Theor. Exp. Plant Physiol. (2014) 26:177–187
DOI 10.1007/s40626-014-0025-z

Arabidopsis *gun4* mutant have greater light energy transfer efficiency in photosystem II despite low chlorophyll content

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Received: 24 June 2014 / Accepted: 10 November 2014 / Published online: 20 November 2014

Abstract Arabidopsis *gun4* mutant is defected in retrograde signaling and is characterized by reduced levels of chlorophyll. However, the impact of this mutation on plant photosynthetic performance remains unclear. We carried out a physiological characterization of *gun4* in order to investigate the role of GUN4 for plant photosynthetic performance under light stress. The *gun4* plants showed reduced minimal fluorescence in dark-adapted leaves and quantum yield of unregulated energy dissipation of photosystem II (PSII) under non-light-stress condition. The effective quantum yield of the PSII (Φ_{PSII}) and photochemical quenching (qL) were higher in *gun4* plants. Higher values of Φ_{PSII} were also observed in *gun4* under different light intensities. However, the rate of net carbon assimilation and stomatal conductance were lower in *gun4*. No differences were detected between the genotypes in the total absorption of $^{14}CO_2$ as well as in the percentage of ^{14}C flux to basic amino acids, sugars, starch, and cell wall. After light stress, the potential quantum yield of PSII decreased only in wild type and the non-photochemical quenching was higher in *gun4*. Taken together, the results suggest that *gun4* transfers more efficiently the excess of light energy absorbed despite a reduction in chlorophyll and carotenoids content and has greater capacity to dissipate the excess of energy absorbed.

Keywords *gun4* · Light stress · Light energy transfer · Light harvesting complex II · Photosystem II

Abbreviations

Φ_{PSII}	Effective quantum yield of PSII
GUN	Genome uncoupled

Electronic supplementary material The online version of this article (doi:10.1007/s40626-014-0025-z) contains supplementary material, which is available to authorized users.

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gun4	Genome uncoupled 4 mutant
LHCB	Light harvesting chlorophyll binding proteins
LHC	Light harvesting complex
LHCII	Light harvesting complex of the complex II
F_m	Maximal fluorescence in dark adapted leaves
F_o	Minimal fluorescence in dark adapted leaves
MgChl	Mg-chelatase enzyme
Mg-ProtoIX	Mg-protoporphyrinIX
A	Net photosynthetic rate
qN	Non-photochemical quenching
Φ_P	Photochemical quantum yield of PSII
qL	Photochemical quenching of lake model
PSII	Photosystem II
F_v/F_m	Potential quantum yield of PSII
ProtoIX	ProtoporphyrinIX
Φ_{NO}	Quantum yield of non-regulated non-photochemical energy loss in PSII
g_s	Stomatal conductance
C_i	Substomatal CO_2 concentration
E	Transpiration
WT	Wild type

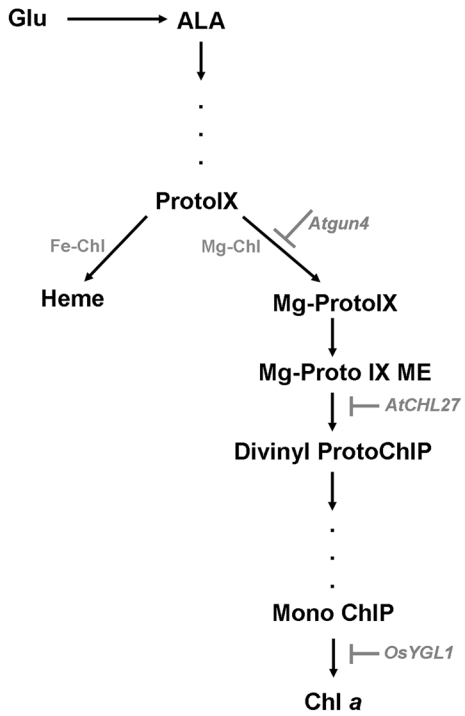

Fig. 1 Schematic representation of a summarized tetrapyrrole and chlorophyll biosynthetic pathway showing the local of GUN4 mutation. *Glu* L-glutamate, *ALA* 5-aminolevulinic acid, *ProtoIX* nprotoporphyrin IX, *MgChl* magnesium chelatase, *FeChl* ferrochelatase, *Mg-ProtoIX* Mg-protoporphyrin IX, *Mg-Proto IX ME* Mg-protoporphyrin IX monomethyl ester, *Divinyl ProtoChlP* divinyl Protochlorophyllide, *Mono ChlP* monovinyl protohlorophylide; Chl a—Chlorophyll a; Heme—heme groups

