Alterations in photosynthesis and antioxidant enzyme activity in winter wheat subjected to post-anthesis water-logging

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Abstract

Winter wheat (*Triticum aestivum* L.) cultivars Yangmai 9 (water-logging tolerant) and Yumai 34 (water-logging sensitive) were subjected to water-logging (WL) from 7 d after anthesis to determine the responses of photosynthesis and anti-oxidative enzyme activities in flag leaf. At 15 d after treatment (DAT), net photosynthetic rate under WL was only 3.7 and 8.9 μmol(CO_2) m^{-2} s^{-1} in Yumai 34 and Yangmai 9, respectively, which was much lower than in the control. Ratios of variable to maximum and variable to initial fluorescence, actual photosynthetic efficiency, and photochemical quenching were much lower, while initial fluorescence and non-photochemical quenching were much higher under WL than in control, indicating damage to photosystem 2. WL decreased activities of superoxide dismutase and catalase in both cultivars, and activity of peroxidase (POD) in Yumai 34, while POD activity in Yangmai 9 was mostly increased. The obvious decrease in the amount of post-anthesis accumulated dry matter, which was redistributed to grains, also contributed to the grain yield loss under WL.

Additional key words: catalase; chlorophyll fluorescence; dry matter redistribution; net photosynthetic rate; peroxidase; photosystem 2; superoxide dismutase; *Triticum aestivum*; yield.

Introduction

Along with the global climate change, water-logging (WL) is becoming a worldwide extreme event. Both the magnitude and frequency of WL are predicted to increase, especially in high and mid-latitude regions, where increasing excessive wetness will be associated with high precipitation (Schumacher and Johnson 2006, García *et al.* 2007). WL severely reduces grain yield of aerobic crops by 20–50 % in winter wheat (Belford and Cannell 1979, Cannell *et al.* 1984, Musgrave 1994, Zhang *et al.* 2006). The downstream area of Yangtse River accounts for more than 15 % of the total wheat growth area in China, that also suffers severe WL with an average rainfall of about 500–800 mm during the wheat growth season which mostly concentrates during the period between anthesis and maturity. Since this period is critical for grain yield formation (Blacklow and Incoll 1981, Nicolas *et al.* 1985, Borrell *et al.* 1989), post-anthesis WL becomes the major limiting factor for wheat yield improvement in this area.

WL decreases crop yield by destructing pigments, depressing photosynthesis, and promoting leaf senescence (Cannell *et al.* 1984, Huang *et al.* 1994, Musgrave 1994, Brisson *et al.* 2002, Smethurst and Shabala 2003) and then affecting plant growth, dry matter accumulation and distribution (Brisson *et al.* 2002, Jiang and Wang 2006).

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Abbreviations: APA – amount of post-anthesis accumulated dry matter transfer into grain; CAPA – contribution of APA to grain mass; CAT – catalase; Chl – chlorophyll; CRAP – contribution of RAP to grain mass; DAA – days after anthesis; DAT – days after treatment; DM – dry matter; F_0 – original fluorescence; F_v/F_m – maximum photochemical efficiency; F_v/F_0 – ratio of variable fluorescence to original fluorescence; GM – grain mass; MDA – malondialdehyde; NPQ – non-photochemical quenching; PAR – photosynthetically active radiation; PBS – phosphate buffer solution; P_N – net photosynthetic rate; POD – peroxidase; PRAP – redistribution percentage of redistributed pre-anthesis stored dry matter from vegetative organs to grain; PS – photosystem; q_P – photochemical quenching; RAP – redistribution amount of pre-anthesis stored dry matter from vegetative organs to grain; RC – reaction centre; SOD – superoxide dismutase; SPAD – soil plant analysis development; Φ_{PS2} – actual photosynthetic efficiency.
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Photosystem (PS) 2 plays a key role in the responses of leaf photosynthesis to environmental perturbations (Baker 1991), and its malfunction is easily detected by the chlorophyll (Chl) *a* fluorescence, which is the subtle reflection of primary reactions of photosynthesis (Sayed 2003). Chl fluorescence responses have been broadly investigated in wheat exposed to different adversities, such as drought, heat, excessive irradiance, heavy metals, *etc*. (Behera and Choudhury 2003, Soja and Soja 2005, Zhao and Tan 2005, Hassan 2006). However, knowledge on Chl fluorescence response to WL is very limited and does not fully explain the photosynthetic response mechanism under WL stress.

