

Changes in photosynthetic pigments and chlorophyll fluorescence parameters in the super-high-yielding rice hybrid Peiai64S/E32 during senescence

L.D. ZENG^{*,†}, Q.L. ZHANG^{**,†}, M.L. CAI^{**}, W.S. CHOW^{***}, and C.L. PENG^{**,+}

*School of Life Science, Huizhou University, 516007 Huizhou, Guangdong Province, China**
*Guangdong Provincial Key Laboratory of Biotechnology for Plant Development, School of Life Sciences, South China Normal University, 510631 Guangzhou, China***
*Division of Plant Sciences, Research School of Biology, College of Medicine, Biology and Environment, The Australian National University, Acton, Australian Capital Territory 2601, Australia****

Abstract

The hybrid rice (*Oryza sativa* L.) cultivar Peiai64S/E32, which is a hybrid of E32 and Peiai64S, has a super high yield compared with that of its parents. Changes in photosynthetic pigments and chlorophyll (Chl) fluorescence parameters were investigated in the flag leaves during senescence. The content of Chl and carotenoids in the flag leaves of Peiai64S/E32 was more stable than those of their parents during senescence. Furthermore, Peiai64S/E32 had a more stable PSII activity and a lower nonphotochemical quenching and deepoxidation state than that of its parents in the later stage of senescence. These results demonstrated that Peiai64S/E32 functioned longer than its parents did during the leaf senescence and grain-filling period. This occurred because there was more excitation energy to drive photosynthetic CO₂ fixation in Peiai64S/E32, as the pigments supplying the energy survived longer in the high-yielding rice compared to the parents.

Additional key words: crop; light absorption; photoprotection; photosynthesis.

Introduction

Rice (*Oryza sativa* L.) is one of the most important food crop species worldwide, and more than half of the global population consumes rice as a staple food (Dorward 2012). As important photosynthetic source organs of rice, leaves provide the majority of energy and organic matter for plants. The yield of rice mainly originates from photosynthesis of leaves. With the development of rice grains, the leaves enter the senescence stage (Gregersen *et al.* 2013). The pigments related to photosynthesis degrade rapidly during the process of leaf senescence (Zhang *et al.* 2019), which leads to a gradual decrease in the photosynthetic rate (Sakuraba *et al.* 2012).

Chl and carotenoids (Car) are two major pigments involved in photosynthesis in the leaves of higher plants (Rodríguez *et al.* 2016, Zhang *et al.* 2018). A decline of the pigment contents is usually regarded as a reliable index of leaf senescence (Zhang *et al.* 2019, Zheng *et al.* 2019).

The function of Chl is to absorb and transfer light energy, and Chl is an important component of the antenna complex system (Han *et al.* 2017). The Chl content of rice leaves decreased gradually during the process of senescence (Zhang *et al.* 2019). The Chl found in plants are Chl *a* and *b* and their roles are somewhat different. Chl *a* and *b* are both present in all light-harvesting complexes but the core or reaction centers contain only Chl *a*. The Chl *a/b* ratio and the Chl (*a+b*) content can reflect the state of quantum capture and the utilization of the whole system (Dittami *et al.* 2010). The Car present in plants are normally β -Car and xanthophylls, such as violaxanthin (V), antheraxanthin (A), zeaxanthin (Z), lutein (L), and neoxanthin (N). V, A, and Z are interconverted during the xanthophyll cycle, which increases thermal dissipation and reduces damage by light (Demmig-Adams and Adams 1992). This cycle is one of the mechanisms in plants for protection against photoinhibition (Niyogi *et al.* 2001).

Chl fluorescence in plants mainly occurs because of

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⁺Corresponding author; phone: +86-20-85217612, fax: +86-20-85215535, e-mail: pengchl@scib.ac.cn

Abbreviations: A – antheraxanthin; Car – carotenoids; Chl – chlorophyll; DAT – days after transplanting; DES – deepoxidation state; F₀ – minimal fluorescence yield of the dark-adapted state; F₀' – minimal fluorescence yield of the light-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; F_v/F_m – maximal quantum yield of PSII photochemistry; L – lutein; N – neoxanthin; NPQ – nonphotochemical quenching; q_N – nonphotochemical quenching coefficient; q_P – photochemical quenching coefficient; RCII – reaction center of PSII; V – violaxanthin; Z – zeaxanthin.

