N-glycosylation regulates photosynthetic efficiency of *Arabidopsis thaliana*

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Abstract

N-glycosylation is one of the most important protein modifications in eukaryotes. It has been well established that N-glycosylation plays multiple roles in regulating stress tolerance of plants. However, the effects and mechanism of N-glycosylation on photosynthesis have not been well understood. In the present study, an obvious decrease in photosynthetic capacity and dry mass were detected in *alg3-3* and *cgl1-1*, two typical mutants in N-glycosylation process. The maximal photochemical efficiency of PSII decreased significantly in *cgl1-1*. The values of effective quantum yield of PSII photochemistry, rate of photosynthetic electron transport through PSII, and photochemical quenching coefficient, which reflected the photochemical efficiency of plants, decreased as well, while the values of quantum yield of nonregulated energy dissipation of PSII showed obvious enhancement, the similar tendency was also observed in *alg3-3*. Furthermore, we found that N-glycosylation was also required to maintain the stability of a chloroplast-located protein CAH1, which was closely related to photosynthesis. These results suggest that N-glycosylation plays crucial roles in maintaining photosynthetic efficiency.

Additional key words: asparagine-linked glycosylation; biomass; carbonic anhydrase; chlorophyll fluorescence.

Introduction

Asparagine-linked glycosylation (N-glycosylation) is an essential process occurring in the lumen of the endoplasmic reticulum (ER) and the secretory system of eukaryotic organisms (Silberstein and Gilmore 1996, Helenius and Aebi 2001). It is critical for protein folding, quality control, and transport of the secretory proteins, which plays crucial roles in plant adaptive responses to environmental stresses, such as plant immunity (Kang *et al.* 2015), temperature tolerance (Zhang *et al.* 2009), and salt sensitivity (Koiwa *et al.* 2003, Liu *et al.* 2018). More than one thousand proteins containing different N-glycosylation sites have been found in the model plant *Arabidopsis thaliana* (Song *et al.* 2013). As one of the major and most widespread protein posttranslational modifications in eukaryotes, the core processes are evolutionarily strongly conserved.

The initial synthesis of the lipid-linked oligosaccharide occurs on the cytoplasmic side of the ER and is mainly carried out by a series of glycosyltransferases of the ALG (asparagine-linked glycosylation) family (Bickel *et al.* 2005). The precursor is then transported across the ER membrane by a flippase-like protein for further synthesis to finally form an oligosaccharide containing three molecules of glucose (Glc), two molecules of N-acetylglucosamine

(GlcNAc), and nine molecules of mannose (Man) in the luminal side of the ER (Higgins 1994, Stagljar *et al.* 1994, Reiss *et al.* 1996, Larkin and Imperiali 2011, Schoberer *et al.* 2018). Subsequently, the completed oligosaccharide (Glc₃Man₉GlcNAc₂) is transferred by the oligosaccharyltransferase (OST) complex onto the asparagine in the Asn-Xaa-Ser/Thr consensus motif (Xaa represents any amino acid except proline) of the nascent polypeptides (Silberstein and Gilmore 1996, Helenius and Aebi 2001). The mannose glycans are gradually trimmed as part of ERQC (ER-associated quality control compartment) system (Aebi *et al.* 2010). Then, the correctly folded glycoproteins are transported to the Golgi apparatus and further modified by Golgi resident glycosyltransferases and glycosidases for the formation of glycoproteins with mature oligosaccharide (Song *et al.* 2013, Strasser 2016).

