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BRIEF COMMUNICATION

Changes in the non-photochemical quenching of chlorophyll fluorescence during aging of wheat flag leaves

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Abstract

Non-photochemical quenching of chlorophyll fluorescence (q_N) and its three components (q_{Nf}, q_{Nm}, and q_{Ns}) in the flag leaves of wheat grown in the field were studied by a fluorometer *PAM-2000* on clear days. The diurnal variation patterns of q_N in just fully extended (JFEL) and aging leaves (AL) were similar, but q_{Nm} declined markedly in JFEL while it remained at a relatively high level in AL under strong sunlight at noon. Furthermore, at midday q_{Nf} was higher than q_{Ns} in JFEL, but much lower in AL. The results show the relative contributions of different mechanisms in preventing the photosynthetic apparatus from photodamage change during leaf development.

Additional key words: diurnal variation; photoinhibition; photosystem 2 reaction centres; state transition; trans-thylakoid membrane proton gradient; *Triticum aestivum*; xanthophyll cycle.

The parameters of chlorophyll (Chl) *a* fluorescence induction are sensitive indices for diagnosing the state of operation of the photosynthetic apparatus. Yet their magnitude changes with leaf age (for review see Šesták and Šiffel 1997, for more recent papers see also Behera and Chodury 1997, De Almeida *et al.* 1998, Santos *et al.* 1998).

Non-photochemical quenching (q_N) of Chl fluorescence, caused by some non-photochemical processes, is often analyzed in studies on the mechanisms of response and acclimation of the photosynthetic apparatus to environmental stress such as strong irradiance and low temperature. According to its relaxation time in darkness, q_N can be divided into three components: the fast phase (q_{Nf}, 1-2 min) related to the trans-thylakoid-membrane proton gradient (ΔpH), the middle phase (q_{Nm}, 10-15 min)

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attributed to state transition, and the slow phase (q_{Ns}, more than 20 min) induced by photoinhibition. Most studies of the three components of q_N were done indoors (Demmig and Winter 1988, Horton and Hague 1988, Quick and Stitt 1989). A few reports were focused on the relative contributions of the three components of q_N under field conditions (Wu *et al.* 1997, Hong and Xu 1999). In the study with wheat leaves reported here, we found the regular changes of the three components of q_N during leaf aging, indicating that the predominant mechanism in preventing the photosynthetic apparatus against photodamage changes with the stage of leaf development.

Wheat (*Triticum aestivum* L. cv. Gaoyuan 338) plants were grown in pots with soil from February to June. They were placed under natural sunlight in the field, adequately watered and fertilized. The just fully expanded (JFEL) and aging (AL) flag leaves (a 26 d gap was between them, from the end of April to the late May) were used for measurement of Chl fluorescence, Chl content, and relative membrane permeability.

All measurements of Chl fluorescence were performed with a portable modulation fluorometer *PAM-2000* (*Walz*, Effeltrich, Germany). Before each measurement, wheat leaves were dark-adapted for at least 2 h in the morning. To determine the initial fluorescence F_0, the measuring radiation (photon flux density, PFD, 0.1 μmol m^{-2} s^{-1}) was turned on and F_0 was recorded. Then the leaf samples were exposed to a saturating pulse (about 3 000 μmol m^{-2} s^{-1}, 0.8 s) to obtain maximal fluorescence F_m. The ratio of variable to maximal fluorescence, F_v/F_m, in dark-adapted leaves could be thus calculated as $F_v/F_m = (F_m - F_0)/F_m$. Non-photochemical quenching (q_N) and its three components were detected in the same leaf for a whole day and calculated according to Quick and Stitt (1989). Diurnal variations in q_N and its three components (one of three replicates measured in different dates) are shown in Fig. 1. Although these curves of diurnal variations were not identical, their basic features were the same. Chl contents in the JFEL and the AL were determined with 80 % acetone according to Lichtenthaler (1987). Relative membrane permeability of wheat leaves was measured with a *DDS-11A* electrical conductivity system (Shanghai, China) according to Yang *et al.* (1996).