1 Introduction

Pathways that communicate chloroplast to nucleus, called retrograde signaling, has been revealed through *gun* (*genome uncoupled*) mutants of *Arabidopsis thaliana*. Retrograde signaling involves plastids signals that induce nuclear gene expression. There are five genes GUN in Arabidopsis (GUN 1-5), which four of these (GUN2-5) encode proteins related to tetrapyrrole biosynthetic pathway (Susek and Chory 1992; Susek et al. 1993; Mochizuki et al. 2001; Larkin et al. 2003). GUN4 encodes a chloroplast protein that activates Mg-chelatase enzyme (MgChl), responsible by protoporphyrinIX (ProtoIX) to Mg-protoporphyrinIX (Mg-ProtoIX) conversion in the chlorophyll biosynthetic pathway (Fig. 1) (Tanaka and Tanaka 2007). Furthermore, GUN4 is required for posttranslational control of tetrapyrrole biosynthesis for promoting the interaction between H-subunit of MgChl enzyme (MgChl-H) and chloroplast membranes (Adhikari et al. 2009; Peter and Grimm 2009). The phenotype of Arabidopsis *gun4* mutant was characterized by reduced levels of Mg-ProtoIX, chlorophyll and carotenoids (Larkin et al. 2003). In the other hand, the overexpression of GUN4 in tobacco increased chlorophyll and Mg-ProtoIX levels as well as increased MgChl enzyme activity (Peter and Grimm 2009). Interestingly, GUN4 mutation in cyanobacteria leads to reduced both Fe-chelatase enzyme activity and Heme levels. It suggests that GUN4 is also involved in the biosynthesis of Heme groups, controlling the flow of substrate for chlorophyll or Heme biosynthetic pathway (Wilde et al. 2004; Masuda and Fujita 2008). Although the function of GUN4 and their precursors- and- products for both retrograde signaling and chlorophyll synthesis has been previously demonstrated in Chlamydomonas and Arabidopsis (Koussevitzky et al. 2007; Czarnecki et al. 2012; Formighieri et al. 2012; Zhou et al. 2012), the mechanisms by which GUN4 are involved in retrograde signaling and the impact of GUN4 mutation on

photosynthetic process remains poorly understood (Brzezowski et al. 2014).

Pathways that communicate organelles (plastids and mitochondria) to nucleus are important once coordinate the development and function of the organelles with the whole plant cell (Pfannschmidt 2010). The communication between chloroplast and nucleus involves the exportation of signaling factors from chloroplast to cytosol where the signaling pathway transduces the signal to the nucleus (Kleine et al. 2009). It has been reported that changes in the chloroplast redox potential, the accumulation of reactive oxygen species, Mg-ProtoIX as well as 5-aminolevulinic acid (ALA) biosynthesis could be involved in the retrograde signaling from chloroplast to nucleus (Nott et al. 2006; Zhang 2007; Larkin and Ruckle 2008; Czarnecki et al. 2012). The accumulation of Mg-ProtoIX was reported as a possible signal from chloroplast that regulate light harvesting chlorophyll binding protein-II (LHCBII) genes in the nucleus (Strand et al. 2003; Larkin et al. 2003; Koussevitzky et al. 2007; Larkin and Ruckle 2008). However, strongly evidences suggest that this retrograde signaling cannot be related to Mg-ProtoIX accumulation, indicating that other unknown signal is involved in the chloroplast to nucleus signaling (Mochizuki et al. 2008; Moulin et al. 2008). The current idea is that there is no only one signal in retrograde signaling but rather the result of continuously sensed metabolic and genetic activities in the organelles (Czarnecki et al. 2012). Given these controversies concerning retrograde signaling a more carefully characterization of *gun* mutants are needed to understand the function of these genes in retrograde signaling and photosynthetic process, especially under light stress conditions.