The significance of N-glycosylation modifications in mammals has been well exemplified by the severe congenital disorders resulting from mutations of the N-glycosylation pathway (Schollen *et al.* 2005). However, no visible change was found in the *Arabidopsis* N-glycosylation mutant *alg3*, which is defective in the biosynthesis of the Dol-PP-linked glycans; it seems reasonable to suggest that the glycans in the structure of mannose type were shown to be nonessential (Henquet *et al.*

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Abbreviations: ETR – rate of photosynthetic electron transport through PSII; F_v/F_m – maximal photochemical efficiency of PSII; q_N – nonphotochemical quenching coefficient; q_P – photochemical quenching coefficient; SD – standard deviation; $Y_{(NO)}$ – quantum yield of nonregulated energy dissipation of PSII; $Y_{(NPQ)}$ – quantum yield of regulated energy dissipation of PSII; Φ_{PSII} – effective quantum yield of PSII photochemistry.

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2008). Also, another mutation of N-glycosylation pathway in *Arabidopsis*, the *complex glycan less* mutant (*cgl1-1*), which completely lacks the complex N-glycans, has also no visible developmental disorders and only shows some changes in salt-stress response (Kang *et al.* 2008). The phenotype ambiguity of N-glycosylation mutants in ‘ideal’ greenhouses may be the reason why this process is rarely studied in plants (Zielinska *et al.* 2012). Nevertheless, the N-glycosylation abnormalities result in unfolded protein response (von Schaewen *et al.* 1993, Henquet *et al.* 2008). This reminds us that many unknown abnormalities are still to be discovered. It is well known that photosynthesis in plants and algae occurs in chloroplasts, and this process is the basis of plant growth and development. In addition, most chloroplast-located proteins are synthesized in the ER and then transported to the chloroplast through the secretory pathway. CAH1, alpha-type carbonic anhydrase (EC 4.2.1.1), is one of the few plant proteins known to be targeted to the chloroplast, and which is essential for efficient photosynthesis (Samuelsson and Karlsson 2001, Burén *et al.* 2011, Kupriyanova *et al.* 2017). The mature CAH1 harboring multiple glycoforms of N-glycans (Burén *et al.* 2011) might be a good substrate to study the relation between N-glycosylation and photosynthesis.

It has long been known that chlorophyll (Chl) fluorescence induction kinetics of leaves from plants provides an indicator of plant photosynthetic capacity. The fluorescence parameters are directly related to the energy transport, the photosynthetic carbon dioxide assimilation rate, and the formation of primary photosynthetic sugars in plants (Harbinson *et al.* 1990, Murchie and Lawson 2013). As rapid and noninvasive technique for detecting subtle differences of leaf metabolism or developmental disorders of the seedlings (Barbagallo *et al.* 2003), Chl fluorescence induction kinetics has received increasing attention in the fields of plant physiology, molecular biology, and molecular genetics.

In the present study, we analyzed the phenotype of *ALG3* and *CGL* knockout mutant plants. We showed that the mutants grow normally under greenhouse conditions. In order to investigate whether N-glycosylation could affect the photosynthetic capacity, we also examined if chlorophyll fluorescence parameters could reveal the possible alterations in the PSII photochemistry in the *alg3-3* and *cgl1-1* mutants. Our data showed that the photosynthetic capacity of *alg3-3* and *cgl1-1* decreased to different degrees. Moreover, the underlying molecular mechanisms of photosynthetic capacity changes were also analyzed.

Materials and methods

Plant materials and growth conditions: *Arabidopsis thaliana*, ecotype Columbia (Col-0), was the parental line for mutant plants and the generation of transgenic plants. Seeds of *alg3-3* (SALK_046061) and *cgl1-1* (CS6192) were obtained from the Arabidopsis Biological Resource Center (ABRC) at Ohio State University. The seeds were surface sterilized with 70% ethanol and then stratified at 4°C for 2 d in darkness. The stratified seeds were placed on plates

containing 1/2 MS medium supplemented with 0.3% gelrite and 1% sucrose. The plates were then placed for seed germination and plant growth in a growth chamber (MLR-352H-PC, Panasonic, Japan) at 22°C under irradiance of $100 \pm 5 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ at long-day conditions with a 16-h light/8-h dark cycle, and 50% relative humidity.