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[†]These authors contributed equally to this work.

the reaction center of PSII. Fluorescence is closely related to photosynthesis and can provide information concerning photosynthesis (Fu *et al.* 2012, Murchie and Lawson 2013, Zhang *et al.* 2019). The value of the maximal quantum yield of PSII photochemistry (F_v/F_m) in different plant leaves ranges from 0.75 to 0.85 under normal conditions (Maxwell and Johnson 2000), but it decreases significantly during the aging process (Ou *et al.* 2003). Moreover, F_v/F_m is an important indicator of the degree of senescence. The hybrid rice (*Oryza sativa* L.) cultivar Peiai64S/E32 has a super high yield compared to its parents (Yuan 2000, Ou *et al.* 2005). This paper investigated the changes in photosynthetic pigments and chlorophyll fluorescence in the flag leaves of the super-high-yielding rice hybrid Peiai64S/E32 and its parents during senescence, and the reasons for the high yield of Peiai64S/E32 were analyzed.

Materials and methods

Plant material: Seeds of the super-high-yielding rice hybrid Peiai64S/E32 (Peiai64S×32, cv. Peiai64S/E32, HY) were supplied by the Hunan Hybrid Rice Center, and the seeds of its paternal line 'E32' (cultivar E32, Japonica rice, Fath) and its maternal line 'Peiai64S' (cultivar 64S, which simultaneously contains components of Hsien rice, Japonica rice, and Javan rice, Moth) were supplied by the Heredity Breeding Group of the South China Institute of Botany of the Chinese Academy of Sciences. The seeds were sown in an experimental field of the South China Institute of Botany; the plants were managed and fertilized in accordance with routine practices. Experiments were carried out at the later development stage: from 47 d after transplanting (DAT) to 84 DAT.

HPLC analysis of Car composition: Samples were taken at 13:00 h on a sunny day in the field and quickly immersed in liquid nitrogen. Fresh leaves (0.5 g) were ground in a mortar with a small amount of CaCO₃ powder, quartz sand, and 5 mL of acetone. The homogenate was then centrifuged at 13,000 × g at 4°C for 10 min. The supernatant was subsequently filtered through a membrane with microholes (FA of 0.05 μm) and then analyzed by HPLC. The sample was then treated *via* a C₁₈ chromatograph column (5 μm, 210 × 4.6 mm) and linear gradient separation, with 10 μL per sample (Waters 2695, Waters, USA). Pigment content was estimated according to the methods of Gilmore and Yamamoto (1991). The deepoxidation state (DES) was defined as $(Z+0.5A)/(V+A+Z)$.

Measurements for Chl fluorescence parameters: F_v/F_m values need to be measured under low light condition after dark adaptation. The leaves were wrapped with wet gauze for 15 min in the dark. The parameters of the Chl fluorescence kinetics of the rice leaves *in vitro* were measured by a pulse-modulated fluorescence PAM 101/102/103 fluorometer (Heinz Walz, Effeltrich, Germany) at 25°C. The minimal fluorescence yield of the light-adapted state (F_0') was recorded after adaptation to the intensity of light [300 μmol(photon) m⁻² s⁻¹]. The detached leaves were dark

adapted for 15 min before measurement, and the minimal fluorescence yield of the dark-adapted state (F_0) was measured with a low-intensity modulated beam [1.6 kHz, 0.5 μmol(photon) m⁻² s⁻¹]. The maximal fluorescence yield of the dark-adapted state (F_m) was induced by saturated light [Schott lamp KL 1500 FL 103; 6,000 μmol(photon) m⁻² s⁻¹, 2 s] followed by treatment with a lower intensity of light [300 μmol(photon) m⁻² s⁻¹] and with light at 100 kHz supplied by the PAM 102 instrument to improve the ratio of signal to noise and to stabilize the output signals of fluorescence. The primary photochemical efficiency of PSII [$F_v/F_m = (F_m - F_0)/F_m$], the activity of PSII [$F_v/F_0 = (F_m - F_0)/F_0$], the photochemical quenching coefficient (q_p) and the nonphotochemical quenching coefficient (q_n) were calculated according to the methods of Schreiber *et al.* (1986) and Genty *et al.* (1990), and all the Chl fluorescence parameters were reported as relative values.

