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# Photosynthetic decline in flag leaves of two field-grown spring wheat cultivars with different senescence properties

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## Abstract

Photosynthetic characteristics were compared between two field-grown spring wheat (*Triticum aestivum* L.) cultivars, Ningmai 8 (NM8) and its half-father sibling Ningmai 9 (NM9), to investigate the differences in photosynthetic decline of flag leaves between these two cultivars with different senescent appearance after emergence through senescence. Maximum photosynthetic activities for these two cultivars were observed at around 10 days after emergence (DAE). Photosynthetically, therefore, 10 DAE was taken to define the point at which leaf senescence was initiated. During the photosynthetic rapid decline occurring after 27 DAE, as compared with NM8, NM9 showed a significantly higher Chl content, a higher Chl *a/b* ratio, light-saturated photosynthetic rate ( $P_{Ca=360}$ ) and light- and CO<sub>2</sub>-saturated photosynthetic rate ( $P_{max}$ ), carboxylation efficiency (CE), but a significantly lower dark respiration rate ( $R_D$ ) and CO<sub>2</sub> compensation point ( $C_{comp}$ ). Thylakoid membranes in NM9 also showed a slightly higher electron transport activity of PS II, but similar absorption spectra properties measured at room temperature as compared to NM8. The slower photosynthetic decline may contribute some to its higher grain yield in NM9. Due to the slower decrease of Chl, CE and  $P_{Ca=360}$ , and the lower  $R_D$  in NM9, flag leaf senescence was delayed in comparison to the earlier senescent NM8. Compared with its half-father sibling NM8, NM9 had a later onset and a slower rate of flag leaf senescence, which may be partly responsible for its higher grain yield. © 2005 SAAB. Published by Elsevier B.V. All rights reserved.

**Keywords:** Flag leaf; *Triticum aestivum* L.; Senescence

## 1. Introduction

Photosynthesis is the primary source of dry matter production and grain yield in crop plants. The improvements of leaf photosynthesis have occurred with the advance of breeding high-yielding cultivars (Jiang et al., 2002). Among the 18 cultivars of winter wheat released in the period between 1945 and 1995 in the area of Beijing, China, an increase in grain yield was found associated with the elevation of leaf photosynthetic rate over the past 50 years (Jiang et al., 2003). Therefore, increasing single-leaf photosynthesis could be the main way to substantially enhance grain yield.

However, because of the close correlation between leaf area during maturation and grain yield in wheat, early senescing which seriously restricts the potential for heterotic vigour remains a significant physiological problem (Jiao et al., 2003).

Wheat genotypes vary in the timing of senescence initiation and also in the subsequent rate of leaf senescence. The quest of the causes of differences in leaf photosynthetic rate among interspecies and/or intraspecies of crops may be one of the important strategies of crop engineering (Jiang et al., 2002). So delaying leaf senescence has become an agronomically desirable trait (Grover, 1993; Quirino et al., 2000; Subhan and Murthy, 2001). Flag leaf photosynthesis in wheat contributes about 30–50% of the assimilates for grain filling (Sylvester-Bradley et al., 1990) and initiation of grain filling coincides with the onset of senescence, therefore, photosynthesis of the flag leaf is the most important basis of the formation of grain yield, and the onset and rate of senescence are important factors for determining grain yield.

Historically, research on biochemical changes that occur during leaf senescence has focused on loss of photosynthetic pigments, degradation of protein, and re-absorption of mineral nutrients. The drastic decline in activities of PS II, PS I and whole chain electron transport has also been reported in several

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senescing systems (see review in Subhan and Murthy, 2001), indicating that the photochemical activity inhibits photosynthesis during leaf senescence (Harding et al., 1990). Most of these studies were conducted under controlled conditions, however, only a few reports are related to senescence of field-grown plants (see review in He et al., 2003). Moreover, few reports are comprehensive considering of the in vitro photochemical and biochemical properties of thylakoid membranes and in vivo photosynthetic properties derived from gas exchange measured in the field. Additionally, the changing direction of Chl *a/b* ratio is still controversial during natural leaf senescence and therefore is worth to note further.

Ningmai 8 (NM8, Yang86-17 × Xifeng) and Ningmai 9 (NM9, Yang86-17 × Yangmai 5) are two spring wheat cultivars with half-father but with different senescent appearance and grain yield. Cultivar NM9 has a higher grain yield and is later senescent than NM8 (Zhang et al., 2005a). Our previous work has showed that NM9 is more effective in the utilization of low light than its half-father cultivar NM8, supported by higher apparent quantum yield of photosynthesis and lower light compensation point (Zhang et al., 20005b) and more drought resistance (Zhang et al., 2005a). Since these two cultivars have similar plant type and half-father origin, it is useful as senescent materials to study differences in photosynthesis and related physiological characteristics, which would contribute some to wheat physiological breeding.

## 2. Materials and methods

Field experiments were carried out at the Experimental Fields of the Institute of Agricultural Sciences of Jiangsu Nanjing, China (32°03'N, 118°47'E). Nanjing is located in the monsoon climate area of north subtropical zone, with very distinctive four seasons; its annual average temperature is 15 ± 2.7 °C and the annual precipitation 989.3 ± 156 mm (Fan and Chen, 1997). The soil types are clay loam with 1.25 g cm<sup>-1</sup> bulk density. During the period from 1 April (10 days before the first sampling) to 17 May (the last sampling date), mean daily temperature is 19.63 ± 0.66 °C, mean daily maximal temperature 24.67 ± 0.91 °C, minimum daily temperature 15.32 ± 0.65 °C, mean daily precipitation 9.50 ± 3.90 mm, mean daily relative humidity 75.50 ± 3.02%.