Chl fluorescence parameters were measured using a portable fluorometer (Mini PAM, Walz, Germany), as described previously by Demmig-Adams *et al.* (1996). The PSII maximal photochemical efficiency (F_v/F_m) was defined as $(F_m - F_0)/F_m$, where F_m is the maximum fluorescence emission from the dark-adapted state measured with a pulse of saturating light, and F_0 is the minimal fluorescence from the dark-adapted state. The effective quantum yield of PSII photochemistry (Φ_{PSII}) was defined as $(F_m' - F_s)/F_m'$, where F_m' is the maximum fluorescence emission from the light-adapted state measured with a pulse of saturating light and F_s is the steady-state level of fluorescence emission at the given irradiance. The coefficient of nonphotochemical quenching (q_N) was defined as $(F_m - F_m')/(F_m - F_0')$, where F_0' is minimal fluorescence of the light-adapted state measured with a far-red pulse. The NPQ parameter was defined according to the equation: $\text{NPQ} = (F_m - F_m')/F_m'$. The coefficient of photochemical quenching (q_P) was defined as $(F_m' - F_s)/(F_m' - F_0')$ according to Lazár (2015). As Φ_{PSII} represents the number of electrons transferred per photons absorbed by PSII, the ETR (the rate of photosynthetic electron transport through PSII) can be calculated as $\text{ETR} = \Phi_{\text{PSII}} \times \text{PAR} \times 0.5 \times 0.84$ according to Genty *et al.* (1989). The quantum yield of nonregulated energy dissipation of PSII ($Y_{(\text{NO})}$) and the quantum yield of regulated energy dissipation of PSII ($Y_{(\text{NPQ})}$) was also defined according to Kramer *et al.* (2004).

Dry biomass and leaf Chl content: For dry biomass measuring, shoots of 4-week-old plants were detached and oven-dried at 80°C for 24 h; the shoots were then weighed separately. The leaf Chl content was measured spectrophotometrically as described previously with minor modifications (Liang *et al.* 2017). Fresh leaves were weighed and incubated at room temperature with 10 mL of solution (acetone: ethanol = 1:1) for at least 24 h, then clarified by centrifugation for 5 min at $13,000 \times g$, while this process was protected from light. The absorbance of the supernatant was measured at wavelengths 645, 652, and 663 nm (A_{645} , A_{652} , and A_{663}) with the microplate reader (FL-600, BioTek, USA). Chl concentration was estimated using following equations: $\text{Chl } a [\text{mg g}^{-1}] = (12.7 \times A_{663} - 2.69 \times A_{645}) \times V/(1,000 \times W)$, $\text{Chl } b [\text{mg g}^{-1}] = (22.9 \times A_{645} - 4.68 \times A_{663}) \times V/(1,000 \times W)$, total Chl $[\text{mg g}^{-1}] = (A_{652} \times V)/(34.5 \times W)$, where V represents the volume [mL] of the extracted solution, and W represents the fresh mass [g] of the sample.

Identification of the homozygous mutant plants: Genomic DNA was extracted from the leaves as the template for PCR reaction. To identify the genotype of the T-DNA insertion *alg3-3* mutant, the DNA was amplified with the forward primer *alg3-F1* GATGCAATCCTAGTCGCACT;

reverse primer *alg3-R1* CCTGAGCAGGGTAGACTTCC, and the T-DNA left border primer *LBa1* TGGTTCACG-TAGTGGGCCATCG. To identify the genotype of the *cgl1-1* mutant, the primer *cgl1-dcaps-F2* TATCAAAC-TCCGTTGCTTC, and primer *cgl1-dcaps-R2* GACAGC-TTGATCAGATCGAT were used for PCR amplification. The PCR products were digested with *HinfI* (TaKaRa Bio, Japan) and then electrophoresed.

