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Genetic decrease in fatty acid unsaturation of phosphatidylglycerol increased photoinhibition of photosystem I at low temperature in tobacco leaves ☆

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ABSTRACT

Leaves of transgenic tobacco plants with decreased levels of fatty acid unsaturation in phosphatidylglycerol (PG) exhibited a slightly lower level of the steady state oxidation of the photosystem I (PSI) reaction center P700 ($P700^+$) than wild-type plants. The PSI photochemistry of wild-type plants was only marginally affected by high light treatments. Surprisingly, all plants of transgenic lines exhibited much higher susceptibility to photoinhibition of PSI than wild-type plants. This was accompanied by a 2.5-fold faster re-reduction rate of $P700^+$ in the dark, indicating a higher capacity for cyclic electron flow around PSI in high light treated transgenic leaves. This was associated with a much higher intersystem electron pool size suggesting over-reduction of the PQ pool in tobacco transgenic lines with altered PG unsaturation compared to wild-type plants. The physiological role of PG unsaturation in PSI down-regulation and modulation of the capacity of PSI-dependent cyclic electron flows and distribution of excitation light energy in tobacco plants under photoinhibitory conditions at low temperatures is discussed. This article is part of a Special Issue entitled: Photosynthesis Research for Sustainability: from Natural to Artificial.

1. Introduction

The unique and highly conserved lipid composition of thylakoid membranes is dominated by two uncharged galactolipids, monogalactosyldiacylglycerol (MGDG) and digalactosyl-diacylglycerol (DGDG), comprising approximately 50 and 20% of the total acyl lipids in thylakoid membranes respectively. The remaining lipids are distributed between the negatively charged phosphatidylglycerol (PG) and a sulfolipid sulfoquinovosyldiacylglycerol (SQDG). PG is the phospholipid that is present in thylakoid membranes and although its amount in thylakoid membranes is only 10% of the total chloroplast lipids it accounts for approximately 85% of the total PG in plant leaves [1–4].

The essential role of acyl lipids in thylakoid membranes as one of the important factors controlling the structural organization and functional activities in photosynthetic membranes has been well characterized and reviewed [4–7]. Moreover, since PG is the only phospholipid within the thylakoid membranes, its specific role in the chloroplast development, functional activities and assembly of the photosynthetic apparatus has been extensively studied [7,3,8]. Reduced content of PG in the *pgp1* mutant of *Arabidopsis* caused retardation of plant growth and chloroplast differentiation, and a decrease in photosynthetic activity [9,10]. PG has been also identified as indispensable for photoautotrophic growth of *Synechocystis* sp. PCC 6803 [11,12]. Furthermore, the presence of PG in photosynthetic membranes in higher-plant leaves plays a significant role in modulating the susceptibility of PSII to photoinhibition and acclimation to low temperatures [13–15].

In addition, it has been demonstrated that the acclimation and sensitivity of various photoautotrophs to low temperature conditions depend not only on lipid composition, but also on unsaturation level of thylakoid lipids, which is accompanied by an increase in the fatty acid unsaturation. High levels of unsaturated fatty acids in chloroplast lipids are important to maintain plant growth, chloroplast structure, photosynthetic capacity/stability at low temperatures [16–18], altered the tolerance of cyanobacteria to salt stress [19,20] and stabilized the photosynthetic apparatus against photoinhibition at low temperature in transgenic tobacco plants [21]. It has been demonstrated that not only the overall unsaturation level of chloroplast lipids, but specifically the degree of unsaturation of fatty acids in PG in plastid membranes strongly correlates with the chilling sensitivity of higher plants [19,22,23]. A decreased level of fatty acid unsaturation of PG in thylakoid

Abbreviations: PSI, photosystem I; PSII, photosystem II; P700, reaction center pigment of PSI; $P700^+$, oxidized form of the reaction center pigment of PSI; PG, phosphatidylglycerol

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membranes of genetically modified tobacco plants resulted in reduced growth at low temperatures and increased sensitivity to cold stress [24]. In contrast, increased levels of *cis*-unsaturated fatty acids in PG of thylakoid membranes, alleviated chilling stress-induced inhibition of photosynthetic rates in transgenic rice seedlings [25] and tomato plants [26]. It has been also shown that the structural consequences of genetically engineered saturation of the fatty acids of PG in tobacco thylakoid membranes result in a rigid lipid domain existing at temperatures below 25 °C [27].