Measurement of Chl a and b content: Fresh leaf material was extracted in 80% acetone at 4°C in the dark for 24 h. Taking 80% acetone as a blank control, the absorption spectra of Chl at 663, 645, and 470 nm wavelengths were recorded using a UV-2450 spectrophotometer (Shimadzu, Tokyo, Japan). The total content of Chl and Car was subsequently measured according to the methods of Lichtenthaler (1987).

Statistical analysis: Values were expressed as means ± standard error (SE). Statistical significance was determined by one-way or two-way analysis of variance (ANOVA) with Duncan's multiple range test using SPSS Statistics 19.0 (IBM, NY, USA). Means were considered to be significantly different at the level $p < 0.05$. Data were processed by using the SigmaPlot software (version 10.0, SYSTAT Software Inc., Richmond, CA, USA).

Results

Chl and Car content: Chl is an important pigment that is a component of photosystem reaction centers and adjacent antennae. Chl *a* is a component of the reaction centers and is a key substance in light energy transformation. The content of Chl *a*, Chl *b*, and Car in the flag leaves declined during senescence (Table 1). Chl *a* declined more quickly than did Chl *b*, and Chl decreased more quickly than did Car in HY and its parents (Fig. 1). The ratio of Chl *a/b* was nearly stable before 74 DAT, and the content of Chl *a* declined quicker than did that of Chl *b* after 74 DAT. The results showed that Chl *a/b* and Chl (*a+b*) declined and that Car/Chl increased in the flag leaves during senescence. Car/Chl in the two parents was more stable before 63 DAT but later increased quickly. Car/Chl in HY increased quickly after 74 DAT. The ratio of β-Car/ΣX increased first but then decreased with the flag leaf senescence (Fig. 1C). The content of Chl and Car was stable in the HY during leaf senescence and was significantly higher than those of the parents in the later stage of leaf senescence. Table 2 showed that lines and dates have significant effect on the changes of Chl and Car content, and there was significant

Table 1. Changes in the chlorophyll (Chl) and carotenoid (Car) of the flag leaves of HY rice and its parents during senescence. $\Sigma\text{Car} = \beta\text{-Car} + \text{L} + \text{N} + \text{V} + \text{A} + \text{Z}$. A – antheraxanthin; L – lutein; N – neoxanthin; V – violaxanthin; Z – zeaxanthin. The values are the means ($n = 5$). The different letters indicate statistical significance ($p < 0.05$). Fath – paternal line of super high-yielding hybrid rice Peiai64S/E32; Moth – maternal line of super high-yielding hybrid rice Peiai64S/E32; HY – the super high-yielding hybrid rice Peiai64S/E32.

Content [nmol g ⁻¹ (FM)]	Line	DAT [d]			
		47	63	74	84
Chl <i>a</i>	Fath	3,028 ± 245 ^a	3,396 ± 252 ^a	1,825 ± 138 ^a	923 ± 88 ^b
	Moth	2,596 ± 231 ^{ab}	2,908 ± 188 ^a	860 ± 92 ^b	698 ± 57 ^c
	HY	3,450 ± 316 ^a	2,476 ± 175 ^b	1,599 ± 167 ^a	1,225 ± 93 ^a
Chl <i>b</i>	Fath	833 ± 86 ^a	996 ± 84 ^a	568 ± 64 ^a	345 ± 37 ^a
	Moth	665 ± 67 ^b	755 ± 62 ^b	227 ± 30 ^b	237 ± 35 ^b
	HY	913 ± 87 ^a	651 ± 66 ^b	434 ± 37 ^a	387 ± 44 ^a
Chl (<i>a+b</i>)	Fath	3,862 ± 245 ^{ab}	4,393 ± 416 ^a	2,393 ± 262 ^a	1,269 ± 95 ^b
	Moth	3,262 ± 264 ^b	3,664 ± 310 ^b	1,087 ± 111 ^b	936 ± 82 ^c
	HY	4,364 ± 317 ^a	3,128 ± 241 ^{bc}	2,034 ± 195 ^a	1,612 ± 117 ^a
ΣCar	Fath	1,293 ± 149 ^{ab}	784 ± 93 ^b	1,580 ± 203 ^a	824 ± 101 ^b
	Moth	1,332 ± 146 ^a	1,288 ± 103 ^a	699 ± 94 ^c	792 ± 71 ^b
	HY	1,676 ± 187 ^a	1,218 ± 102 ^a	1,202 ± 123 ^b	1,088 ± 97 ^a