Transgenic plant construct: *pCAH1: GFP* was generated from *pPZP212-BRI1: BRI1-GFP* as follows. The primers 5'-ATGCCTGCAGGTCGACATATGCAGTGGCCATAT TCTGAGG-3'/5'-TTTACTCATGGGATCATTGGGTTTT TTCTTTTTGTTACCG-3' were used to amplify a 4.3-kb genomic fragment consisting of ≈ 1.5 kb of the *CAH1* promoter region, which was inserted *via* the *Sall* and *BamHI* restriction sites. The resulting construct carries a kanamycin-resistant gene as the selectable marker for plant transformation.

Western blot analyses: *Arabidopsis* seeds were germinated on 1/2 MS medium. To inhibit N-glycosylation, 2-week-old seedlings were transferred to 1/2 MS medium containing 30 $\mu\text{g mL}^{-1}$ tunicamycin (*Sigma*) for continued growth for another 24 h in the growth chamber as described previously with minor modifications (McCormack *et al.* 2015). The samples were prepared for loading by adding 2 $\times$ sample buffer [0.125 M Tris (pH 6.8), 4.0% SDS, 20% glycerol, 0.2 M DTT, 0.02% bromophenol blue], and heating the samples at 95°C for 10 min. Then the supernatants were used for SDS-PAGE on 8.0% Tris-acetate (Liu *et al.* 2018). Next, proteins in the gel were electrophoretically transferred to PVDF membrane (Roche, Switzerland). Membranes were incubated with primary antibodies in 5.0% milk at room temperature. The primary antibody used in this study was mouse anti-GFP (*Abcam*); the secondary antibody was HRP-conjugated goat anti-mouse IgG (*D110087*, *BBi*). Rubisco small sub-unit (RbcS) was used as a loading control. The SDS-PAGE containing RbcS was stained with *Coomassie Brilliant Blue R-250* (0.05% CBB, 10.0% acetic acid, and 5.0% methanol) for 2 h and then destained by destaining solution (7.0% acetic acid and 5.0% methanol). The relative amount of proteins was determined by stained RbcS bands separated by SDS-PAGE (Dyballa and Metzger 2009).

Statistical analysis: The results were expressed as the mean $\pm$ standard deviation. The data were statistically evaluated with one-way analysis of variance (*ANOVA*) between the different groups and by *Tukey's* multiple comparisons test. The difference was considered to be statistically significant when $P \leq 0.05$.

Results

***alg3-3* and *cgl1-1* showed inconspicuous phenotype under optimal conditions in greenhouse:** *alg3-3* is a T-DNA insertion mutant identified in *Arabidopsis* (Fig. 1A), it derives from incidental splicing of the third exon carrying the T-DNAs, which blocks the first mannose

adjunction step on Dol-PP-linked glycans in ER (Fig. 1C). Besides, we also investigated another *Arabidopsis cgl1-1* mutant that disrupts adding a GlcNAc residue to the Man₅GlcNAc₂ glycan in Golgi body (Fig. 1B,C). Both of these homozygous plants did not show any visible changes in their phenotype under normal laboratory conditions (Fig. 1C). Nevertheless, a dry biomass accumulation decreased significantly in the mutant plants compared with the wild type (WT) (Fig. 1S, *supplement*).

N-glycosylation defects induce changes in F_v/F_m : The Chl fluorescence parameter, F_v/F_m , reflects the maximum quantum efficiency of PSII photochemistry in the dark-adapted state and it has been widely used for early stress detection in plants (Schansker *et al.* 2014, Magyar *et al.* 2018). Our results showed that the value of F_v/F_m declined differently in the typical N-glycosylation-deficient *alg3-3* and *cgl1-1* mutant plants compared with the WT plants, especially in the *cgl1-1* mutant, whose values were significantly lower than those in the leaves of WT (Fig. 2). Thus, the above data suggested that their photosynthetic capacity was damaged to a certain degree.