Two newly developed spring wheat (*Triticum aestivum* L.) cultivars Ningmai 8 and Ningmai 9 were grown in 2003–2004 in a field. N-P-K fertilizers were applied at 99, 20.2 and 145.1 mg kg<sup>-1</sup>, respectively, 2 days before sowing on 20 October 2003. The plant density was about 7 plants per m<sup>2</sup> (space: 0.6 × 0.24 m). Nutrients and water were supplied sufficiently throughout and thus potential nutrients stress and drought stress were avoided. Ditches were dug to drain excessive water to prevent from flooding due to heavy rainfall. Sampling started from the emergence (10 April 2004) through advanced senescence (17 May 2004) of flag leaves on main culm in the morning (07:30–11:30 h) on sunny days at 7–10 day intervals depending on weather.

Photosynthetic responses to CO<sub>2</sub> ( $P_n/C_i$  curve) of intact flag leaves were made in the field using a portable photo-

synthesis system (CIRAS-2, PP SYSTEMS, UK) at morning between 07:30 and 11:30 h to avoid photoinhibition potentially resulting from high light stress at midday. Measurements were made under saturating photosynthetic photon flux densities (1500 μmol m<sup>-2</sup> s<sup>-1</sup> PFD) from a LED light source, cuvette relative humidity was tracked ambient, and leaf temperature was controlled at 20 ± 0.5 °C, similar to mean daily growth temperature (19.63 ± 0.66 °C) during the sampling period. Flow rate was 197 ± 3 ml min<sup>-1</sup>. Different CO<sub>2</sub> concentration was supplied by a fresh CO<sub>2</sub> cartridge. Flag leaves were firstly exposed to 150 μmol mol<sup>-1</sup> of ambient CO<sub>2</sub> concentration ( $C_a$ ) until equilibration (for around 5 min) to stimulate stomatal opening, then ambient CO<sub>2</sub> concentration ( $C_a$ ) in the cuvette was changed from 0, 50, 100, 200, 300, 450, 600, 750, 900, 1100, 1300, up to 1500 μmol mol<sup>-1</sup>. Data were recorded automatically at an interval of 2 min between two CO<sub>2</sub> levels with an automatic programmed software (CIRAS-2, PP SYSTEMS, UK). When the fluctuation in  $P_n$  (net photosynthetic rate) was less than 0.5 μmol m<sup>-2</sup> s<sup>-1</sup>, six repeats at each  $C_a$  level were recorded at an interval of 3.0 s. 2 min was enough to be stable for  $P_n$  at each  $C_a$  according to preliminary experiments. Responses were determined in three flag leaves from three different plants for each species each sampling date.

The non-linear adjustment of  $P_n/C_i$  curves followed the model described by Prado and Moraes (1997) and Zhang et al. (2003)

$$P_n = P_1 * \left(1 - e^{-K*(x - C_{comp})}\right) \quad (1)$$

where  $P_n$  is the net photosynthetic rate (μmol m<sup>-2</sup> s<sup>-1</sup>),  $P_1$  is the CO<sub>2</sub>- and light-saturated net photosynthetic rate ( $P_{max}$ , μmol m<sup>-2</sup> s<sup>-1</sup>),  $K$  is an empirical constant (0.0001),  $x$  is the ambient or intercellular CO<sub>2</sub> concentration ( $C_a$  or  $C_i$ , μmol mol<sup>-1</sup>),  $C_{comp}$  is the CO<sub>2</sub> compensation point (μmol mol<sup>-1</sup>), and  $e$  is the base of the natural logarithm. Leaf respiration in the day ( $R_D$ , μmol m<sup>-2</sup> s<sup>-1</sup>) can be calculated at  $x=0$ , namely CO<sub>2</sub>-evolution under the CO<sub>2</sub>-free air condition. The estimation of carboxylation efficiency (CE, mol m<sup>-2</sup> s<sup>-1</sup>) of CO<sub>2</sub> assimilation was obtained from the following equation derived from the inclination of the straight line in the first linear phase of the curve obtained from Eq. (1).

$$CE = P_1 * K * e^{K * C_{comp}} \quad (2)$$

To analyze chlorophyll content of flag leaves, leaf samples were taken and immediately frozen in liquid nitrogen. Leaf samples were extracted in ice-cold 80% acetone. The extract was centrifuged at 3000 × *g* for 5 min. The upper solution was measured with a UV-754 spectrophotometer (Shanghai, China) at 645 and 663 nm. Chl content and Chl *a/b* ratio were calculated as described by Arnon (1949).

To investigate photochemical properties of thylakoid membrane, thylakoid membranes were isolated as described by Dunahay et al. (1984) and Chen et al. (2004) with some modifications. 2 g of flag leaves after removal the midrib was kept in refrigerator at 4 °C for 30 min. Then, the leaves were cut into pieces and were triturated in a *Triturator* with 250 ml of cool extracting medium (50 mM Tris-HCl, pH 7.6, 5 mM

MgCl<sub>2</sub>, 10 mM NaCl, 0.4 M sucrose, 0.1% bovine serum albumin, BSA). The homogenate was squeezed through four layers of nylon cloth, and the filtrate was centrifuged at 300×g for 1 min to remove large debris. The upper solution was re-centrifuged at 4000×g for 10 min. After removing the upper solution, the precipitate was supplemented with a buffer (0.15 M sucrose, 5 mM MgCl<sub>2</sub>, 0.2% BSA, 20 mM Tris–HCl, pH 8.0) and then was re-centrifuged. The final precipitate containing chloroplasts was mixed with a buffer (15 mM NaCl, 5 mM MgCl<sub>2</sub>, 20 mM 2-(*N*-mopholino) ethanesulfonic acid, pH 6.5). Chlorophyll concentration was diluted at about 20 mg ml<sup>-1</sup>.