When PS2 is damaged, excessive excited photon energy by the light reaction systems is thought to produce reactive oxygen species (ROS) under stress, which cause severe damage to cell membranes, DNA, and proteins (Ahmed *et al.* 2002). Superoxide dismutase (SOD), catalase (CAT), and peroxidases (POD) are antioxidative enzymes scavenging these ROS (Ahmed *et al.* 2002, Monneveux *et al.* 2003, Stepien and Klobus 2005). In wheat, many studies have focused on the production and scavenging process under adversities of drought, heat, and salinity (Zhang and Kirkham 1994, Mandhania *et al.* 2006, Zhao *et al.* 2007), heavy metals (Gajewska and Sklodowska 2007a,b), *etc*., while seldom under WL (Kalashnikov *et al.* 1999), especially under post-anthesis WL.

We selected two winter wheat cultivars to investigate the alterations in photosynthesis, Chl fluorescence, and membrane lipid peroxidation in flag leaf of wheat subjected to post-anthesis WL. The knowledge will help understand the physiological mechanism of yield loss in wheat by WL.

Materials and methods

Plants: Wheat cultivars Yangmai 9 and Yumai 34 were grown in plastic pots (22 cm in height and 25 cm in diameter) filled with 7.5 kg of clay soil. The soil contained 12.1 g kg^{-1} organic matter, 1.2 g kg^{-1} total N, 82.3 mg kg^{-1} available N, 30.9 mg kg^{-1} Olsen-P, 126.7 mg kg^{-1} available K, and was mixed with 0.9 g N, 0.36 g P_2O_5, and 0.9 g K_2O per pot before being filled into the pot. Another 0.3 g N per pot was fertilized at jointing. Two water treatments were continuously established for each cultivar from 7 d after anthesis (DAA), *i.e.* soil relative water content maintaining at 70–80 % as control, and maintaining 1–2 cm water layer above the soil as WL treatment. The experiment was a completely random design, with four replications for each treatment.

P_N of flag leaf was measured using a portable photosynthesis system (*LI-6400*, *LI-Cor*, USA) with an open system and logged at CO_2 concentration of about 370 μmol mol^{-1} in the leaf chamber, and at the photosynthetically active radiation of 1 100 μmol m^{-2} s^{-1}. All measurements were conducted in sunny days from 09:00 to 11:00, starting from 7 DAA and ending at 22 DAA (15 d after treatment, DAT), when flag leaves obviously turned aged and P_N was very low in both cultivars. Ten leaves were taken for each treatment at each measurement.

Chlorophyll (Chl) content was measured with a Chl Meter (*SPAD-502*, *Minolta*, Japan) using the same flag leaf as used for P_N measurement. For each measurement, five SPAD (soil plant analysis development) readings were taken at the position around two thirds of the leaf length and averaged as the Chl content for the leaf.

Chl fluorescence: The same leaf as for P_N measurement was taken for Chl fluorescence measurement with a portable pulse amplitude modulation fluorometer (*FMS2*, *Hansatech*, King's Lynn, Norfolk, UK). The maximum and initial fluorescence (F_m and F_0) were taken after a 20-min dark-adaptation. Non-photochemical quenching (NPQ), actual photosynthetic efficiency (Φ_{PS2}), and photochemical quenching (q_P) were estimated using "actinic light" of 1 100 μmol m^{-2} s^{-1} for 10 min and an 1-s pulse of saturating radiation of 4 000 μmol m^{-2} s^{-1}. The parameters were calculated according to Li *et al.* (2007).