The maintenance of plants growth under excessive amounts of light has been reported mainly as result of increased photosynthetic capacity of the plant and/or through the activation of process that dissipate the excess of energy absorbed in order to reduce the susceptibility of photo-oxidative damage (Apel and Hirt 2004; Walters 2005; Li et al. 2009). In this context, mutations in GUN genes can induce the overexpression of light harvesting chlorophyll binding (LHCB) proteins especially under stress conditions (Mochizuki et al. 2001; Peter and Grimm 2009; Brzezowski et al. 2014). LHCB are important proteins related to the energy transfer from light harvest complex (LHC) of photosystem II (PSII) to the reaction center of PSII (Teramoto et al. 2001). Given the importance of GUN4 for biosynthesis of photosynthetic pigments and the strong connection between GUN genes and LHCB proteins it's surprisingly that the effect of GUN4 mutation on light energy transfer in LHCII and plant photochemical performance is still unclear. Therefore, we carried out a photosynthetic characterization of *gun4* mutant in order to investigate the role of GUN4 gene for the plant photosynthetic performance under light stress conditions.

2 Materials and methods

2.1 Plant material and growth conditions

All *Arabidopsis thaliana* plants used in this study were of the columbia ecotype (Col-0). The *gun4* knockout mutant (Gene ID: AT3G59400) (SALK_011461) were previously described (Mochizuki et al. 2001; Larkin et al. 2003). Seeds of wild type (WT) and *gun4* mutant (homozygous) were surface-decontaminated by shaking 70 % ethanol for 5 min, rinsed with sterile distilled H_2O. In sequence treated with 100 % ethanol for 1 min, rinsed three times with sterile distilled H_2O. The seeds were then allowed to germinate in Petri dishes containing MS medium (25 cm^3) (Murashige and Skoog 1962) and cultivated under photoperiod of 14 h illumination and light intensity of 120 µmol m^{-2} s^{-1}, with day/night temperatures of 20 ± 3 °C. After 7 days the plants were transferred to recipient (0.1 L) containing commercial soil mixture, keeping in the room with the same conditions described above. We carried out a photosynthetic curve under different light intensities in completely expanded leaves of WT Arabidopsis plants in order to choose the level of light stress would be imposed to the plants. After this, five-week-old plants were subjected to light stress for 0, 14 or 28 h under 800 µmol photons m^{-2} s^{-1} using white lamps of 1,000 W in ambient with controlled temperature. It was disposed a water layer among the plants and light source in order to avoid heat excess. Unless otherwise described the fully expanded 9th–12th rosette leaves of six-week-old plants were harvested for subsequent analysis.

2.2 Gas exchange analysis and chlorophyll a fluorescence measurements

The gas exchange analysis were carried out in completely expanded leaves of WT and *gun4* mutant

using infra-red gas analyzer (IRGA, Licor 6400, Lincoln, USA) according Flexas et al. (2007). It was estimated net photosynthetic rate (*A,* μmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}), substomatal CO_2 concentration (C_i μmol CO_2 mol^{-1}), and transpiration rate (*E,* mmol m^{-2} s^{-1}) at 0 and 14 h of light stress.

The chlorophyll *a* fluorescence measurements were performed in completely expanded leaf of WT and *gun4* mutant by modulated light fluorometer system (MINI-PAM, Walz, Effeltrich, Germany). For established fluorescence parameters, initially measured minimal (F_o) and maximal fluorescence (F_m) in dark adapted leaves. It was estimated maximal quantum yield of PSII (F_v/F_m) according to Kitajima and Butler (1975). After, it was measured the parameters of light adapted leaves which was used to estimate effective quantum yield of PSII (Φ_{PSII}) (Genty et al. 1989), photochemical quenching of lake model (qL) ($qL = (F_m' - F) \times F_o'/(F_m' - F_o') \times F$) (Kramer et al. 2004), non-photochemical quenching ($qN = (F_m - F_m')/(F_m - F_o')$ (Bilger and Schreiber 1986), quantum yield of non-regulated non-photochemical energy loss in PSII ($\Phi_{NO} = F/F_m$), quantum yield of regulated non-photochemical energy loss in PSII ($\Phi_{NPQ} = (F/F_m') - (F/F_m)$). The parameters Φ_{PSII}, Φ_{NO}, and Φ_{NPQ} were estimated according Hendrickson et al. (2004) in which $\Phi_{PSII} + \Phi_{NO} + \Phi_{NPQ} = 1$. The chlorophyll *a* fluorescence parameters were also determined in light responses curves. The parameters were estimated from different light intensities (0, 80, 160, 260, 370, 560, 800, 1,060 e 1,660 μmol of photons m^{-2} s^{-1}).