Activities of electron chain transport (PS I, PS II and whole electron chain transport) were measured polarographically with a Clark-type liquid-phase electrode (Chlorolab-2, Hansatech, UK) fitted with a circulating water jacket at 20±0.5 °C, similar to mean daily growth temperature (19.63±0.66 °C) during the sampling period. Actinic light from a slide projector was inserted into the side of the electrode chamber and the light intensity was 1500 μmol m<sup>-2</sup> s<sup>-1</sup>. 2 ml reaction buffer (pH 7.6–7.8) contained 25 mM Tricine, 5 mM NaCl, 0.2 M sucrose, 5 mM MgCl<sub>2</sub>, supplemented with 150 μM methyl viologen (MV), 2 mM NaN<sub>3</sub>, 0.2 mM 2,6-dichlorophenol indophenol (DCPIP), 5 mM NH<sub>4</sub>Cl, 0.05 mM DCMU and 5 mM ascorbate for oxygen uptake (DCPIP→MV, PS I activity), and with 5 mM K<sub>3</sub>F(CN)<sub>6</sub>, 1 mM *p*-phenylene diamine for oxygen evolution (H<sub>2</sub>O→K<sub>3</sub>F(CN)<sub>6</sub>, PS II activity), and with 2 mM MV, 2 mM NaN<sub>3</sub> and 5 mM NH<sub>4</sub>Cl for oxygen uptake (H<sub>2</sub>O→MV, whole electron chain transport).

Absorption spectra of thylakoid membranes at room temperature between 360 and 780 nm were performed on a UV-754 spectrophotometer (Shanghai, China) and all samples were adjusted to a Chl concentration of 20 μg ml<sup>-1</sup> before measurement. Room-temperature fluorescence emission at 680 nm was obtained by filtering the emission spectra (600 to 800 nm) with a broad band red filter with maximal transmission at 680 nm. The samples were excited at 435 nm in a luminescence spectrometer (LS50B, Perkin Elmer). The sample medium was 50 mM Tris–HCl (pH 7.8) and the chlorophyll concentration was adjusted to 4 μg ml<sup>-1</sup> for each measurement.

All data were analyzed on Microcal Origin (version 7.0) and significant differences were performed by one-way ANOVA at  $P \leq 0.05$ .

### 3. Results

Loss of chlorophyll (Chl) is an index of progress in leaf senescence. Fig. 1A shows the time courses of changes in Chl content in flag leaves in two spring wheat cultivars. Chl content increased after emergence, peaked at full expansion, then maintained relatively constant between 10 and 20 days after emergence (DAE), thereafter significantly declined with flag leaf senescing. There were not significant differences in Chl content between two wheat cultivars before 27 DAE, but after that, NM9 showed a higher chlorophyll concentration than NM8 and the difference in Chl content increased between the two cultivars, from 6.6% at 27 DAE to 15.6% at 42 DAE.

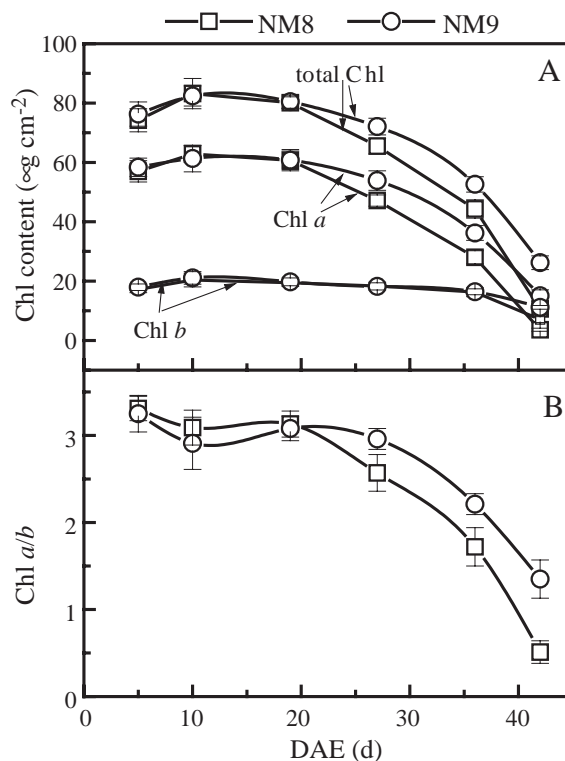


Fig. 1. Total chlorophyll, Chl *a* and *b* content (A) and Chl *a/b* ratio (B) from flag leaf emergence through senescence of two spring wheat cultivars grown in the field. Values are means±S.E. of three independent samples at the same time.

As shown in Fig. 1B, the ratio of Chl *a/b* decreased slightly after emergence of flag leaves for both cultivars, and maintained stable values between 10 and 19 DAE for NM8 but between 10 and 27 DAE for NM9. After that, the ratio started to decrease significantly. The alteration in Chl *a/b* ratio indicated that there was a change in the photosynthetic pigment stoichiometry during flag leaf late senescence, due mainly to a decrease in the Chl *a* concentration. The onset of the significant decline in the ratio was about 8 days earlier in NM8 than in NM9.