The specific role of PG in acclimation to low temperatures and the specific enrichment in PG of various PSII preparations (PSII membranes, PSII core complexes) and light-harvesting pigment–protein complexes of PSII (LHCII) in higher plants and cyanobacteria [28–31] directed most of the research in establishing the role of PG in PSII structural organization and functions (for reviews see: [7,4,32,72]). It has been demonstrated that specific binding of PG with D1 protein stabilizes the PSII reaction centers [33] and is required for dimerization of PSII [34]. The important role of PG in stabilizing the acceptor side of PSII (Q_B side) as well as the manganese binding in oxygen-evolving complex has been also established [35,36]. Stability of LHCII trimers is dependent on the molecular species of PG, especially its 16:1 (3t) content [37,38]. Hobe et al. [39,40] identified the specific PG binding motif at the N-terminus of LHCII apo-proteins required for LHCII trimerization.

Our recent studies have demonstrated a mechanism of photoinhibition of PSII in which the extent of photoinhibition is a result of balance between the rate of photodamage and the rate of repair [41,42]. Analysis of the photoinhibition in mutants and transformants of *Synechocystis* has revealed that the unsaturation of fatty acids stimulates the repair but does not affect the rate of photodamage [43,44]. Furthermore, transgenic tobacco, in which the unsaturation of fatty acids in PG had been reduced, demonstrated that the unsaturation of fatty acids stimulates the repair but had no effect on the damage also in photosynthesis of higher plants [21]. Thus, the contribution of unsaturation of fatty acids in PG and other lipids to photoinhibition of PSII has been well characterized in details [23].

By contrast, the contribution of unsaturation of fatty acids in membrane lipids to photoinhibition to PSI has not been investigated. The presence of two PG molecules localized in peripheral and one in the central core of PSI reaction center in cyanobacteria [45] implies that PG may play an important role not only in the function of PS I, but also presumably in the assembly of the PS I core complex [45,15]. In fact, an earlier study has revealed that the presence of only MGDG and PG with the molar ratio of 2:1 in PSI preparations from tobacco plants and PG was only associated with the PSI core complex [46]. It has been demonstrated that PG is essential for oligomerization of PSI reaction centers in cyanobacteria [47,48] and is also required for the translation of PSI subunits such as PsaA and PsaB, in cyanobacteria [49]. Deprivation of PG in the *cdsA*- or *pgsA*-mutants reduced the level of the PSI complex, but not of the PSII complex [12,35] and the loss of PG inhibited the synthesis of the PSI complex rather than its stability [50]. In addition, PG-dependent changes in the molecular organization of pigment–protein complexes of both the external antenna LHCI and PSI core complex have been also reported [51].

The purpose of this study was to clarify the role of unsaturation of fatty acids in PG on the PSI photochemistry under photoinhibitory conditions at low temperatures. We report that PSI was more sensitive to photoinhibition at low temperature in transgenic plants of tobacco with genetically reduced levels of unsaturated fatty acids in PG.

2. Materials and methods

2.1. Plant materials and growth conditions

Wild-type and transgenic tobacco plants were obtained as described previously [13]. Plants were grown in pots at 25 °C under fluorescent light (230 μmol photons m^{-2} s^{-1}) and 16/8 h light/dark cycle and were supplied with 0.1% (v/v) Hyponex (Hyponex Corporation, Marysville, OH, USA) on a regular basis[13]. For high-light treatment, leaves from wild-type and three transgenic lines of plants were exposed to photosynthetically active radiation of 1500 μmol photons m^{-2} s^{-1} treatments at 3 °C.

2.2. Analysis of fatty acid composition of PG in thylakoid membranes

Chloroplasts were isolated from wild-type and transgenic tobacco plants as described in [52]. Lipids were extracted from isolated thylakoid membranes according to the method of Bligh & Dyer [53]. PG was fractionated by ion-exchange column chromatography and then purified by thin-layer chromatography on silica gel as described previously [22]. Purified PG was subjected to methanolysis and the resultant methyl esters were analyzed by gas–liquid chromatography [22].