Lipid peroxidation was estimated by measuring the content of 2-thiobarbituric acid-reactive substances in leaf homogenates, prepared in 20 % trichloracetic acid containing 0.5 % 2-thiobarbituric acid, and heated at 95 °C for 25 min. Malondialdehyde (MDA) content was then determined spectrophotometrically (*UV-2401*, *Shimadzu Corp.*, Japan) at A_{532} and corrected for non-specific turbidity at A_{600}.

Activities of antioxidant enzymes: 0.5 g of leaves was homogenized in 5 cm^3 of respective extraction buffer (50 mM pH 7.0 PBS+0.4 % PVP) in a pre-chilled mortar and pestle on ice. The homogenate was centrifuged at 10 000×*g* for 30 min at 4 °C and the supernatant was collected as crude enzyme extraction.

Total SOD activity was assayed by measuring the ability of inhibiting the photochemical reduction of nitro blue tetrazolium (NBT). The reaction mixture (3 cm^3) contained 130 mM methionine, 750 μM NBT, 100 μM EDTA, and 0.05 cm^3 of enzyme extract in 50 mM PBS (pH 7.8). The reaction was started with 20 μM riboflavin by exposing the cuvette to a 15-W circular "white light" tube for 10 min. The reaction mixture was measured at A_{560}. One unit of SOD activity was defined as the amount of enzyme per fresh mass (FM) sample causing 50 % inhibition of the photochemical reduction of NBT.

100 mm^3 of enzyme extract and 4 cm^3 of 50 μM PBS (pH 7.0) were mixed and incubated at 30 °C for 10 min. The reaction was started with adding 1 cm^3 of 50 μM

H_2O_2, and terminated after 1 min by adding 2 cm^3 of 10 % H_2SO_4. CAT activity was then determined by estimating the residual H_2O_2 in the reaction solution using 10 mM $KMnO_4$ titration to pink.

POD activity was measured according to the change in absorption at 470 nm due to guaiacol oxidation. Total 3 cm^3 of reaction solution contained 0.2 M PBS (pH 6.0), 29 % H_2O_2, and guaiacol. The reaction was started by adding 10 mm^3 of enzyme extract. Changes in readings at A_{470} were then recorded within 3 min after the start of the reaction at 1-min intervals.

Dry matter (DM) distribution and redistribution: Redistribution amount of pre-anthesis stored DM from vegetative organs to grain (RAP) was calculated by the difference in dry matter in vegetative organs between anthesis and maturity. Redistribution percentage of redistributed pre-anthesis stored DM from vegetative organs to grain (PRAP) was the percentage of RAP to DM in vegetative organs at anthesis. Contribution of RAP to grain mass (CRAP) was the percentage of RAP to grain DM. Amount of post-anthesis transfer of accumulated DM into grain (APA) was given by the difference between final grain matter at maturity and RAP, while its contribution to grain matter (CAPA) was the percentage of APA to grain mass at maturity.

Statistical analysis: The one-way ANOVA was used to test the significance between WL and control treatment, and between the two cultivars.

Results

Chl content: WL significantly decreased SPAD value in both wheat cultivars, especially after 10 DAT (Fig. 1*A*). Yumai 34 was more sensitive to WL, since the SPAD in flag leaf decreased much faster than in Yangmai 9. At 15 DAT, SPAD value in flag leaf decreased by 16.2 and 10.1 %, respectively, in Yumai 34 and Yangmai 9 under WL, compared with that under control.

***P*N** of flag leaf declined rapidly since starting of WL in both cultivars, as compared with the control (Fig. 1*B*). P_N was also more sensitive in Yumai 34 than Yangmai 9 in response to WL, and decreased by 73.6 and 40.7 %, respectively, at 15 DAT. Hence WL obviously promoted wheat flag leaf senescence by destructing Chl and depressing leaf P_N. Yumai 34 was a more sensitive cultivar to WL than Yangmai 9.