The changes in the net photosynthetic rate of flag leaves as a function of the intercellular CO<sub>2</sub> concentration ( $C_i$ ) were shown in Fig. 2. It can be seen that with leaf maturation, the light- and CO<sub>2</sub>-saturated net photosynthetic rate ( $P_{max}$ ) increased in 10 DAE, then kept relatively stable higher values between 10 and 27 DAE, and after that decreased markedly (Figs. 2 and 3). To examine the actual net photosynthetic ( $P_{Ca=360}$ ) at current ambient CO<sub>2</sub> (around 360 μmol mol<sup>-1</sup>) and light-saturated conditions, values of  $P_{Ca=360}$  were calculated from  $P_n/C_a$  curves (data not shown). As shown in Fig. 3A,  $P_{Ca=360}$  decreased earlier than  $P_{max}$  for two cultivars, namely a noticeable decrease at 19 DAE for  $P_{Ca=360}$  and 27 DAE for  $P_{max}$ , respectively.

The initial slope of  $P_n/C_i$  curves, calculated from Eq. (2), a measure of CE of Rubisco, showed a similar changing pattern to that of  $P_{Ca=360}$  from emergence through senescence of flag leaves (Figs. 2 and 3). However, the  $K_{C_i}$  value ( $K_{C_i}$ , Michaelis–Menten constant) for the CO<sub>2</sub> concentration at the half maximal

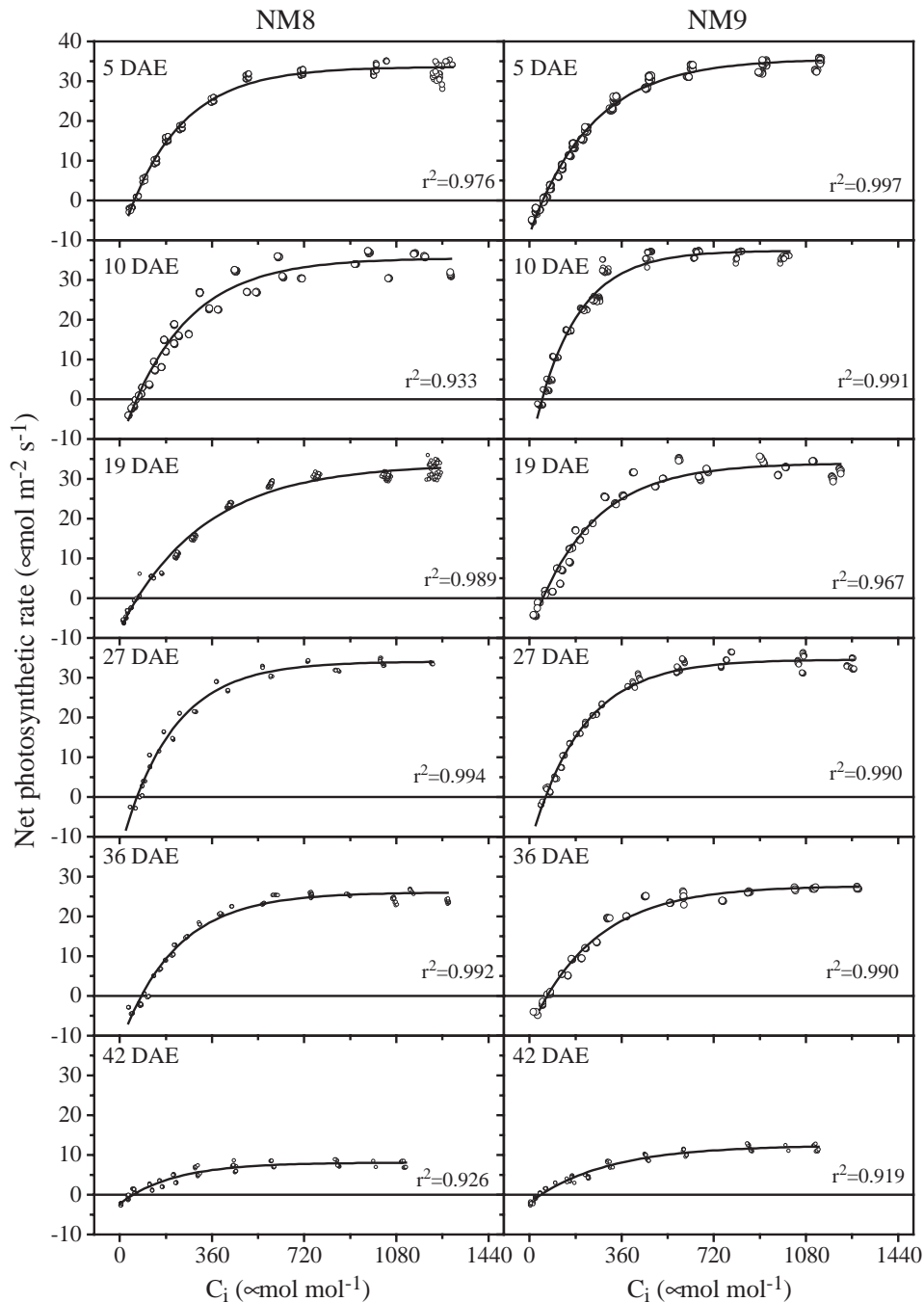


Fig. 2. Photosynthetic responses of flag leaves to intercellular  $\text{CO}_2$  concentration ( $C_i$ ) in flag leaves of two spring cultivars grown in the field. Each regression curve was obtained by fitting at two or three replicate data.  $r^2$  denotes determination coefficient between observed and simulated values.