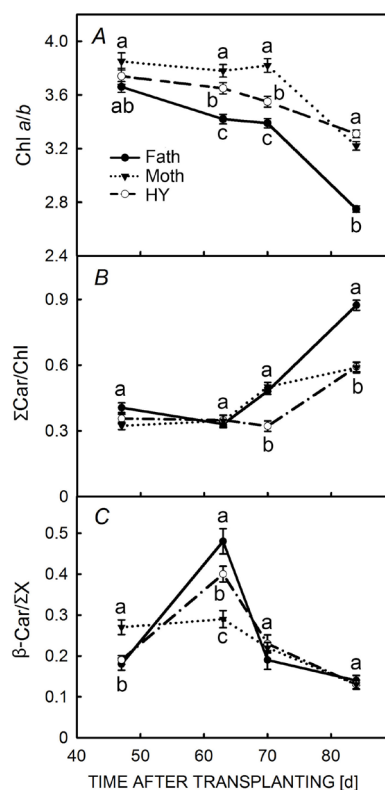


Fig. 1. Changes in chlorophyll *a/b* (Chl *a/b*) (A), $\Sigma\text{Car}/\text{Chl}$ (B) and $\beta\text{-Car}/\Sigma\text{X}$ (C) of the flag leaves of HY rice and its parents during senescence. $\Sigma\text{Car} = \beta\text{-Car} + \text{L} + \text{N} + \text{V} + \text{A} + \text{Z}$. $\Sigma\text{X} = \text{V} + \text{A} + \text{Z} + \text{L} + \text{N}$. A – antheraxanthin; L – lutein; N – neoxanthin; V – violaxanthin; Z – zeaxanthin. The values are the means ($n = 5$). The different letters indicate statistical significance ($p < 0.05$). Fath – paternal line of super high-yielding hybrid rice Peiai64S/E32; Moth – maternal line of super high-yielding hybrid rice Peiai64S/E32; HY – the super high-yielding hybrid rice Peiai64S/E32.

interaction between lines and dates.

Xanthophyll cycle sink components and Car compositions: The content of A and Z increased during flag leaf senescence, and the content of V decreased (Table 3). The content of V, A, and Z was the highest in HY at the later stage (84 DAT) of flag leaf senescence. The content of $\beta\text{-Car}$, L, and N decreased during flag leaf senescence, and they were the highest in HY at 84 DAT. Compared to those in the parents, the content of Car compounds (V, A, Z, L, N, and $\beta\text{-Car}$) in HY notably exhibited moderate parental dominance at 74 DAT and substantial parental dominance at 84 DAT. Heterosis was not obvious in the former stage of senescence. Table 2 showed that lines and dates have significant effect on the changes of xanthophyll cycle sink components (lines have no significant effect on the changes of Z) and Car compositions, and there was significant interaction between lines and dates.

Xanthophyll cycle and Chl fluorescence parameters:

The values of F_v/F_m in HY and its parents were stable before 74 DAT but decreased quickly after 74 DAT (Fig. 2A). The value of Φ_{PSII} in the two parents decreased quickly from 63 DAT, and this decrease was lower in HY during flag leaf senescence (Fig. 2B). F_0 increased, and F_0' declined gradually (Fig. 2C,D). The value of nonphotochemical quenching (NPQ) in the two parents decreased before 63 DAT but increased after 63 DAT. However, the value of NPQ in HY decreased before 74 DAT but increased after 74 DAT. The NPQ was lowest in HY after 74 DAT (Fig. 2E). The value of DES increased gradually with increased flag leaf senescence and was highest in the Moth plants (Fig. 2F). Lines and dates have significant effect on the changes of F_v/F_m , Φ_{PSII} , F_0 , F_0' , NPQ, and DES, and there was significant interaction (except F_0') between lines and dates (Table 2).