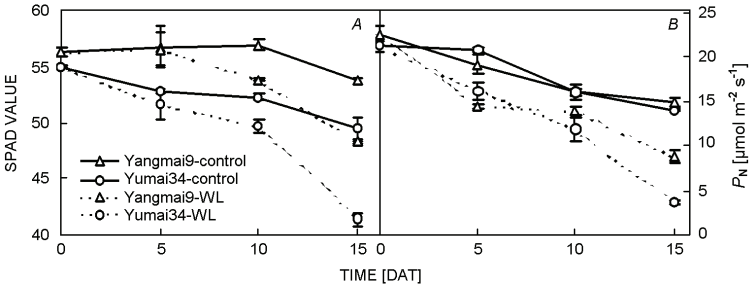

Fig. 1. Post-anthesis water-logging (WL) effects on SPAD value (*A*) and net photosynthetic rate, P_N (*B*) of wheat flag leaf.

Chl fluorescence: Very similar changing pattern of F_0 in response to WL was observed in the two cultivars (Fig. 2*A*). Under WL, F_0 slightly decreased during the first 5 DAT, then rapidly increased between 5 and 10 DAT, and decreased again ever since till 15 DAT, while in the control F_0 straight decreased with grain filling. WL generally increased F_0 in both cultivars, especially between 10 and 15 DAT, compared with control.

F_v/F_m and F_v/F_0 decreased during the grain filling under both WL and control in both cultivars, but the decrease was much faster under WL than in the control (Fig. 2*B,D*). Consequently, F_v/F_m and F_v/F_0 were much higher under WL than in the control between 5 and 15 DAT. Yangmai 9 showed much higher F_v/F_m than Yumai 34 during the whole measurement period under both WL and control conditions.

Φ_{PS2} and q_P showed decline patterns similar to F_v/F_m under both water treatments in both cultivars during the whole measurement period (Fig. 2*D,E*). WL again obviously decreased both Φ_{PS2} and q_P, especially between 10 and 15 DAT. NPQ showed reverse pattern to q_P and increased during the whole measurement period (Fig. 2*F*). Under WL, NPQ was 28.1 and 32.9 % higher than under control in Yangmai 9 and Yumai 34, respectively, at 15 DAT. This indicated that much more excess photon energy absorbed by Chl *a* in flag leaf was dissipated under WL than that in the control.

These results show that potential damage of PS2 and increase in non-photochemical dissipation of photon energy in wheat flag leaf happened especially at 10 to 15 DAT.

Lipid peroxidation and antioxidant enzymatic activities: MDA content in wheat flag leaf increased from 0 to 15 DAT under both treatments in both cultivars (Fig. 3*A*). WL obviously increased MDA content in both cultivars during the whole measurement period. At 15 DAT, MDA content was about 15.3 and 22.1 % higher

under WL than that in the control in Yangmai 9 and Yumai 34, respectively. This indicated Yumai 34 suffered from more severe damage of lipid peroxidation in flag leaf than Yangmai 9 by WL, since content of MDA is well accepted as production of membrane lipid peroxidation in organisms.

Activities of SOD and CAT obviously decreased along with the grain filling in both control and WL treatment in both cultivars (Fig. 3*B,C*). However, the decreases in SOD and CAT were much faster under WL than in the control in the two cultivars. Compared with control at 15 DAT, SOD activity in flag leaves of Yangmai 9 and Yumai 34 was decreased by 26.6 and 29.8 % in the WL variant, while CAT activity by 51.3 and 19.5 %, respectively. POD activity also declined during the whole measurement period, while the changing pattern with respect to WL varied with cultivar (Fig. 3*D*). In Yumai 34, the POD activity was generally reduced by WL and at 15 DAT was about 13.5 % lower under WL than in the control. In Yangmai 9, however, WL generally increased POD activity except at 10 DAT. At 15 DAT, POD activity was about 10.1 % higher under WL than in the control.