$\text{CO}_2$  fixation rates displayed a negative pattern (Fig. 3C), indicating that a lower carboxylation efficiency is correlated with a higher  $\text{CO}_2$  requirement for half saturation of the enzyme substrate complex. CE had a lower value after emergence and then increased when maturing at around 10 DAE. There was a steady decrease from 10 to 42 DAE, especially markedly at the late senescing stage. Compared with NM8, NM9 had higher values in  $P_{C_a=360}$ , while no significant difference in  $P_{\max}$  for both cultivars, with the only exception for the values at 19 DAE.

The changing pattern of leaf respiration in the light ( $R_D$ ) under  $\text{CO}_2$ -free air condition was similar to that of the value of

the  $\text{CO}_2$  compensation point ( $C_{\text{comp}}$ ) (Table 1). Both  $R_D$  and  $C_{\text{comp}}$  were increased after emergence and peaked at full anthesis (around 12 DAE), then slightly decreased at 19 DAE, and after that increased again until 36 DAE, but profoundly reduced at 42 DAE. NM9 had lower values in  $R_D$  during the whole developmental stage of flag leaves, only with the exception of the value at 42 DAE ( $3.2 \mu\text{mol m}^{-2} \text{s}^{-1}$  vs. 5.3, for NM8 and NM9, respectively).

To further elucidate changes in primary photochemical reactions in photosynthesis during senescence, photosynthetic electron transport activities were investigated using thylakoids

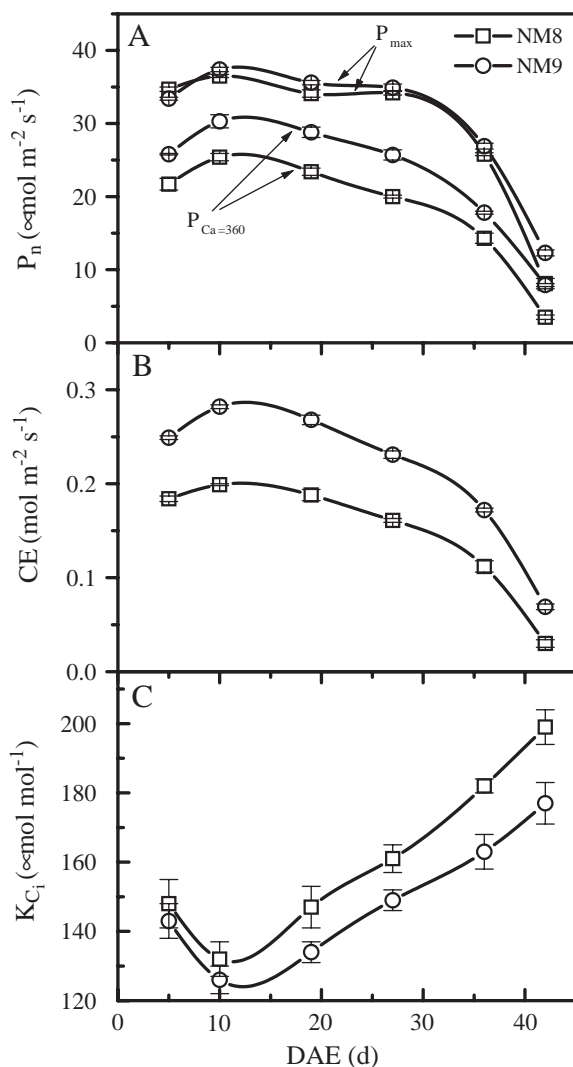


Fig. 3. Light- and  $\text{CO}_2$ -saturated ( $P_{\text{max}}$ , A), ambient  $\text{CO}_2$  ( $P_{\text{Ca}=360}$ , A) photosynthetic rates ( $P_n$ ), carboxylation efficiency (CE, B) and the  $\text{CO}_2$  concentration for the half-maximal  $\text{CO}_2$  fixation rates ( $K_{\text{Ci}}$ , C) (Michaelis–Menten constant) during flag leaf senescence of two spring wheat cultivars grown in the field.

isolated from flag leaves. As shown in Table 2, PS II electron transport activity decreased more than those of PS I and the whole electron chain. There were relative stable values for PS I and whole electron chain between 10 and 19 DAE, while PS II started to decline significantly after 10 DAE. For example, PS II electron transport activity was reduced about 19–20%,

47–50% and 76–82% at 27, 36 and 42 DAE for both cultivars, respectively. However, the PS I mediated electron transport activity was decreased only by 14%, 34–47% and 54–61% at 27, 36 and 42 DAE, respectively. A similar pattern of PS I activity was also observed for whole chain electron transport activity. NM8 showed earlier and larger decreases in electron transport activities than NM9.

As shown in Fig. 4, at room temperature the fluorescence emission for the two cultivars increased from emergence to full expansion in 10 DAE, and then maintained relatively stable values between 10 and 19 DAE, and then significantly decreased with flag leaf aging. There was also a slight shift in the spectral peaks, from 677.5 nm at 5 DAE to 680.5 nm at 42 DAE for both cultivars, indicating that the thylakoid organization had changed during flag leaf senescence. Before 19 DAE, NM9 had similar spectrum of room-temperature fluorescence emission in the red wavelength range as that of NM8, but with leaf aging, there was an increasing difference in the intensity of emission between the two cultivars.