2.3 Determination of photosynthetic pigments

Immediately after stress treatment, the rosettes of Arabidopsis were frozen at liquid N_2 and used for determination of photosynthetic pigments. Total chlorophylls and carotenoids were extracted in acetone:water 80 % (v/v) and their concentration determined spectrophotometrically following Lichtenthaler (1987).

2.4 $^{14}CO_2$ feeding experiment

The redistribution of the photosynthetic metabolic flux was determined in leaves exposed to $^{14}CO_2$. The $^{14}CO_2$ was imposed to leaves using oxygen electrode (Hansatech, Kings Lynn, UK) under 400 μmol photons m^{-2} s^{-1}, at 25 °C, for 60 min. The preparation of the radioactive solution containing ^{14}C as well as the exposition to leaves was realized according DaMatta et al. (2008). After the exposition, leaves were frozen in liquid N_2 and store at −80 °C. The extractions as well as the flux distribution between starch, cell wall, basic amino acids, organic acids, and sugars were realized according DaMatta et al. (2008). The incorporated radioactivity was determined using a liquid scintillation analyzer (Beckman LS 6500; Beckman Instruments, Fullerton, CA, EUA).

2.5 Co-expression analysis

The analyses of the co-expression network were performed using microarray data from on line platform ATTED-II (http://atted.jp/) according Obayashi et al. (2011). The network of GUN4 (At3g59400) co-expressed genes was obtained using CoexViewer tool of ATTED-II 5.2 according Obayashi et al. (2009). The data are available in http://atted.jp/top_draw.shtml#CoexViewer. A complete list of genes co-expressed with GUN4 gene is available in ATTED-II on line platform (http://atted.jp/). Further information's about the significance of the mutual rank values for the gene co-expression can be found in Obayashi and Kinoshita (2009).

2.6 Statistical analysis

To analyze the effect of light stress in each genotype, the data were statistically compared by ANOVA and Dunnet test at 5 % of significance ($P < 0.05$). Between genotypes, in the same stress treatment, the data were statistically compared by Student *t* test ($P < 0.05$). All statistical analyses were performed using the Saeg system version 9.1. The number of replicates (n) is indicated in each figure.

3 Results

3.1 Photosynthetic pigments content are reduced in *gun4* plants

The *gun4* mutant showed a phenotype with light green/yellowish leaves (Figure S1) according with lower chlorophyll and carotenoids content in the

Table 1 Mean values and standard error of chlorophyll (Chl) and carotenoids (Carot) content (g kg^{-1} fresh weight) in wild type (WT) and *gun4* mutant of *Arabidopsis thaliana* under 0, 14, or 28 h of light stress

	WT			*gun4*		
	0	14	28	0	14	28
Chl-a	**5.86 ± 0.19** a	4.10 ± 0.37 a	2.04 ± 0.23 b	4.73 ± 0.34 a	4.53 ± 0.33 a	2.81 ± 0.56 b
Chl-b	**2.37 ± 0.10** a	**2.22 ± 0.25** a	1.17 ± 0.12 b	1.56 ± 0.12 a	0.95 ± 0.13 a	0.94 ± 0.18 a
Chl-a + b	**8.23 ± 0.29** a	**7.06 ± 0.52** a	**5.36 ± 0.49** b	6.30 ± 0.4 a	5.48 ± 0.30 a	3.76 ± 0.49 b
Carot	**1.13 ± 0.04** a	**1.27 ± 0.2** a	0.90 ± 0.36 a	0.83 ± 0.05 a	0.60 ± 0.20 a	0.79 ± 0.25 a

Values followed by the same letter in each genotype and different light stress conditions are not statistically different by the Dunnet test ($P < 0.05$)

Values in bold and underline type indicate statistic difference between the genotypes in the same light treatment by Student *t* test ($P < 0.05$) (n = 6)

absence of light stress (Table 1). After 14 h of light stress, no significant differences in pigments content were observed in each genotype. However, the *gun4* mutant still showed lower total chlorophyll, chlorophyll *b* and carotenoids content than WT (Table 1).