Decrease of the Chl content is an apparent sign of leaf aging under natural conditions without any nutrient deficiency. The Chl content of the AL was about half that of the JFEL (Table 1). Furthermore, the relative membrane permeability of the AL was more than twice that of the JFEL. In both types of leaves at different development stages, the F_0 decreased at midday, indicating the enhanced operation of some heat dissipation mechanisms in the light-harvesting complex. Nevertheless, the photochemical efficiency of photosystem 2 (PS2), F_v/F_m, of the AL was lower than that of the JFEL. The difference in the F_v/F_0 between them was more obvious (Table 1), and hence the AL was more seriously photoinhibited than the JFEL at midday on clear days.

For flag leaves at different development stages, the diurnal variation patterns of q_N were similar, but those of the three components of q_N differed significantly in the following two aspects (Fig. 1). First, by comparison with that at medium PFD in the morning or afternoon, q_{Nm} declined markedly at midday in the JFEL. On the contrary, it increased slightly at midday in the AL. Second, in the JFEL, both q_{Nf} and q_{Ns} increased with the increasing of sun irradiance in the morning and reached their maximum values at midday, with q_{Nf} much larger than q_{Ns}. However, in AL the q_{Ns} increased while q_{Nf} decreased a little at midday, and q_{Nf} was much smaller than q_{Ns}. These results indicate that during leaf development a regular change occurs in the magnitude of relative contribution of the mechanisms that protect the photosynthetic apparatus from photodamage. Namely, with the aging of leaves the contribution of the heat dissipation dependent on ΔpH (q_{Nf}) decreases, while the importance of state transition (q_{Nm}) and the heat dissipation dependent on xanthophyll cycle (q_{Ns}) increases.