DM distribution and redistribution: Significant decreases were observed in redistribution amount of pre-

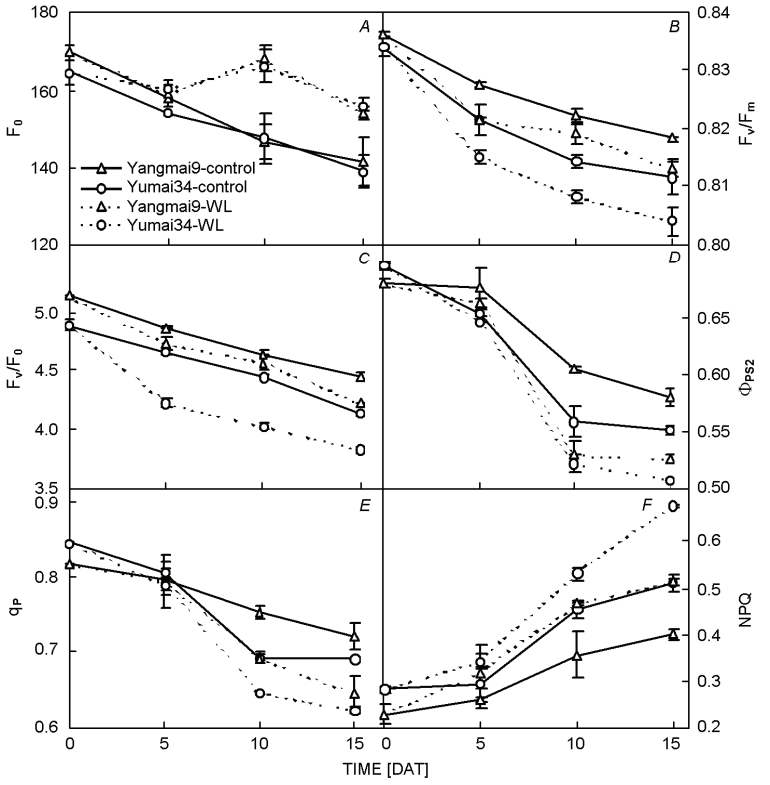

Fig. 2. Post-anthesis water-logging (WL) effects on (*A*) original fluorescence (F_0), (*B*) maximum photochemical efficiency (F_v/F_m), (*C*) F_v/F_0, (*D*) actual photosynthetic efficiency (Φ_{PS2}), (*E*) photochemical quenching (q_P), and (*F*) non-photochemical quenching (NPQ) of wheat flag leaf.

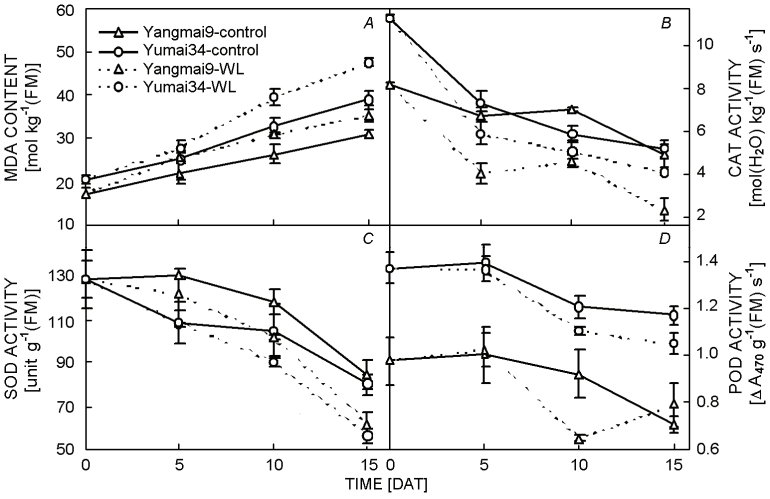

Fig. 3. Post-anthesis water-logging (WL) effects on (*A*) malondialdehyde (MDA) content and activities of (*B*) superoxide dismutase (SOD), (*C*) catalase (CAT), and (*D*) peroxidase (POD) in wheat flag leaf.