2.3. P700 measurements

The redox state of P700 was determined *in vivo* under ambient CO_2 conditions using a PAM-101 modulated fluorometer equipped with ED-800T emitter-detector and PAM-102 units according to [54] as described in [55]. Far red light (FR, $\lambda_{max} = 715$ nm, 25 W m^{-2}, Schott filter RG 715) was provided by the 102-FR light source. MT (multiple turnover — 50 ms) and ST (single turnover — half peak width 14 μs) saturating flashes were applied with a XMT-103 and XST-103 power/control units respectively. The redox state of P700 was evaluated as the absorbance change around 820 nm in a custom designed cuvette. The multi-branched fiber optic system connected to the emitter-detector unit was attached to the adaxial side of the leaf and measurements were made at the growth temperature of 20 °C. The signals were recorded using an ADC-12BN4 data acquisition card and a FIP-Version 4.3 fluorescence induction program (Q_A-Data© 1995, Turku, Finland) installed on an IBM-compatible personal computer. The complementary area between the oxidation curve of P700 after ST and MT excitation and the stationary level of $P700^+$ under FR representing the ST- and MT-areas respectively were calculated using a Microcal™ Origin™ Version 4.1 software (Microcal Software Inc., Northampton, MA, USA) and were used for estimations of the functional pool size of intersystem electrons on a P700 reaction center basis which was determined as [56]: $e^-/P700 =$ MT-area/ST-area.

3. Results and discussion

In agreement with previous results, transformation of tobacco plants with a binary plasmid pSQ resulted in considerably altered fatty acid composition of PG in thylakoid membranes [13,24]. The relative amounts of hexadecanoic (16:0) and octadecanoic (18:0) acids in wild-type plants of tobacco were 29 and 2 mol%, respectively. As expected, a 2-fold higher amount (mol%) of hexadecanoic acid was observed in all transgenic plants compared to the wild-type plants (Table 1). The relative amount of octadecanoic acid was also significantly increased (3–4 fold). Concomitantly, and in accordance with previous results [20,34], the content of *cis*-unsaturated fatty acids (18:1 + 18:2 + 18:3) decreased from 37.9 mol% in wild-type plants to 12–18 mol% in the transgenic plants (Table 1).

Selective inhibition of PSI-related photochemical activities under low temperatures and either high light or moderate/weak illumination has been reported [55,57–61]. The extent of PSI photooxidation ($P700^+$) was assessed *in vivo* by measuring the extent of far-red (FR) light-induced absorbance change at 820 nm ($\Delta I_{820}/I_{820}$) [54,55,62]. Illumination of dark adapted tobacco leaf discs with saturating FR light caused a fast absorbance change resulting in a new steady state level of I_{820}, which reflects the oxidation of P700 to $P700^+$. Since under these conditions only the cyclic electron flow

Table 1
Fatty acid composition (mole percentage) of PG from leaves of wild-type and three pSQ lines of transgenic tobacco plants.

	Transgenic lines			
	Wild type	pSQ7-05-2	pSQ8-03-1	pSQ8-03-2
16:0	29	49	56	57
16:1t[a]	31	25	21	23
18:0	2	7	7	7
18:1	5	3	3	2
18:2	11	4	4	3
18:3	23	11	8	7
18:1 + 18:2 + 18:3	38	18	16	12
Σ*cis*-MS[b]	74	36	31	25

[a] 16:1t, Δ3-*trans*-hexadecenoic acid.
[b] Sum of the *cis*-unsaturated molecular species (Σ*cis*-MS) was calculated from the sum of unsaturated fatty acids (18:1 + 18:2 + 18:3) as described previously [20,34].

around PSI is supposed to be active, the increase in $\Delta I_{820}/I_{820}$ is a measure of the new steady state reflecting the net balance between the rates of FR light-induced oxidation of P700 and counteracting dark reduction of $P700^+$ by electron donation *via* cyclic electron flow (Fig. 1A). Application of single turnover (ST) flash of saturating white light during the steady state oxidation of P700 caused a partial reduction of $P700^+$, while the multiple turnover (MT) flash resulted in almost complete reduction of $P700^+$. It should be noted that the reduction of $P700^+$ induced by ST and MT flashes was completely inhibited in DCMU-treated leaves (data not shown), thus demonstrating that the reduction of $P700^+$ to P700 in the presence of FR light in response to ST or MT flashes in the absence of DCMU was due to activation of PSII-dependent electron flow (Fig. 1A).

