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Research article

Reduced glutamine synthetase activity plays a role in control of photosynthetic responses to high light in barley leaves

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ABSTRACT

The chloroplastic glutamine synthetase (GS, EC 6.3.1.2) activity was previously shown to be the limiting step of photorespiratory pathway. In our experiment, we examined the photosynthetic high-light responses of the *GS2*-mutant of barley (*Hordeum vulgare* L.) with reduced GS activity, in comparison to wild type (*WT*). The biophysical methods based on slow and fast chlorophyll fluorescence induction, P700 absorbance, and gas exchange measurements were employed. Despite the *GS2* plants had high basal fluorescence (F_0) and low maximum quantum yield (F_v/F_m), the CO_2 assimilation rate, the PSII and PSI actual quantum yields were normal. On the other hand, in high light conditions the *GS2* had much higher non-photochemical quenching (NPQ), caused both by enhanced capacity of energy-dependent quenching and disconnection of PSII antennae from reaction centers (RC). *GS2* leaves also maintained the PSII redox poise ($Q_{\bar{A}}/Q_A$ total) at very low level; probably this was reason why the observed photoinhibitory damage was not significantly above *WT*. The analysis of fast chlorophyll fluorescence induction uncovered in *GS2* leaves substantially lower RC to antenna ratio (*RC/ABS*), low PSII/PSI ratio (confirmed by P700 records) as well as low PSII excitonic connectivity.

1. Introduction

Plants convert the light energy into chemical energy (ATP and NADPH) through the photosynthetic electron transport on thylakoid membranes in chloroplast. This energy can be used for fixation of CO_2 in Calvin cycle. However, plants are exposed to extremely variable conditions (e.g. excess of incident light, CO_2 shortage due to closed stomata, variable temperature, stresses) inevitably causing imbalances between supply and demand of energy at different levels. The excess of energy can result to damages of molecular structures in chloroplast (photoinhibition). Therefore, the complex of precisely regulated protective mechanisms acts to keep the functionality of the photosynthetic apparatus (Melis, 1999; Allakhverdiev et al., 2008; Vass, 2012).

The photorespiration is generally considered a "safety valve" of photosynthesis (Osmond and Grace, 1995). The photorespiratory pathway starts with the oxygenase reaction catalyzed by ribulose-1,5-bisphosphate carboxylase/oxygenase enzyme (Rubisco), producing glycollate-2-phosphate, which is then metabolized to form the Calvin cycle intermediate glycerate-3-phosphate. During this metabolic process, ATP and reducing equivalents are consumed. Thus, the pathway represents the energy sink, which enables to prevent the over-reduction of the photosynthetic electron transport chain, especially under stress conditions leading to low CO_2 assimilation rates (Wingler et al., 2000; Foyer et al., 2009).

Although photorespiration includes many metabolic steps which are performed across chloroplasts, mitochondria and peroxisomes, several studies suggest that the rate-limiting step is the reassimilation of ammonia catalyzed by chloroplastic glutamine synthetase (*GS2*) (Wallsgrove et al., 1987; Häusler et al., 1994a). Kozaki and Takeba (1996) have demonstrated that a transgenic tobacco plant over-expressing chloroplastic glutamine synthetase had increased photorespiration capacity.

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Abbreviations[1]

A_{CO2}	CO_2 assimilation rate
ChlF	Chlorophyll Fluorescence
GS	glutamine synthetase
GS2	chloroplastic glutamine synthetase
LED	light emitting diode
LHC	light harvesting complex
NPQ	non-photochemical quenching
P700	primary electron donor of PSI (reduced form)
$P700^+$	primary electron donor of PSI (oxidized form)
PAR	photosynthetic active radiation
PQ	plastoquinone
PSI	Photosystem I
PSII	Photosystem II
Q_A	primary PSII acceptor
qE	pH dependent energy dissipation
RuBP	Ribulose 1,5-bisphosphate
WT	Wild Type
ΔpH	Transthylakoid pH gradient

In plants, *GS2* together with ferredoxin-dependent glutamate synthase (Fd-GOGAT) plays a major role in re-assimilation of ammonium liberated in mitochondria by the glycine decarboxylase, in the pathway known as glutamine synthetase/glutamate synthase (GS/GOGAT) cycle in chloroplasts. Moreover, the product of this cycle, glutamate, is required for one of the peroxisomal transamination reactions. The GS/GOGAT cycle runs in chloroplast and it is directly associated with photosynthetic electron transport as it consumes electrons from taken from reduced ferredoxin at the acceptor side of photosystem I (PSI) and ATP (Hodges et al., 2013).

Thus, the activity of *GS2* is essential for the process of photorespiration and decrease in the *GS2* enzymatic activity leads to multiple effect, including decrease of ammonium re-assimilation, accumulation of metabolic intermediates due to interruption of photorespiratory cycle beyond Rubisco as well as by the direct effects on the redox poise of electron carriers in chloroplast, as it was demonstrated on *GS2* mutant studies (Wingler et al., 2000).