Table 1. Post-anthesis water-logging (WL) effects on distribution and redistribution of dry matter in wheat. APA – amount of transfer of post-anthesis accumulated dry matter into grains [g stem^{-1}]; CAPA – contribution of APA to grain mass [%]; CRAP – contribution of RAP to grain mass [%]; FRAP – fraction of redistributed pre-anthesis stored dry matter from vegetative organs to grains [%]; GM – grain mass at maturity [g spike^{-1}]; RAP – redistribution amount of pre-anthesis stored dry matter from vegetative organs to grains [g stem^{-1}]. **, *, and NS – significant differences between cultivars at 1 %, 5 %, and no significance, respectively.

Cultivar	Treatment	RAP	FRAP	GM	CRAP	APA	CAPA
Yangmai 9	Control	0.49 a	25 a	2.14 a	23 a	1.65 a	77 a
	WL	0.38 b	19 b	1.62 b	18 b	1.32 b	82 a
Yumai 34	Control	0.58 a	29 a	1.89 a	31 a	1.31 a	69 b
	WL	0.21 b	10 b	1.32 b	19 b	1.07 b	81 a
F value of cultivar		NS	10.80*	13.13*	NS	23.26*	NS

Table 2. Post-anthesis water-logging (WL) effects on the yield and yield components of wheat. **, *, and NS – significant differences between cultivars at 1 %, 5 %, and no significance, respectively.

Cultivar	Treatment	Spikes per pot	Kernels per spike	1000-kernel mass [g]	Biomass [g pot^{-1}]	Kernel yield [g pot^{-1}]	Harvest index
Yangmai 9	Control	21a	57.1 a	45.5 a	3.58 a	54.6 a	0.72 a
	WL	20 a	60.6 a	30.5 b	3.19 b	36.9 b	0.58 b
Yumai 34	Control	23 a	44.1 a	49.5 a	3.34 a	50.2 a	0.65 a
	WL	24 a	41.4 a	33.7 b	2.97 b	33.0 b	0.46 b
F value of cultivar		6.42*	99.36**	70.06**	NS	68.02**	170.07**

anthesis stored DM from vegetative organs to grains, amount of post-anthesis accumulated dry matter transfer into grains (APA), fraction of RAP to total pre-anthesis stored dry matter (FRAP), and consequently in final grain mass per spike in both cultivars under WL as compared with control (Table 1). This indicated that post-anthesis WL decreased saccharide supply for grain filling. Contribution of RAP to grain mass (CRAP) also decreased, while contribution of APA to grain mass (CAPA) increased under WL. This inferred that most part of the grain mass depended on the post-anthesis accumulation of photosynthates, while the contribution of RAP to grain mass was more unreliable for grain mass formation in wheat under WL, when compared with control.

Yield and yield components: Significant loss in grain yield was observed, which mostly attributed to sharp decrease in 1 000-kernel mass, since no significant difference in kernel number per spike were observed in both cultivars under WL, compared with control (Table 2). Obvious decrease in harvest index was also observed contributing to grain loss under WL. This was consistent with the much lower RAP, FRAP, and CRAP under WL than in the control (Table 1).

Discussion

WL promotes plant leaf senescence *via* chlorosis (Huang *et al.* 1994, Smethurst and Shabala 2003), which was also found in our study of wheat under post-anthesis WL. In the study of Smethurst and Shabala (2003), however, leaf Chl content increased during the first six days after WL in lucerne, which is inconsistent with our finding of significant decrease in leaf P_N under post-anthesis WL that agrees with the results for other plant species under WL at various growth stages (Malik *et al.* 2001, Ahmed *et al.* 2002).