The typical traces illustrating the FR light-induced P700 transients presented in Fig. 1 and the data summarized in Table 2 indicate lower capacity for P700 photooxidation ($\Delta I_{820}/I_{820}$, $P700^+$) in all pSQ-transgenic lines compared with the $P700^+$ values in wild-type tobacco leaves (Table 2). Kinetic measurements of dark re-reduction of $P700^+$ after turning off the FR light (Fig. 1), which is thought to reflect the extent of cyclic electron flow around PSI [63,64] and/or the interaction of stromal components with the intersystem electron transport chain [56] indicated significantly accelerated re-reduction of $P700^+$ in all transgenic lines of plants compared with wild-type plants (Fig. 2).

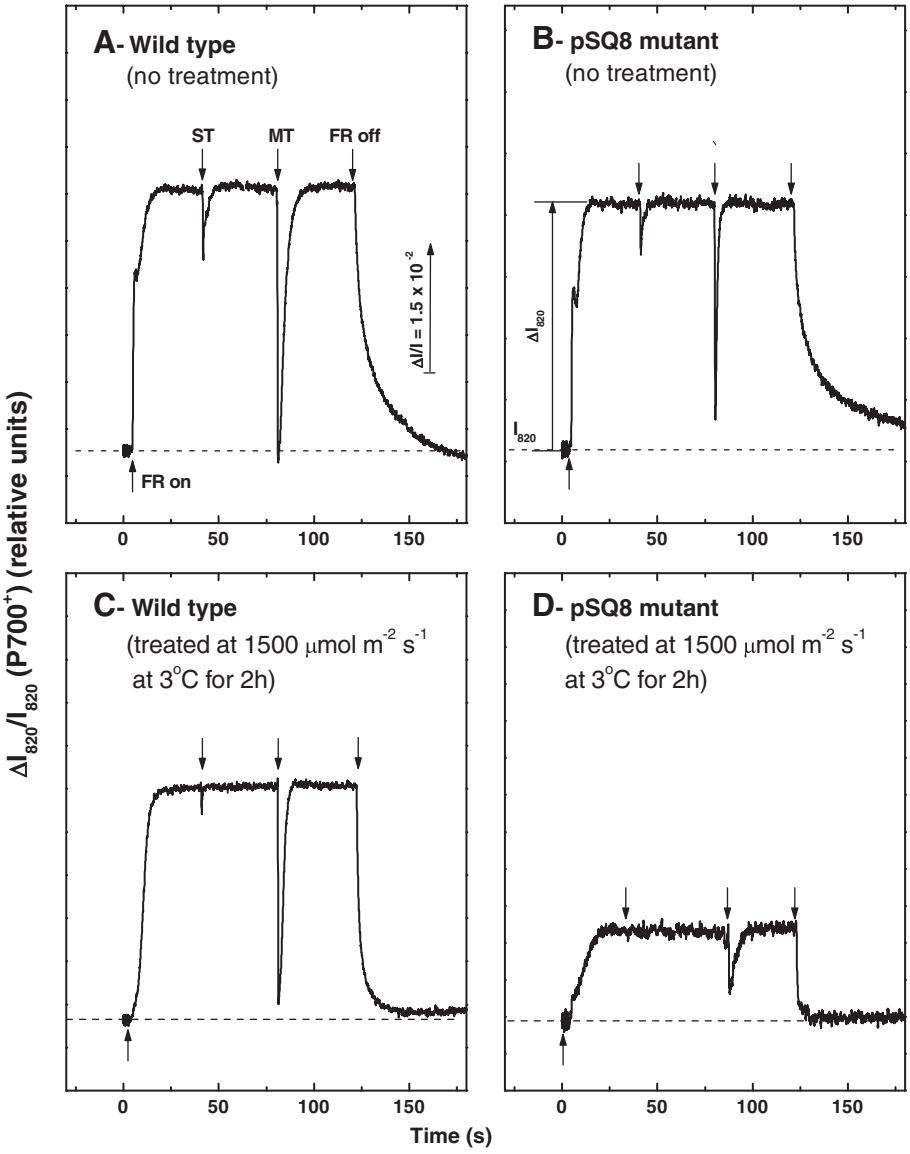

Fig. 1. Typical traces of *in vivo* measurements of the redox state of P700 in wild type (A, C) and pSQ8 transgenic (B, D) tobacco leaves. After reaching a steady state level of $P700^+$ by FR light, single turnover (ST) and multiple turnover (MT) pulses of white light were applied. ↑, light on; ↓, light off. A, B — Control leaves. C, D — High light (1500 μmol photons m^{-2} s^{-1}, 2 h, 3 °C) treated leaves. The measurements were performed at the growth temperature of 25 °C. Signals of $\Delta I_{820}/I_{820}$ are normalized in the same way in A–D.