Table 2. Two-way analysis of variance of photosynthetic pigments and chlorophyll fluorescence parameters. A – antheraxanthin; Car – carotenoid; Chl – chlorophyll; DES – deepoxidation state; F_0 – minimal fluorescence yield of the dark-adapted state; F_0' – minimal fluorescence yield of the light-adapted state; F_v/F_m – maximal quantum yield of PSII photochemistry; L – lutein; N – neoxanthin; NPQ – nonphotochemical quenching; V – violaxanthin; Z – zeaxanthin; Φ_{PSII} – effective quantum yield of PSII photochemistry. $\Sigma X = V + A + Z + L + N$. $\Sigma Car = \beta\text{-Car} + L + N + V + A + Z$. * – $p < 0.05$, ** – $p < 0.01$, *** – $p < 0.001$, ns – no significant difference at $p = 0.05$ ($n = 5$).

Source	F-value		
	Lines	Dates	Lines × Dates
Chl a [nmol g ⁻¹ (FM)]	1,295.06***	13,795.50***	570.44***
Chl b [nmol g ⁻¹ (FM)]	64.01***	325.33***	22.56***
Chl (a+b) [nmol g ⁻¹ (FM)]	537.52***	5,026.41***	226.24***
ΣCar [nmol g ⁻¹ (FM)]	91.95***	157.48***	54.75***
Chl a/b	7.97**	11.10***	3.70*
$\Sigma Car/Chl$	11.06***	304.42***	8.16***
$\beta\text{-Car}/\Sigma X$	51.05***	358.82***	52.74***
V [nmol g ⁻¹ (FM)]	55.23***	1,074.06***	30.63***
A [nmol g ⁻¹ (FM)]	23.05***	136.53***	52.66***
Z [nmol g ⁻¹ (FM)]	2.85 ^{ns}	287.63***	36.04***
VAZ [nmol g ⁻¹ (FM)]	23.68***	22.41***	39.47***
$\beta\text{-Car}$ [nmol g ⁻¹ (FM)]	23.88***	295.73***	29.04***
L [nmol g ⁻¹ (FM)]	202.09***	178.23***	102.35***
N [nmol g ⁻¹ (FM)]	56.96***	196.17***	13.29***
F_v/F_m	49.86***	13,599.71***	44.97***
Φ_{PSII}	5.03*	5.97**	3.91**
F_0	125.72***	300.05***	43.68***
F_0'	66.75***	352.67***	0.98 ^{ns}
NPQ	22.36***	346.99***	23.31***
DES	125.42***	4,417.24***	24.01***

Discussion

Chl is an important structural substance through which plants capture light energy. A high content of Chl is the basis of high photosynthetic rate. The content of Chl in the flag leaves decreased gradually during senescence (47–84 DAT). HY exhibited hybrid dominance at 47 DAT (flowering period) and at 84 DAT (the fully ripe stage). The Chl content was more stable in the HY than that in the parents during senescence. The higher Chl content is beneficial for increasing rice yield (Zhang *et al.* 2019). Car play two important roles in photosynthesis: Car can capture light energy and have the capability of providing photoprotection. Car can transfer absorbed excited energy to Chl and can also remove singlet oxygen to prevent photosynthetic organisms from damage (Domonkos *et al.* 2013, Niyogi and Truong 2013, Hashimoto *et al.* 2016). Two-way ANOVA showed that lines and dates have significant effect on the changes of Chl and Car content (Table 2). The content of Car in the parents fluctuated greatly during the aging process, but the content in HY was stable. The content in the HY was the highest in the later stage (84 DAT) of senescence. The results of the Chl and Car showed that super-high-yielding rice not only had enough auxiliary pigments to capture light but also had a stronger light protection capacity, which helps avoid photooxidation caused by free radicals (Croce *et al.*

2007). This protection is also helpful for keeping the photosynthetic apparatus stable.