Photochemical efficiency decreased in *alg3-3* and *cgl1-1*: In order to investigate further the effects of N-glycosylation on actual photochemical efficiency of plants, correlation parameters for photochemical efficiency were measured and analyzed. The Φ_{PSII} denotes the effective quantum yield of PSII photochemistry for the light-adapted state, also denoted as effective quantum yield of PSII photochemistry, which is an indicator of the real photochemical activity (Genty *et al.* 1989). In the present study, the data clearly showed that the values of Φ_{PSII} decreased obviously with the increase of light intensity both in the mutant plants and the WT (Fig. 3A). However, it is worth mentioning that the mutants always exhibited lower Φ_{PSII} values than that of the WT under the same irradiance (Fig. 3A). The q_P (Fig. 3C), which estimates a fraction of 'open' PSII reaction centers based on a puddle model (Stirbet 2013, Lazár 2015), had the same tendency of change as that of the Φ_{PSII} . Similarly, the photochemical efficiency and the rate of photosynthetic electron transport through PSII (ETR) also showed a downward trend in the mutants (Fig. 3B), compared with that of WT. These changes could give a good indication that the defective of N-glycosylation led to a decrease in the photosynthetic capacity of plants.

Nonphotochemical quenching in *alg3-3* and *cgl1-1*: Nonphotochemical quenching parameter (NPQ) equals the ratio of the quantum yield of regulated energy dissipation to the quantum yield of nonregulated energy dissipation of PSII (Lazár 2015). In order to further investigate whether nonphotochemical processes were affected in the mutant plants, the coefficient of nonphotochemical quenching (q_N), the NPQ parameter (Fig. 4A,B), and the quantum yield of regulated energy dissipation in PSII, $Y_{(\text{NPQ})}$ (Fig. 4C), were analyzed. The nonphotochemical processes rose along with the increase of light intensity, slight changes still could be found in q_N and $Y_{(\text{NPQ})}$, while the NPQ values were always lower than their counterparts in WT,

Fig. 1. (A) PCR analysis of *Arabidopsis* wild type (WT) and N-glycosylation mutants *alg3-3* and *cgl1-1* seedlings. Genomic DNA was extracted from the leaves of *alg3-3* seedlings, primers *alg3-F1* (F), *alg3-R1* (R), and *LBa1* were used to analyze the genotypes of *alg3-3*. (B) The genotype analysis of *cgl1-1*, primers *cgl1-dcaps-F2*, and *cgl1-dcaps-R2* were used for PCR, and digested by *Hinf I*. DNA from WT was used as positive control for the analysis. (C) Schematic representations of the major N-glycan structures of *alg3-3* and *cgl1-1* (top), photos of the seedlings after 21 d of growth in the growth chamber, scale bar = 1.0 cm.

Fig. 2. The maximum quantum yield of PSII (F_v/F_m) of *Arabidopsis* wild type (WT) and N-glycosylation mutants *alg3-3* and *cgl1-1*. The data were statistically evaluated with one-way ANOVA multiple comparisons using Tukey's test methods. Different letters above each bar indicate significant differences at $P \leq 0.05$ ($n = 4$).

especially, in the *cgl1-1* mutant (Fig. 4B). These results indicated that N-glycosylation also affected nonphotochemical processes.

$Y_{(NO)}$ reflects the yield for other energy losses (Lazár 2015); in most cases, high $Y_{(NO)}$ value indicates that

both photochemical energy conversion and protective regulatory mechanisms are inefficient. In other words, plants have serious problems to cope with the incident radiation, either it had been already damaged or it would be photodamaged upon further irradiation (Kramer *et al.* 2004). Thus, contrary to previous results, the values of $Y_{(NO)}$ showed a clear increase in the mutants, especially in *cgl1-1* (Fig. 4D). These results indicated that the N-glycosylation defect mutants suffered more of PSII damage than WT.