Table 2
Photochemical efficiency of steady state oxidation of P700 measured as $\Delta I_{820}/I_{820}$ in leaves of wild-type and three pSQ lines of transgenic tobacco plants. All measurements were performed at the growth temperature. Mean ± SE values were calculated from 5 to 7 measurements in 3–5 independent experiments.

Transgenic lines	$\Delta I_{820}/I_{820}$ ($\times 10^{-2}$)	%
Wild type	3.1 ± 0.1	100
pSQ7-05-2	2.8 ± 0.2	90
pSQ8-03-2	2.0 ± 0.1	64
pSQ8-03-1	2.6 ± 0.3	83

Concomitant with the lower level of oxidized P700 ($P700^+$) (Table 2), exposure of pSQ-transgenic leaves to high light at low temperature caused greater loss of PSI activity measured by $\Delta I_{820}/I_{820}$ during the 4-h measuring period than in wild-type leaves (Figs. 1 and 2A). Moreover, the extent of PSI photoinhibition exhibited a very good relationship with the amount of unsaturated fatty acids (mol%) in wild-type and all pSQ-transgenic lines of plants tested (Fig. 3). In parallel, the rates of $P700^+$ reduction in the dark ($t_{1/2}$ $P700^{+\,decay}$) were significantly faster in all transgenic lines of plants exposed to high light stress during the same measuring period (Fig. 2B). This implies that transgenic plants can develop higher capacity for PSI-dependent cyclic electron flow (CEF) in response to high light stress than wild-type tobacco. This up-regulated CEF is consistent with the higher necessity to compensate the more impaired linear electron transfer in the transgenic plants compared to wild-type plants [65]. Furthermore, PSI cyclic electron transport is accelerated when PSI is exposed to acceptor-side limitation [66,67] or under conditions of elevated reduced state of PSII occurring as a result of high light treatment [59,55].

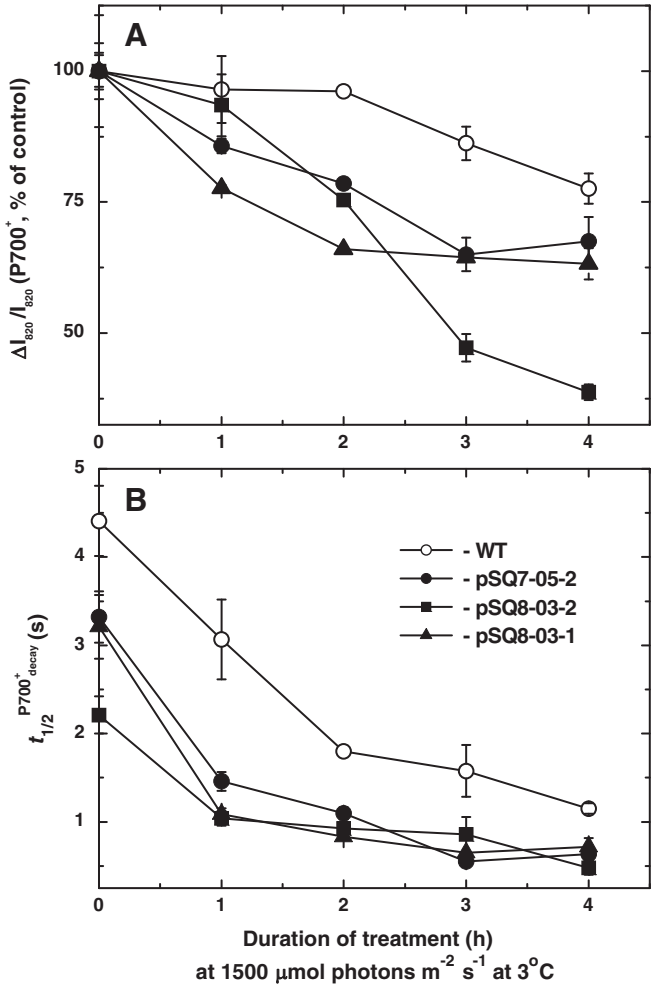

Fig. 2. Time courses of the steady state level of P700 photooxidation measured by $\Delta I_{820}/I_{820}$ (A) and half-times of $P700^+$ reduction ($t_{1/2}^{P700^+_{decay}}$) (B) during exposure to high light (1500 µmol photons m^{-2} s^{-1}) treatments at 3 °C in wild-type and three pSQ lines of transgenic tobacco leaves.