Mutants of the photorespiratory cycle have contributed significantly to the understanding of this biochemical pathway and its links to other physiological processes (Leegood et al., 1995; Somerville, 2001; Reumann and Weber, 2006). The changes in phenotypes of these mutants compared to wild type have been assumed to be due to the depletion of photosynthetic carbon and nitrogen cycle intermediates and perhaps to the accumulation of toxic photorespiratory intermediates (Cousins et al., 2008). While the mutants with completely reduced activity of particular photorespiratory enzymes (i.e. the homozygous photorespiratory mutants) are not able to survive at ambient CO_2 concentrations, the heterozygous mutants with only partially reduced enzyme activities can be grown well in air (Wingler et al., 2000). The *GS2* mutant of barley was shown to have normal rates of photosynthesis in moderate light and ambient CO_2, but lower rates when photorespiratory flux was increased in high light and low CO_2 (Häusler et al., 1994a; Wingler et al., 1999). Wingler et al. (2000) suggest three ways how the reduced photorespiratory enzyme activity may affect photosynthesis in mutant plants. First, an impairment of the recycling of the carbon in the photorespiratory pathway could result in a depletion of Calvin cycle metabolites. The supply of glutamine to a *GS2* mutant of barley restored photosynthetic activity (Blackwell et al., 1987). However, the pools of RuBP was shown to be almost unaffected by mutation of GS-2 (Leegood et al., 1995; Wingler et al., 1999).

The second possible effect could be an impairment of photorespiratory nitrogen re-assimilation resulting in a decline in the leaf nitrogen and protein content. In the *GS2* mutants, the problem with NH_3 loss can be expected (Häusler et al., 1994a). However, this effect can be partially diminished by alternative pathway, where the mutants bypass the normal photorespiratory pathway by oxidative decarboxylation of glyoxylate and formation of serine from formate. The advantage of this alternative photorespiratory pathway is the absence of NH_3 loss (Häusler et al., 1996). The lower leaf protein content was shown be a factor of lower importance in *GS2* mutants (Wingler et al., 2000).

The third possible constraint affecting photosynthesis could be accumulation of photorespiratory metabolites having a feedback effect on Calvin cycle activity (Leegood et al., 1995, 1996). NH_3 accumulation has probably only negligible direct negative effect on photosynthetic electron transport (Blackwell et al., 1987). Accumulation of serine is also unlikely to inhibit photosynthesis (Wingler et al., 2000). The more probable is the regulatory feedback effects of some metabolites on enzyme activities, e.g. changes glyoxylate content in *GS2* mutants influenced the activation state of Rubisco (Campbell and Ogren, 1990).

In our study, we have examined photosynthetic responses of *GS2* mutant of barley with reduced activity of chloroplastic glutamine synthetase, employing mainly the non-destructive biophysical tools. In addition to conventional saturation pulse method of chlorophyll fluorescence (Schreiber, 1986) measured simultaneously with gas exchange or P700 absorbance, we applied also the analysis of fast chlorophyll *a* fluorescence induction. This analytical tool can serve as a valid examination of environmental effects on the photosynthetic apparatus (Živčák et al., 2008; Kalaji et al., 2012; Brestič et al., 2012). Our results indicate that the mutation of the enzyme not directly associated with conversion of light energy led to surprisingly significant modifications of structure and function of photosystems.

2. Materials and methods

2.1. Plant material

As a plant material, the genotypes of barley (*Hordeum vulgare* L.) were used. We examined the "*wild type*" barley cv. Kompakt (hereinafter labeled *WT*) and *GS2*-mutant of barley, i.e. the heterozygous photorespiratory mutant with reduced activity of chloroplastic glutamine synthetase (*GS2*, EC 6.3.1.2), provided by P. Lea, University of Lancaster, UK. This mutant was obtained by crossing mutant deficient in chloroplastic GS activity with non-mutant barley line. The heterozygous mutant were characterized with app. 66% *GS2* activity compared to WT (Häusler et al., 1994b).

2.2. Cultivation of plants

In outdoor experiments, the plants were grown in the pots (18 l pots with soil substrate and 40 plants per one pot) under natural light. Plants were watered twice a day (according to their demands) and supplied by inorganic fertilizers.

In laboratory experiments, the plants were cultivated in middle-size pots (4 l) in standard peat substrate with neutral pH (9 plants per pot). The pots were regularly irrigated and occasionally fertilized using standard liquid fertilizer with micronutrients. Plants were grown in a growth chamber with artificial light provided by fluorescent tubes (Osram Fluora) with maxima in red and blue spectral region; the incident PAR at leaf level was app. 250 μmol

[1] See Table 1 for other symbols representing chlorophyll fluorescence and P700 parameters.