We found a damaged light reaction system. All parameters that indicate function of PS2 including F_0, the efficiency to capture excitation energy by open PS2 reaction centre (RC), the potential maximum photochemical efficiency of PS2 (F_v/F_m), and the actual quantum yield of PS2 obviously decreased under WL in comparison with those in the control. Meanwhile, q_P significantly decreased, while NPQ dramatically increased, indicating much more absorbed and excited photon energy was dissipated along with the malfunction of PS2 in flag leaf of wheat under WL than in the control. This inferred that the conversion efficiency of captured photon energy by PS2 decreased and the following carbon assimilation process could also be depressed by post-anthesis WL, which is consistent with the decreased P_N of flag leaf. This also agrees with the finding of Schlüter and Crawford (2001) that significant reduction in the photosynthetic capacity occurred due to damage of PS2 RC and subsequent electron transport in leaves of *Acorus calamus* and *Iris pseudacorus* in anoxic conditions. Our results are also consistent with the researches in lucerne (Smethurst and Shabala 2003,

Smethurst *et al.* 2005) and barley (Pang *et al.* 2004) under WL and wheat under water deficit and/or heat (Khan 2005, Hassan 2006), high irradiance (Behera and Choudhury 2003, Monneveux *et al.* 2003), cold (Rizza *et al.* 2001), and salinity (Stepien and Klobus 2005). However, Guidi and Soldatini (1997) observed that the F_v/F_m ratio remained unchanged while photosynthesis continued to decrease in soybean leaf after 6 d of flooding. The cause of this disagreement is not clear, but the difference of species used could be partly involved.

The malfunction of PS2 that reduces the efficiency of electron transport for photosynthetic reaction could result in substantive accumulation of ROS, since chloroplast is one of the main ROS production sites in plant cells (Garnczarska *et al.* 2004). ROS can be generated by the direct transfer of the excitation energy from Chl to produce singlet oxygen or by oxygen reduction in the Mehler reaction in chloroplast (Stepien and Klobus 2005), and then promotes the membrane lipid peroxidation in cell. Content of MDA, the product of lipid peroxidation significantly increased, while activities of ROS scavenging enzymes of SOD and CAT dramatically decreased under post-anthesis WL. This indicated that severe lipid peroxidation occurred, at least to a great extent, as a sequence of malfunction of PS2 in wheat flag leaf.

However, activity of another ROS scavenger, POD, showed diverse patterns between cultivars in responding to WL. This could be related to the fact that POD in plant plays multiple functions, because of its high number of iso-forms (Passardi *et al.* 2005). POD can act both as ROS scavenger catalysing the reduction of H_2O_2 (Hiraga *et al.* 2000) and as generator of H_2O_2, $^{\bullet}OH$, and $HOO^{\bullet}$ (Passardi *et al.* 2005) and polymerise cell well compounds (Passardi *et al.* 2004). They prevent biological and chemical attacks by modulating activity and expression following internal and external stimuli at every plant growth stage (Passardi *et al.* 2005). In our study the role of POD played under post-anthesis WL could be diverse between cultivars and between different days after WL treatment in Yangmai 9. POD activity was higher under WL than in the control in Yangmai 9, except at 10 DAT (Fig. 3*D*). Since Yangmai 9 is a WL-tolerant cultivar in comparison with Yumai 34, POD might play anti-WL stress roles in Yangmai 9, which needs further evidence.

H_2O_2 is a powerful inhibitor of the Calvin cycle in chloroplasts (Takeda *et al.* 1995). The inhibited Calvin cycle in the dark reaction of photosynthesis can show a feedback effect that counteracts the photochemical reaction and depress the function of light RC of PS2, and then re-enhance the production of ROS when there is no effective release of WL damage. Thereafter, a malignant feedback in the bio-pathways of photosynthesis and ROS reaction may occurs and result in the un-reversed senescence of flag leaf under post-anthesis WL.

Besides the decrease in P_N leading to low DM accumulation under WL (Table 2), the redistribution amount and both the pre-anthesis and post-anthesis transfers of stored DM from vegetative organs to grains obviously decreased (Table 1), resulting in the significant single grain mass and harvest index decrease and subsequent great loss in the grain yield.

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