Table 3 showed the peak values in room-temperature absorption spectra of isolated chloroplasts occurring at 430 nm and 680 nm during the whole developmental stage for both cultivars. The spectral peaks increased after emergence, highest at 19 DAE and then decreased significantly with leaf aging, indicating large quantitative changes in pigment contents during flag leaf senescence. The decrease in absorption at the red peak of around 680 nm was greater than that at the blue-violet peak of around 430 nm during leaf senescence, implying that the decrease due to senescence of chlorophyll was quicker than that of carotenoids in the course of leaf senescence. There was no much difference in the peak heights between two cultivars before 36 DAE. Table 3 also showed that during senescence the decrease in absorption at the peaks was less in NM9 than that in NM8.

#### 4. Discussion

The final stage of leaf development is inevitably senescence with a decline in physiological activity. Senescence of flag leaves in two spring wheat cultivars grown in the field was characterized by Chl loss (leaf yellowing), decreases in the Chl *a/b* ratio, photosynthetic rate, CE, absorption properties of thylakoid membranes at room temperature, and activities of photosynthetic electron transport. However,

Table 1

Leaf respiration in the light ( $R_{\text{D}}$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and  $\text{CO}_2$  compensation point ( $C_{\text{comp}}$ ,  $\mu\text{mol mol}^{-1}$ ) in flag leaves of two spring wheat cultivars from flag leaf emergence through senescence

Parameter	Cultivar	DAE (d)					
		5	10	19	27	36	42
$R_{\text{D}}$	NM8	11.3a (0.9)	13.9b (1.4)	11.7a (0.8)	14.3c (0.4)	15.5c (0.4)	3.2d (0.2)
	NM9	7.6a* (0.3)	10.1b (0.2)	8.3a* (0.5)	9.0c* (0.6)	10.7b* (0.8)	5.3d* (0.5)
$C_{\text{comp}}$	NM8	55.0a (2.6)	67.7b (2.4)	65.7b (1.2)	77.3c (1.5)	83.7c (2.7)	51.7d (2.3)
	NM9	41.0a* (1.7)	61.6b (3.5)	52.7c* (2.2)	65.3d* (2.3)	69.3e* (3.2)	41.3f* (1.7)

$R_{\text{D}}$  was derived from  $P_n/C_i$  curves, and  $C_{\text{comp}}$  were calculated from  $P_n/C_a$  curves. Values in parentheses are standard errors for each mean ( $n=3$ ). Different letters in a same row indicate significant difference ( $P \leq 0.05$ ) and \* denotes significant difference between two wheat cultivars at the same period.

Table 2  
Photochemical activities during flag leaf senescence: photosystem II (PS II,  $\text{H}_2\text{O} \rightarrow \text{K}_3\text{F}(\text{CN})_6$ ) [ $\text{mmol} (\text{O}_2 \text{ evolved}) \text{kg}^{-1}(\text{Chl}) \text{s}^{-1}$ ], photosystem I (PS I, DCPIP  $\rightarrow$  MV) [ $\text{mmol} (\text{O}_2 \text{ consumed}) \text{kg}^{-1}(\text{Chl}) \text{s}^{-1}$ ], and whole chain electron transport (whole,  $\text{H}_2\text{O} \rightarrow \text{MV}$ ) [ $\text{mmol} (\text{O}_2 \text{ consumed}) \text{kg}^{-1}(\text{Chl}) \text{s}^{-1}$ ]

Parameter	Cultivar	DAE (d)					
		5	10	19	27	36	42
PS II	NM8	44.4a (3.2)	52.1b (2.4)	50.0c (5.1)	41.6d (3.1)	26.2e (3.3)	9.4f (2.0)
	NM9	51.1a* (2.4)	55.2b* (2.1)	52.2c (3.4)	44.7d* (1.5)	29.1e* (4.0)	13.4f* (2.3)
PS I	NM8	137.2a (9.1)	156.4b (9.4)	149.3b (7.1)	133.8c (5.6)	102.6e (9.1)	61.7f (6.0)
	NM9	151.4a* (3.1)	164.4b* (5.5)	159.7b* (6.1)	140.7c* (3.4)	122.4d* (7.2)	76.0e* (9.1)
Whole	NM8	28.4a (3.0)	31.4b (4.0)	29.4b (6.2)	27.7c (7.2)	21.4d (5.6)	13.1e (4.4)
	NM9	30.2a* (5.1)	32.3b (4.4)	30.5b (5.2)	28.5c (4.2)	22.9d (6.1)	16.7e* (8.3)

Values in parentheses are standard errors for each mean ( $n=4$ ).

these photochemical and biochemical parameters did not show similar changing pattern and senescing behavior from emergence through senescence of flag leaves and there were some differences in these parameters between these two half-father cultivars (NM8 and NM9), which have different senescent appearance.

The well-known phenomenon in senescing leaves is the loss of Chl content. The degree of Chl loss of the wheat cultivars is strongly associated with light-saturated photosynthetic rate during grain filling, as well as being associated with differences in final grain yield (Reynolds et al., 2000). In this study, the Chl content in NM9 was higher than in NM8 during the flag leaf late senescence between 36 and 42 DAE, showing that process of leaf senescence in NM9 was

delayed compared with the earlier senescent cultivar NM8. However, NM9 had higher light-saturated photosynthetic rate  $P_{\text{Ca}=360}$  (Fig. 3A) and lower leaf respiration, including respiration in the light ( $R_{\text{D}}$ ) (Fig. 2 and Table 1) and dark respiration (Zhang et al., 2005b) during the whole developmental stage, only with the exception of  $R_{\text{D}}$  at 42 DAE. In combination with the longer duration of having a higher content of Chl at the late senescence (Fig. 1A), these findings may be partly responsible for the higher grain yield in NM9 than in NM8.