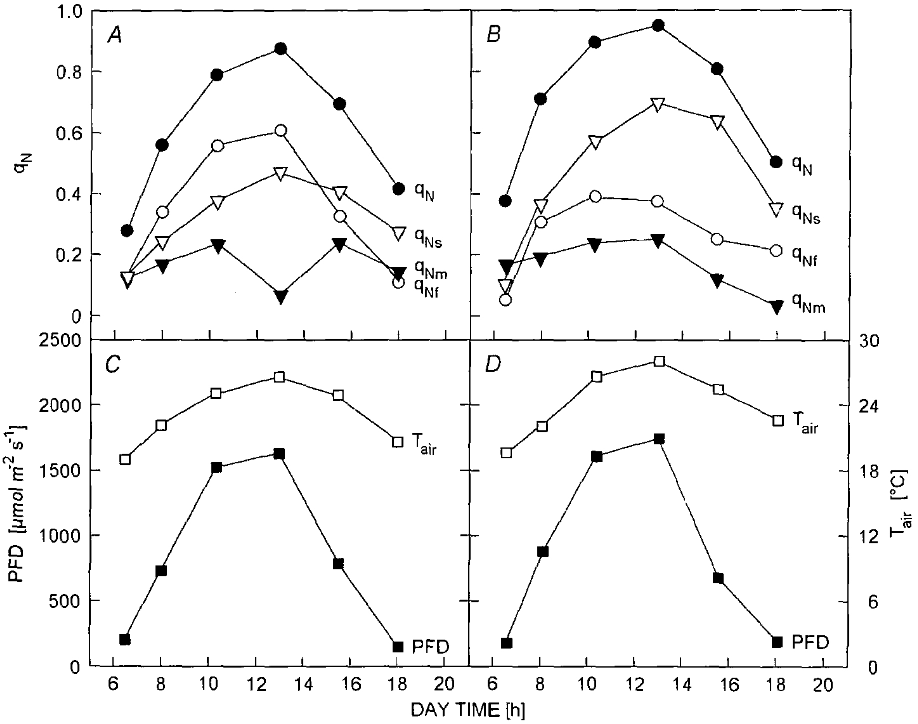

Fig. 1. Diurnal variations in the non-photochemical quenching of chlorophyll fluorescence (q_N) and its three components (q_{Nf}, q_{Nm}, and q_{Ns}) in the just fully expanded (*A*) and the aging (*B*) flag leaf of wheat grown in the field. Three replicates of the observation were made using different flag leaves at different date, but only one of them is shown here. In spite of some differences between them their basic features pointed out by us are identical. As relation units, the respective values of air temperature (T_{air}) and photosynthetic photon flux density (PFD) are shown in lower panels (*C* and *D*).

Certainly, the variation of q_{Ns} is not caused merely by the change in the xanthophyll-cycle-dependent heat dissipation. It is generally thought that q_{Ns} is related to photoinhibition of photosynthesis. Furthermore, photoinhibition may be due to the increase of the xanthophyll-cycle-dependent heat dissipation and/or reversible inactivation or irreversible damage of some PS2 reaction centres (Long *et al.* 1994). If the increase in q_{Ns} under strong irradiance at midday was caused by the irreversible damage of PS2, both q_N and q_{Ns} should remain relatively high in late afternoon. However, this is not the case we observed, so the increase in q_{Ns} under strong irradiance at midday cannot, at least mainly, be attributed to the irreversible damage of the photosynthetic apparatus. The change in the F_0 listed in Table 1 supports this deduction. Several factors contribute to the changes in F_0 under light. An increase in the xanthophyll-cycle-dependent heat dissipation leads to a decrease in F_0, while irreversible damage or reversible inactivation of PS2 reaction centres leads to a rise in F_0 (Demmig *et al.* 1987, Krause and Weis 1991). When these factors act simultaneously (possibly but not necessarily), the direction of F_0 change depends on the predominant one (Xu and Wu 1996). The decline of F_0 in flag leaves of wheat at midday (Table 1) strongly suggests that the increase in q_{Ns} in wheat leaves under strong sun irradiance is not mainly caused by reversible inactivation or irreversible damage of PS2 reaction centres. Instead, it is most likely due to the increased operation of heat dissipation mechanism dependent on the xanthophyll cycle.

Table 1. Changes in chlorophyll (Chl) content, Chl fluorescence parameters (F_0, F_v/F_m, and F_v/F_0), and relative membrane permeability (RMP) in just fully expanded (JFEL) and aging (AL) leaves. Each value is the mean of 5~10 leaves together with standard deviation in parentheses. MOR and MID represent "morning" and "midday", respectively. Level of significance: $^*p<0.05$; $^{**}p<0.01$. The asterisks (**) beside the numbers represent the level of significance between morning and midday, and the asterisks (* or **) below the numbers represent that between JFEL and AL.

	Chl	RMP	F_0		F_v/F_m		F_v/F_0	
	[g m^{-2}]	[%]	MOR	MID	MOR	MID	MOR	MID
JFEL	0.4874	8.594	256.0	217.0^{**}	0.835	0.727^{**}	5.094	2.671^{**}
	(0.0220)	(2.036)	(6.6)	(11.8)	(0.012)	(0.018)	(0.454)	(0.250)
AL	0.2770	19.186	192.0	156.0^{**}	0.813	0.612^{**}	4.361	1.588^{**}
	(0.0200)	(2.964)	(7.8)	(5.3)	(0.008)	(0.027)	(0.223)	(0.181)
	**	**	**	**	*	**	**	**

Then, why q_{Nf} was much lower than q_{Ns} at midday in aging leaves, not like that it was much higher than q_{Ns} in just fully expanded leaves? In aging leaves the increase in the membrane permeability shown in Table 1 is probably disadvantageous for maintenance of high ΔpH across thylakoid membranes. Consequently, the role of ΔpH-dependent heat dissipation weakens in aging leaves, which leads to an enhanced operation of the xanthophyll-cycle and state transition-dependent mechanisms.

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