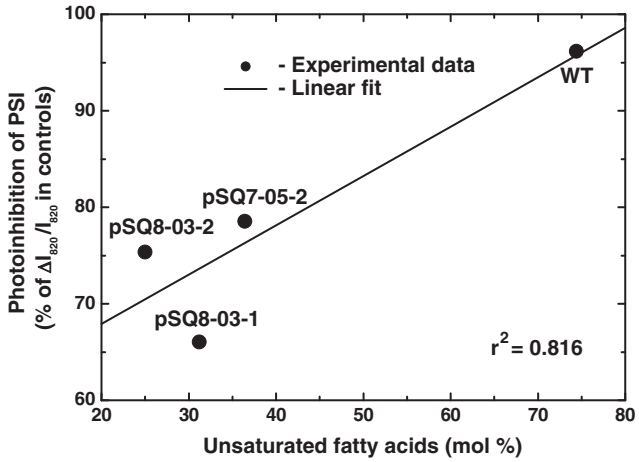

Fig. 3. Correlation between the extent of photoinhibition of PSI and the amount of unsaturated fatty acids in PG in wild-type and three pSQ transgenic lines of tobacco plants. The data represent the extent of photoinhibition of PSI measured as a percentage decrease of $\Delta I_{820}/I_{820}$ after exposure of plants to high light stress (1500 µmol photons m^{-2} s^{-1}) for 2 h, normalized to the initial values of $\Delta I_{820}/I_{820}$ of untreated WT and the three transgenic lines, respectively.

The higher sensitivity of pSQ-transgenic plants to photoinhibition of PSI (Figs. 1 and 2), and the lower capacity for P700 oxidation ($P700^+$) (Table 2) can be explained by either limitations at the acceptor side of PSI [68] or lower abundance of specific components of PSI [69]. Indeed, reduced levels of the PSI complex were reported in PG-deprived mutants [12,35] and the loss of PG inhibited the synthesis of the PSI complex [50]. Furthermore, limitations at PSI acceptor side could also enhance the over-reduction of the intersystem PQ pool observed under photoinhibitory conditions [55,62]. Indeed, 1.5-fold higher increase of the apparent electron pool size (e^-/P700) was registered in photoinhibited transgenic plants compared to WT (Table 3).

It has been reported previously that the lower proportion of unsaturated fatty acids in PG has no significant effect on PSII photochemistry under normal growth conditions [21]. However, leaves of the transgenic plants exhibited higher sensitivity to photoinhibition of PSII than those of WT plants [21]. In this case the higher sensitivity of PSI to photoinhibition could not be attributed to higher proportion of active PSII centers as suggested in [73].

In summary, the results in this report indicate that in addition to the well established effects of altered unsaturation levels of PG in thylakoid membranes on increased chilling sensitivity of photosynthesis

Table 3
Effects of high light (HL) treatment (2 h) at 3 °C on the relative intersystem electron donor pool size to PSI (e^-/P700) in leaves of wild type and three pSQ lines of transgenic tobacco plants. The photon fluence rate of the photoinhibitory light was 1500 µmol photons m^{-2} s^{-1}. All measurements were performed at the growth temperature. Mean ± SE values were calculated from 5 to 7 measurements in 3 independent experiments.

Lines	e^-/P700 (MT-area/ST-area)		
	Control	+HL	%
Wild type	6.5 ± 0.6	13.9 ± 1.2	215
pSQ7-05-2	6.7 ± 0.6	23.8 ± 3.3	349
pSQ8-03-2	5.1 ± 0.6	18.6 ± 1.2	361
pSQ8-03-1	8.4 ± 1.8	24.6 ± 2.7	293

[22–25,70,71] the reduced levels of unsaturated fatty acids in PG in pSQ-transgenic tobacco plants resulted in increased sensitivity of PSI to photoinhibition, which is strongly related to the level of unsaturated fatty acids in PG.

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