Selective loss of pigments denotes structural alterations in individual photosystems (Subhan and Murthy, 2001). The decrease in Chl  $a/b$  ratio during leaf senescence (Fig. 1B) could be further confirmed both by changes in room-temperature absorption and emission properties of thylakoid and by activities of the two photosystems (PS I and PS II). Chl  $a$  as the pigment of the reaction centers and Chl  $b$  as the antenna pigment play an important role in photosynthesis. The decrease at the red peak of around 680 nm, which was greater than that at the blue-violet peak of around 430 nm during leaf senescing (Table 3), indicated that the senescence of photosynthetic core antenna was quicker than that of the LHC antenna system in the course of leaf senescence, which is consistent with the results reported by Wang et al. (2004). Gossauer and Engel (1996) stated that the conversion of Chl  $b$  to Chl  $a$  should precede Chl degradation in higher plants. Therefore, it is easy to conclude that the decreased Chl  $a/b$  ratio implied that the loss of Chl  $a$  may be much greater than Chl  $b$  conversion during leaf aging.

As a useful measure of the two major classes of photosynthetic antennae (with Chl  $a$  only and Chl  $a$  and  $b$ ), the

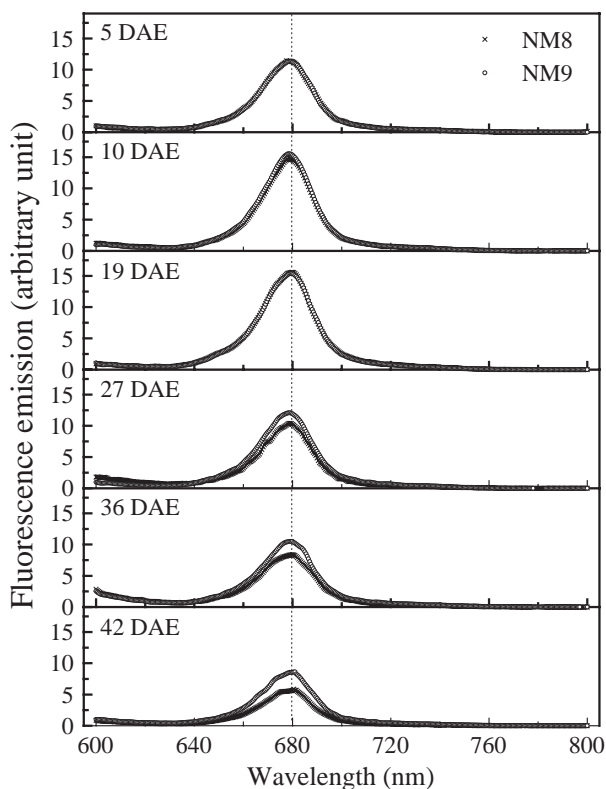


Fig. 4. Room-temperature fluorescence emission spectra through a red band pass filter centered at 680 nm of thylakoid membranes during flag leaf senescence in two spring wheat cultivars grown in the field. All samples were adjusted to a Chl concentration of  $20 \mu\text{g ml}^{-1}$ .

Table 3  
Peak values of room-temperature absorption spectra of thylakoid membrane at 430 nm and 680 nm in flag leaves from emergence through senescence in two spring wheat cultivars grown in the field

DAE	OD <sub>430</sub>		OD <sub>680</sub>		OD <sub>430</sub> /OD <sub>680</sub>	
	NM8	NM9	NM8	NM9	NM8	NM9
5	0.758	0.777	0.485	0.442	1.6	1.8
10	0.923	0.935	0.525	0.520	1.8	1.8
19	0.927	1.055	0.526	0.590	1.8	1.8
27	0.801	0.920	0.431	0.489	1.9	1.9
36	0.665	0.761	0.315	0.396	2.1	1.9
42	0.533	0.626	0.267	0.319	2.0	2.0

change in this ratio can provide an insight into the stability of thylakoid membrane organization during leaf senescence (Subhan and Murthy, 2001). Strangely, it does not always change in a consistent direction, even within a species (Canfield et al., 1995), probably depending on senescence-induced factors. Dark-induced senescence often results in an increased Chl *a/b* ratio (Fang et al., 1998), while natural leaf senescence leads to a decreased Chl *a/b* ratio. Basing on data from 16 field-grown wheat cultivars, Reynolds et al. (2000) reported a declined ratio with leaf aging, similar to the present result (Fig. 1B) and other results showed an increase (see review in Scheumann et al., 1999; Hikosaka and Terashima, 1996; Murchie et al., 2002; Kitajima and Hogan, 2003; Munné-Bosch and Peñuelas, 2003). Lu et al. (2001) reported a relatively stable value in 20 days after anthesis but then a marked increase up to harvest time in a wheat cultivar also grown in the field.

Such a decrease in the Chl *a/b* ratio seems to be a conflict with the fact that Chl *a* is more or less stable during senescence, but chlorophyll *b* is almost labile (Thomas et al., 2002). One possible reason seems to be that there is a cycle of interconversion between Chl *a* and *b* that is particularly significant in senescence, because only pigments with the “*a*” configuration on pyrrole group B are recognized by the Chl degradation pathway (Metile et al., 1999). Thus, the relatively persistent Chl *b* may not be the same Chl *b* that was primarily localized in the antennae of pre-senescent leaves, but instead may be the product of unbalanced operation of the *a*–*b* interconversion cycle (Zavaleta-Mancera et al., 1999). Additionally, the chemical stability of Chl *a* and *b* may be different from their biological stability, that is, Chl *a* in living plant tissues may be less stable than Chl *b*, at least in aging wheat leaves.