Light stress has no effect on carotenoids content in both genotypes. However, the chlorophyll content was significantly reduced in both genotypes after 28 h of light stress, except to chlorophyll *b* content in *gun4* mutant (Table 1).

3.2 GUN4 is co-expressed in a network composed by photosystem II related proteins

We carried out an in silico analysis of GUN4 (At3g59400) gene expression from microarray data available using different web platforms. The Arabidopsis eFP Browser on-line platform (Winter et al. 2007) showed that GUN4 is preferentially expressed in photosynthetic tissues, with high expression in chloroplast of leaf mesophyll cells (data not show). The co-expression network analysis by web platform ATTED-II (Obayashi et al. 2011) showed that there is a high co-expression between GUN4 and genes related to photosynthesis, structure and function of both photosystems I and II, proteins of light harvest complex as well as proteins of chlorophyll biosynthetic pathway (Fig. 2). Among different genes co-expressed with GUN4, highlights to genes of light harvesting chlorophyll binding proteins (LHCB2.3, LHCB3, LHCB4, LHCB4.2, LHCB5, LHCB6, LHCA1, LHCA3), genome uncoupled 5 gene (GUN5), non-photochemical quenching related protein (NPQ4), and subunits of photosystems I and II (PSII protein, PSAD-1, PSAD-2, PSAH1, PSAH2, PSBTN, PSAO, PSAF, PSBO2, PSB28, PSBX, PSBW, PSAL, PASK) (Table S1).

3.3 *gun4* plants has greater photochemical efficiency in photosystem II under non-stress conditions

The *gun4* mutant showed reduced minimal (F_o) and maximal (F_m) fluorescence of dark-adapted leaves (Fig. 3a, b) as well as reduced quantum yield of unregulated energy dissipation of photosystem II (Φ_{NO}) (data not shown) under non-stress conditions. However, *gun4* plants showed increases in non-photochemical quenching (qN) (Fig. 3c), effective quantum yield of PSII (Φ_{PSII}) (Fig. 3e), as well as in photochemical quenching (qL) (Fig. 3f). Higher values of Φ_{PSII} and lower of Φ_{NO} were also found in *gun4* under different light intensities (Fig. 4).

3.4 *gun4* has greater non-photochemical dissipation of light excess

It was observed significant differences between WT and *gun4* in qN (higher values in *gun4*) and F_o and F_m (lower values in *gun4*) independent of the stress level (Fig. 3a–c). Increased qN was observed in both genotypes after 14 h of light stress. However, only WT showed significant increases in F_o and significant reduction in potential quantum yield of the PSII (F_v/F_m) after 14 h of stress (Fig. 3a, d). After 28 h of light stress, all photochemical parameters analyzed were significantly different of the non-stress condition, with exception for qN in WT. However, F_o, F_m, and qN were significantly different between WT and *gun4*