The function of the PSII reaction center irreversibly decreased (declines in F_v/F_m , Φ_{PSII} , and F_0') with increasing flag leaf senescence. F_0 represents the fluorescence level when PSII is completely open. An increase in the value of F_0 means that the reaction center of PSII (RCII) was destroyed or the separation between photosynthetic pigments and RCII caused irreversible denaturation (Calatayud *et al.* 2006, Gorbe and Calatayud 2012). PSII activity can be significantly affected by lines and dates (Table 2). F_0 increased in both HY and its parents during flag leaf senescence, and all kinds of photosynthetic pigments gradually degraded, which showed that RCII was destroyed and that the photosynthetic function gradually diminished. However, the values of F_v/F_m (no significant difference) and Φ_{PSII} were the highest in HY during the senescence of the flag leaves, which showed that HY may have a more stable reaction centers than did its parents. One of its high yield-related characteristics may be the ability to maintain the stability of the photosynthetic apparatus and maintain longer a more efficient functional stage.

Dates have significant effect on the changes of V, A, and Z content (Table 2). During the senescence of rice leaves, the content of V decreased gradually, and the content of A and Z increased continuously (Table 3). V is converted to A and Z under the conditions of excess

Table 3. Changes in the carotenoid (Car) components of the flag leaves of HY rice and its parents during senescence. A – antheraxanthin; L – lutein; N – neoxanthin; V – violaxanthin; Z – zeaxanthin. The values are the means ($n = 5$). The different letters indicate statistical significance ($p < 0.05$). Fath – paternal line of the super-high-yielding rice hybrid Peiai64S/E32; Moth – maternal line of the super-high-yielding rice hybrid Peiai64S/E32; HY – the super-high-yielding rice hybrid Peiai64S/E32.