Concerning the physiological significance of the decreased ratio of Chl *a/b* during leaf senescence, it is still unclear due to contradictive results from different studies. According to the optimum theory, plants itself can adjust photosynthetic pigment composition to obtain maximum resources (e.g. carbon fixation and mineral uptake) at the prime stage. But at the serious senescent stage, plants cannot overcome the functional decline. Decreased Chl *a/b* ratio manifests an increase in the relative amount of LHC system with respect to the core complexes of especially PS II and partly PS I (Björkman, 1981; Lam et al., 1984; Gnojek, 1992; Šprtová and Marek, 1999). Functionally, the decrease in Chl *a/b* ratio improves the capture of far-red radiation and helps to maintain an energy balance between PS I and PS II (Björkman, 1981; Lei and Lechowicz, 1997), resulting in optimal functioning. Therefore, the decrease in Chl *a/b* ratio may be in favor for the plants before late senescence. The faster decrease in this ratio in the earlier senescent cultivar NM8 further implied that Chl *a/b* ratio may play an important role in controlling leaf senescence.

Investigation of  $P_n/C_i$  curves can provide us with information on photochemical versus biochemical properties. Light- and CO<sub>2</sub>-saturated net photosynthetic rate ( $P_{max}$ ) derived from the CO<sub>2</sub> curves reflects the maximum rate of RuBP regener-

ation (assumed to equal maximum rate of coupled photosynthetic electron transport) and the initial slope of the  $P_n/C_i$  curve shows the carboxylation efficiency (CE), i.e. amount of active Rubisco (von Caemmerer and Farquhar, 1981). In the present work,  $P_{max}$  increased in 10 DAE, displayed relatively constant between 10 and 27 DAE for both wheat cultivars, and markedly reduced after 27 DAE, such a changing pattern similar to that of the activity of whole electron transport (Table 2). Statistical analysis showed that they both were closely correlated, with a coefficient of 0.994 ( $P < 0.0001$ ) for NM8 and 0.998 ( $P < 0.0001$ ) for NM9, respectively. This means that the activity of whole electron transport determined the light- and CO<sub>2</sub>-saturated net photosynthetic rate, namely, the capacity of RuBP regeneration. Additionally, the relatively stable capacity of RuBP regeneration before 27 DAE was just in a period with a rapid grain filling rate (data not shown), suggesting that matter production in grain (sink) may be related to RuBP regeneration in leaves (source), which may be controlled by hormone signals (e.g. ABA) from developing grain (Cao et al., 2004).

Although the capacity of RuBP regeneration kept higher values before 27 DAE, the actual net photosynthetic rate  $P_{Ca=360}$  started to decline from 10 DAE for both cultivars (Fig. 3A). Some studies showed that the light-saturated net photosynthetic rate is positively correlated to the activity of Rubisco (Hesketh et al., 1981; Evans, 1986). In the present work, a strong correlation ( $r=0.997$ ,  $P < 0.0001$ ) was also observed for the two cultivars. Therefore, decreased  $P_{Ca=360}$  was attributed partly to a decrease in CE after 10 DAE. Similarly, stronger correlation was observed between  $P_{Ca=360}$  and the activity of PS II ( $r=0.993$  for NM8,  $P < 0.0001$ ;  $r=0.989$  for NM9,  $P < 0.0002$ ). At the late senescence, decreased RuBP regeneration was also an additional factor in the inhibition of the photosynthesis. The stomatal limitation cannot be excluded during leaf senescence, especially at the late senescence (Zhang et al., 2005a), but probably it is not the main reason (Cornish et al., 1991). Therefore, photosynthesis depends not only on the activity of Rubisco, but also on the function of the light-harvesting and electron transport systems within the chloroplast (Spano et al., 2003).

Aliev and his co-workers (1996) reported that the activity of RuBP carboxylation was larger in highly productive wheat genotypes than in the lower productive ones. The similar result was observed here. Compared with NM8, higher yield wheat cultivar NM9 had higher values in CE (Fig. 3B), which resulted in higher  $P_{Ca=360}$ . The significantly higher CE in NM9 (Fig. 3B) indicates that it had a higher amount of activated Rubisco in leaves than NM8. In combination with lower leaf dark respiration (Zhang et al., 2005b), lower  $R_D$  and  $C_{comp}$  means that the high yield wheat cultivar NM9 had a lower C loss, which would be in favor of yield production in grain. Therefore, higher CE and lower  $R_D$  may be selected as a good criterion for a high grain yield wheat variety and it is possible to breed a high grain yield wheat variety with both high CE and low leaf respiration.

In conclusion, all photosynthetic parameters were increased from emergence to full expansion, indicating that the photo-

synthetic apparatus was under functional maturation during this period. After full expansion the primary decline in photosynthesis was due to CE of Rubisco, being accompanied by a decrease in Chl content. Starting with a significantly declined Chl *a/b* ratio, the primary decline in photosynthesis of flag leaves was attributable not only to declined CE, but also to declined electron transport of the photosystems, especially PS II. Compared with the early senescing cultivar NM8, both the higher Chl content and the higher photosynthetic capacity of NM9 became apparent mainly at the late stage of senescence, which indicates that the rate of grain filling at the late stage might play an important role in determining the grain yield of spring wheat cultivars.

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