Carbonic anhydrase degradation after deglycosylation:

It has been well established that carbonic anhydrase (CAH1) influences the activity of carboxylase and decarboxylase, which is essential for efficient photosynthesis capacity of the plant (Badger and Price 1994). The accurate localization and stable existence of protein is the premise of its normal physiological function. Our results showed that the stabilization of CAH1 was influenced by the treatment of tunicamycin (an antibiotic inhibiting N-glycosylation) (Fig. 5A). Our finding suggested that degradation of CAH1 might be the reason, which led to a decrease of photosynthesis in the N-glycosylation defect mutant plants (Fig. 5B).

Fig. 3. The trend of the quantum yield of PSII photochemistry (Φ_{PSII}) (A), the rate of photosynthetic electron transport through PSII (ETR) (B), the coefficient of photochemical quenching (q_p) (C) of *Arabidopsis* wild type (WT) and N-glycosylation mutants *alg3-3* and *cgl1-1* with the increase of light intensity. Each curve represents an average $\pm$ SD of 4–6 independent measurements.

Discussion

As one of the most common and essential protein modification process, N-glycosylation plays multiple roles in eukaryotes. Previous research on this process focused on mammals or yeast, because the mutant plants exhibited rarely any visible phenotype changes under normal conditions. Recently, thousands of different N-glycosylated proteins have been identified in *Arabidopsis thaliana* (Zielinska *et al.* 2012, Song *et al.* 2013), indicating that glycosylation defects have also wide-ranging effects in plants. In recent years, many studies have shown that Chl fluorescence could represent imperceptible changes in the operation and function of the photosynthetic apparatus, even the stress tolerance of plants.

The results of the present study showed a substantial reduction of the photosynthetic capacity in the N-glycosy-

lation mutant plants. However, the PSII activity, reflected by the F_v/F_m , seemed to be affected slightly in *alg3-3* (Fig. 2). This result may suggest that *in vitro* measurements of PSII activity using isolated chloroplasts or thylakoid membranes might not be necessarily equivalent to PSII photochemical activity *in vivo* (Lu and Zhang 1998, Schansker *et al.* 2014). Homozygous *alg3-3* and *cgl1-1* plants (Fig. 1A,B) did not show any visible phenotype alterations under normal laboratory conditions (Fig. 1C). However, many studies have shown that the glycosylation abnormalities result in some disturbance in the cellular level, such as the provoking of the unfolded protein responses in the ER (von Schaewen *et al.* 1993, Henquet *et al.* 2008). This was in agreement with our observation of the significant decrease of dry biomass in both *alg3-3* and *cgl1-1* (Fig. 1S).

The effective photochemical quantum yield of PSII (Φ_{PSII}) and q_p gradually decreased with the increase of light intensity (Fig. 3A,C). These values decreased much faster in the mutants compared to that in WT, while the ETR, q_N , and the NPQ presented a remarkable rise with increasing light intensity and the values of parameters in the mutants were lower than that of the WT at the same light intensity (Fig. 4A,B). These data indicated that capacity of capture (and transfer) of the excitation energy to the photosynthetic reaction centers decreased with increasing light intensity (Green and Parson 2003). However, with the increase of light intensity, the excess excitation energy is dissipated through nonphotochemical quenching (NPQ) of the excited state of Chl *a* in the form of heat (Ort 2001, Demmig-Adams *et al.* 2014). Values of NPQ decreased in both *alg3-3* and *cgl1-1* mutants (especially in *cgl1-1*) compared to the WT (Fig. 4B), indicating the mutant plants may experience damage to PSII (Kozl 2006), either on the (electron) donor or the (electron) acceptor side of PSII (Pospíšil 2009, Hamdani *et al.* 2019).