	Line	DAT [d]			
		47	63	74	84
V [nmol g ⁻¹ (FM)] (% of VAZ pool)	Fath	195.4 ± 8.2 ^b	182.6 ± 11.5 ^a	85.3 ± 9.2 ^a	50.7 ± 7.3 ^{ab}
	(Fath)	(64.7 ± 7.7 ^y)	(63.4 ± 5.2 ^x)	(19.1 ± 2.3 ^x)	(22.5 ± 3.8 ^{xy})
	Moth	200.4 ± 12.2 ^b	144.9 ± 9.3 ^b	37.8 ± 3.5 ^b	36.3 ± 5.5 ^c
	(Moth)	(64.9 ± 4.1 ^y)	(51.7 ± 7.2 ^y)	(16.7 ± 2.1 ^x)	(12.9 ± 1.2 ^z)
	HY	262.6 ± 12.1 ^a	143.6 ± 11.5 ^b	69.1 ± 5.3 ^{ab}	62.0 ± 4.2 ^a
	(HY)	(72.8 ± 7.7 ^x)	(60.1 ± 9.3 ^x)	(19.9 ± 1.3 ^x)	(17.0 ± 1.3 ^y)
A [nmol g ⁻¹ (FM)] (% of VAZ pool)	Fath	30.3 ± 2.4 ^a	39.1 ± 3.2 ^{ab}	102.3 ± 8.9 ^a	44.4 ± 4.1 ^c
	(Fath)	(10.0 ± 1.2 ^x)	(13.6 ± 1.1 ^y)	(22.9 ± 3.3 ^x)	(19.7 ± 2.4 ^{xy})
	Moth	33.7 ± 2.3 ^a	48.0 ± 4.4 ^a	45.7 ± 3.8 ^c	57.7 ± 7.4 ^b
	(Moth)	(10.9 ± 0.9 ^x)	(17.1 ± 1.4 ^x)	(20.2 ± 1.3 ^x)	(20.4 ± 1.6 ^{xy})
	HY	38.5 ± 4.7 ^a	39.9 ± 4.3 ^{ab}	73.0 ± 5.3 ^b	84.0 ± 9.5 ^a
	(HY)	(10.6 ± 1.6 ^x)	(16.7 ± 1.2 ^x)	(21.1 ± 2.2 ^x)	(23.1 ± 2.1 ^y)
Z [nmol g ⁻¹ (FM)] (% of VAZ pool)	Fath	76.2 ± 6.3 ^a	66.1 ± 5.1 ^b	259.3 ± 28.8 ^a	129.8 ± 9.1 ^c
	(Fath)	(25.2 ± 2.4 ^x)	(22.9 ± 2.6 ^y)	(58.0 ± 6.4 ^x)	(57.7 ± 8.0 ^x)
	Moth	74.5 ± 5.8 ^a	87.2 ± 7.7 ^a	142.6 ± 14.7 ^c	188.0 ± 21.0 ^{ab}
	(Moth)	(24.1 ± 2.3 ^x)	(31.1 ± 4.1 ^x)	(63.1 ± 7.3 ^x)	(66.6 ± 6.3 ^x)
	HY	59.7 ± 8.1 ^b	55.2 ± 6.7 ^{bc}	204.9 ± 18.9 ^b	217.6 ± 26.4 ^a
	(HY)	(16.6 ± 1.5 ^y)	(23.1 ± 2.4 ^y)	(59.0 ± 4.1 ^x)	(59.8 ± 7.3 ^x)
VAZ pool [nmol g ⁻¹ (FM)]	Fath	302.0 ± 28.3 ^{ab}	287.9 ± 31.1 ^a	446.9 ± 35.1 ^a	224.9 ± 19.5 ^c
	Moth	308.6 ± 23.3 ^{ab}	280.2 ± 27.3 ^a	226.1 ± 28.2 ^c	282.1 ± 22.3 ^b
	HY	360.8 ± 42.9 ^a	238.8 ± 28.4 ^{ab}	347.1 ± 26.7 ^b	363.7 ± 38.4 ^a
β-Car [nmol g ⁻¹ (FM)]	Fath	193.7 ± 22.3 ^b	255.0 ± 22.7 ^b	191.3 ± 21.2 ^a	100.4 ± 14.3 ^{ab}
	Moth	287.2 ± 33.2 ^a	288.3 ± 31.2 ^b	95.1 ± 11.5 ^b	94.2 ± 8.4 ^b
	HY	243.7 ± 27.2 ^{ab}	385.9 ± 42.3 ^a	159.4 ± 18.5 ^a	124.4 ± 13.6 ^a
L [nmol g ⁻¹ (FM)]	Fath	585.0 ± 60.2 ^b	677.8 ± 63.4 ^a	821.4 ± 81.1 ^a	444.2 ± 50.7 ^{ab}
	Moth	592.9 ± 55.6 ^b	595.7 ± 68.3 ^{ab}	328.6 ± 26.7 ^c	362.4 ± 32.6 ^b
	HY	766.1 ± 81.1 ^a	600.7 ± 56.6 ^{ab}	591.1 ± 65.9 ^b	504.8 ± 62.8 ^a
N [nmol g ⁻¹ (FM)]	Fath	212.7 ± 17.0 ^a	164.1 ± 12.5 ^a	120.6 ± 12.4 ^a	54.6 ± 7.4 ^b
	Moth	143.6 ± 11.2 ^b	123.8 ± 16.8 ^{ab}	50.1 ± 4.3 ^c	53.9 ± 6.5 ^b
	HY	163.9 ± 19.8 ^b	135.4 ± 15.3 ^{ab}	104.7 ± 9.8 ^{ab}	95.1 ± 11.3 ^a

excitation energy by xanthophyll cycle, and the process of the xanthophyll cycle increases thermal dissipation and reduces light damage (Demmig-Adams and Adams 1992, Li *et al.* 2018). Previous results showed that there is a photo-carbon imbalance in the process of rice leaf senescence, which leads to excess excitation energy (Yu *et al.* 2020). V degraded during the senescence stage, which might occur because V in the flag leaves was destroyed by photooxidation during senescence, and V gradually transformed into A and Z, causing a decrease in V and an increase in A and Z (Table 3), and the trend of A and Z was consistent with NPQ. NPQ is positively correlated with the content of A and Z (Li *et al.* 2018), and in the mutants with xanthophyll cycle blocked, NPQ decreased significantly compared with the wild type (Li *et al.* 2016). It showed that xanthophyll cycle can play

a role in the photoprotection of leaves. The content of A and Z increased steadily in the HY, was stable during the senescence stage, and was the highest during the late senescence stage (84 DAT), suggesting that the HY had better photoprotection.

