Kubota 2009 Characteristics of gas exchange and chlorophyll fluorescence during senescence of flag leaf in different rice (*Oryza sativa* L.) cultivars grown under nitrogen-deficient condition. *Plant Prod. Sci.*, **12**: 285–292
- Long, S. P. and J. E. Hallgren 1985 Measurements of CO₂ assimilation by plants in the field and the laboratory. In “Techniques in Bioproductivity and Photosynthesis”, ed. by J. Coombs, D. O. Halls, S. P. Long and J. M. O. Scurlock, Pergamon Press, Oxford, pp. 62–94
- Makino, A., T. Mae and K. Ohira 1984 Relationships between nitrogen and ribulose-1,5-bisphosphate carboxylase in rice leaves from emergence through senescence. *Plant Cell Physiol.*, **25**: 429–437
- Makino, A., T. Mae and K. Ohira 1985 Photosynthesis and ribulose-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
- Murata, Y. and S. Matsushima 1975 Rice. In “Crop Physiology”, ed. by L. T. Evans, Cambridge University Press, Cambridge, pp. 73–99
- Osmond B, M. Badger, K. Maxwell, O. Björkman, R. Leegood 1997 Too many photons: photorespiration, photoinhibition and photooxidation. *Trends Plant Sci.*, **2**: 119–121
- Peng, S. B., G. S. Khush, P. Virk, Q. Tang and Y. Zou 2008 Progress in ideotype breeding to increase rice yield potential. *Field Crops Res.*, **108**: 32–38
- Pham, V. C., S. Murayama, Y. Kawamitsu, K. Motomura and S. Miyagi 2004 Heterosis for photosynthetic and morphological characters in F₁ hybrid rice from a thermo-sensitive genic male sterile line at different growth stages. *Jpn. J. Trop. Agr.*, **48**: 137–148
- van Kooten, O. and J. F. H. Snel 1990 The use of chlorophyll fluorescence nomenclature in plant stress physiology. *Photosynth. Res.*, **25**: 147–150
- Wang, Q., C. Lu and Q. Zhang 2005 Midday photoinhibition of two newly developed super-rice hybrids. *Photosynthetica*, **43**: 277–281
- Wintermans, J. F. G. A. and M. de Mots 1965 Spectrophotometric characteristics of chlorophylls *a* and *b* and their pheophytins in ethanol. *Biochim. Biophys. Acta*, **109**: 448–453
- Yamauchi, M. and S. Yoshida 1985 Heterosis in net photosynthetic rate, leaf area, tillering and some physiological characters on 35 F₁ hybrids. *J. Exp. Bot.*, **36**: 274–280
- Yoshida, S. 1981 *Fundamentals of Rice Crop Science*. Intl. Rice Res. Inst., Manila (Philippines)
- Zhang, C. F., S. B. Peng and R. C. Laza 2003 Senescence of top three leaves in field-grown rice plants. *J. Plant Nutr.*, **26**: 2453–2468
- Zhang, C. J., H. J. Chu, G. X. Chen, D. W. Shi, M. Zuo, J. Wang, C. G. Lu, P. Wang and L. Chen 2007 Photosynthetic and biochemical activities in flag leaves of a newly developed super high-yield hybrid rice (*Oryza sativa*) and its parents during the reproductive stage. *J. Plant Res.*, **120**: 209–217
- Zhang, W. H. and M. Kokubun 2004 Historical changes in grain yield and photosynthetic rate of rice cultivars released in the 20th century in Tohoku region. *Plant Prod. Sci.*, **7**: 36–44
- Zhang, Y., Q. Tang, Y. Zou, D. Li, J. Qin, S. Yang, L. Chen, B. Xia and S. Peng 2009 Yield potential and radiation use efficiency of “super” hybrid rice grown under subtropical conditions. *Field Crops Res.*, **114**: 91–98
- Zhou, X. S., S. Q. Shen, D. X. Wu, J. W. Sun and Q. Y. Shu 2006 Introduction of a xantha mutation for testing and increasing varietal purity in hybrid rice. *Field Crops Res.*, **96**: 71–79