Although plants have developed a number of photoprotection responses such as nonradiative dissipation of energy as heat at the molecular level (Müller *et al.* 2001, Derks *et al.* 2015), too much light can affect PSII and leads to damage and degradation of the reaction center (Barber and Andersson 1992). $Y_{(\text{NO})}$ represents basal (= nonregulated) nonphotochemical quenching, opposite to $Y_{(\text{NPQ})}$, which represents regulated, light-induced nonphotochemical quenching (Lazár 2015). Along with the decreased photosynthetic capacity in the mutant plants, significant growth in $Y_{(\text{NO})}$ was observed in *alg3-3* and *cgl1-1* (Fig. 4D). The results intensely demonstrated that such a decrease in photochemical efficiency was at least partly associated with the significant increase of the nonphotochemical quenching, which was not photoprotective, or with photosystem damage caused by high-light stress.

Chloroplasts are organelles of endosymbiotic origin and the vast majority of chloroplast proteins are synthesized in a precursor form on cytosolic ribosomes, while a unique set of protein transport machinery is responsible for the import of the nuclear-encoded proteins into the chloroplast (Jarvis and Soll 2001, Gutensohn *et al.* 2006). Secretory proteins are mostly modified by N-glycans and

Fig. 4. Nonphotochemical chlorophyll fluorescence quenching analysis of *Arabidopsis* wild type (WT) and N-glycosylation mutants *alg3-3* and *cgl1-1*. The trend of the coefficient of nonphotochemical chlorophyll fluorescence quenching (q_N) (A), the NPQ parameter (B), the quantum yield of regulated energy dissipation in PSII ($Y(NPQ)$) (C), and the trend of the yield for other energy losses with the increase of light intensity ($Y(NO)$) (D). Each curve represents an average $\pm$ SD of 4–6 independent measurements.

Fig. 5. (A) N-glycosylation analysis of CAH1 by migration patterns on SDS-PAGE. (B) A speculative model of CAH1 transport to the chloroplast in the N-glycosylation mutant. CAH1 is synthesized on the rough ER, becomes glycosylated and transported into the chloroplast. CAH1 with abnormality of glycans resulting in the latter degradation by the ERQC/ERAD.

the processing of N-glycans in the ER determines the fate of associated secretory peptides (Helenius and Aebi 2001). It is well documented that abnormal N-glycans in ER are detected by ERQC (ER-associated quality control compartment) system and misfolded proteins are removed via the ERAD (ER-associated degradation) pathway (Hong *et al.* 2009, Aebi 2013, Strasser 2016). Previous reports suggest that a chloroplast-located protein CAH1 becomes N-glycosylated before entering the chloroplast through the secretory pathway (Villarejo *et al.* 2005, Burén *et al.* 2011), indicating that N-glycosylation plays the role in regulation of secretory pathway for chloroplast-located proteins. The well N-glycosylated CAH1 is transported into the chloroplast through the secretory pathway in the WT plants and then participates in the process of photosynthesis. On the other hand, the misfolded or damaged CAH1 (caused by perturbation of N-glycosylation) in the mutant plants might activate the ERQC/ERAD machinery, which may finally lead to the degradation of CAH1 before entering the chloroplast (Fig. 5B). In addition, CAH1 degraded obviously after deglycosylation by tunicamycin (Fig. 5A). These findings suggested that the decline of photosynthetic capacity in the *alg3-3* or *cgl1-1* mutant may be also a consequence of the functional decomposition of CAH1 caused by N-glycosylation defects. Moreover, it is worth mentioning that the calculated significant decrease in dry biomass of *alg3-3* and *cgl1-1* (Fig. 1S) was thus consistent with the findings of the reduced photosynthetic rate, although the Chl content barely changed (Fig. 2S, *supplement*).

N-glycosylation is essential for multicellular life and its complete absence is embryonically lethal. In this work, we found that the obviously decreased photosynthetic capacity in N-glycosylation defective mutants *alg3-3* and *cgl1-1* might be associated with the disturbance of the PSII and impaired electron transfer system. Based on the above results, we also showed that N-glycosylation

regulated the photosynthetic capacity of plants at least in part by CAH1, although the exact mechanism(s) must be confirmed. In conclusion, N-glycosylation is required for plants to maintain the normal function of photosynthesis.

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