NPQ reflects the heat dissipation level, and through NPQ, plants can dissipate excitation energy and decrease the formation of oxidative stress (Ruban 2016). The relatively high Φ_{PSII} and relatively low NPQ in HY at 74 DAT showed that the HY could maintain high PSII activity in the later stage of senescence. The decline in F_0' was considered to reflect the heat dissipation of the PSII antennae depending on the xanthophyll cycle (Khoroshyy *et al.* 2018). The gradual decrease in F_0' in HY and its parents was in line with the increase in Z and DES, which further showed that antenna heat dissipation, depending on

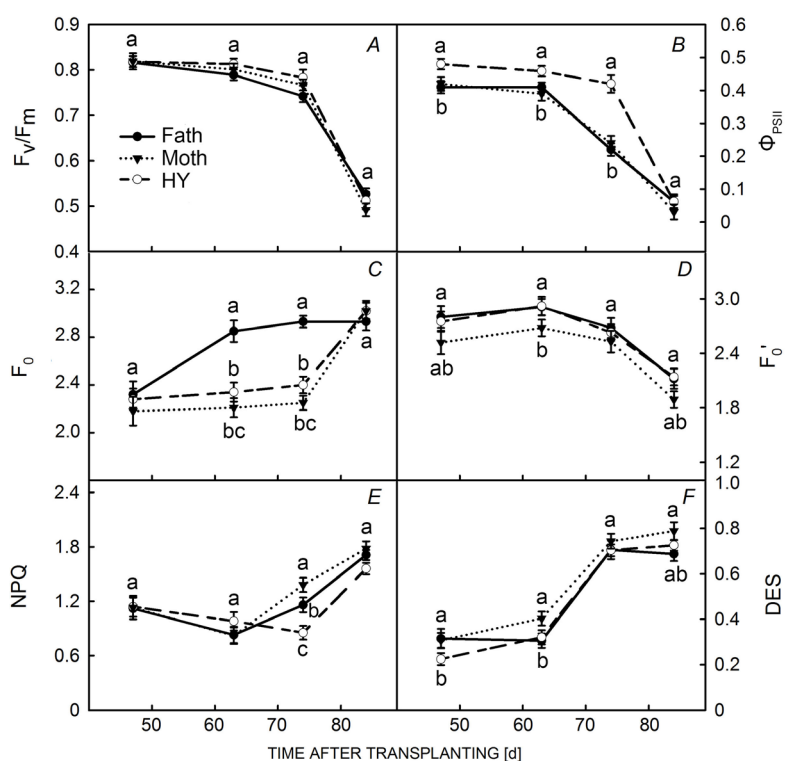


Fig. 2. Changes in the maximal quantum yield of PSII photochemistry (F_v/F_m) (A), effective quantum yield of PSII photochemistry (Φ_{PSII}) (B), minimal fluorescence yield of the dark-adapted state (F_0) (C), minimal fluorescence yield of the light-adapted state (F_0') (D), nonphotochemical quenching (NPQ) (E), and deepoxidation state (DES) (F) in the flag leaves of the super-high-yielding rice hybrid Peiai64S/E32 and its parents during senescence. The values are the means ($n = 5$). The different letters indicate statistical significance ($p < 0.05$). Fath – paternal line of the super-high-yielding rice hybrid Peiai64S/E32; Moth – maternal line of the super-high-yielding rice hybrid Peiai64S/E32; HY – the super-high-yielding rice hybrid Peiai64S/E32.

the xanthophyll cycle, increased during senescence. The PSII activity was higher and more stable in HY than that in the two parents. The results of this study demonstrated that HY functions longer than do its parents during leaf senescence. The ratio of excitation energy distributed to NPQ was lesser in HY than that in its parents during senescence, and there was more excitation energy to supply photosynthetic CO_2 fixation. This may be one of the physiological mechanisms of photosynthesis for the high yield advantage of HY.

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