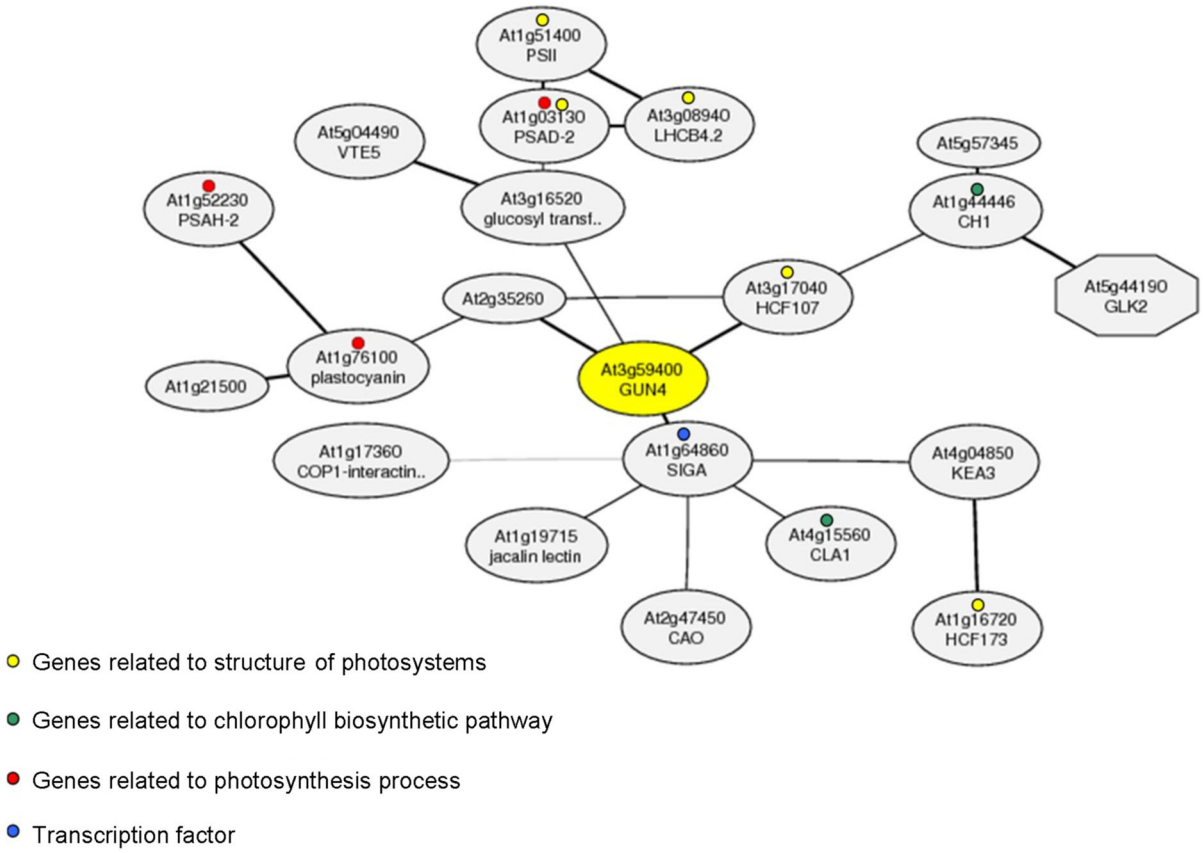

Fig. 2 Co-expression analysis of GUN4 gene (AT3G59400) obtained from microarray data using CoexViewer tool of ATTED-II 5.2. The function of some of these genes is described in the table S1

mutant. After this severe stress, the F_v/F_m values were significantly reduced in both genotypes, but higher decrease was observed in WT (74 %) compared to *gun4* mutant (54 %) (Fig. 3 D). The *gun4* mutant maintains non-photochemical dissipation of light excess through a significant higher qN under light stress (Fig. 3c). Furthermore, there was no difference in F_o between 14 and 28 h of light stress in *gun4* plants (Fig. 3a).

3.5 *gun4* plants has intrinsic water use efficiency increased despite strong reduction in photosynthetic rates

The *gun4* mutant showed decreased net photosynthetic rate (*A*) (43 %), stomatal conductance (g_s) (61 %), and transpiration rate (*E*) (46 %) compared to WT plants. These reductions lead to a phenotype with increased intrinsic water use efficiency (WUE_i), i.e. higher A/g_s relationship (Fig. 5). Due to the mild reductions in *A* and *E* in WT after light stress, no differences were observed in these parameters between WT and *gun4* plants. However, g_s was still lower in *gun4* after light stress which leads to a higher A/g_s relationship in *gun4* (Fig. 5). Given the strong light stress imposed on plants whereas that 28 h of light stress dramatically reduced all photochemical parameters (F_v/F_m, Φ_{PSII}, and qL), no net photosynthetic rate was detected in leaves of both genotypes after 28 h of light stress (data not shown).

3.6 $^{14}CO_2$ incorporation in WT and *gun4* leaves

We carried out a feeding experiment in whole leaves submitted to saturate $^{14}CO_2$ concentration. Under these conditions, total ^{14}C incorporation was not

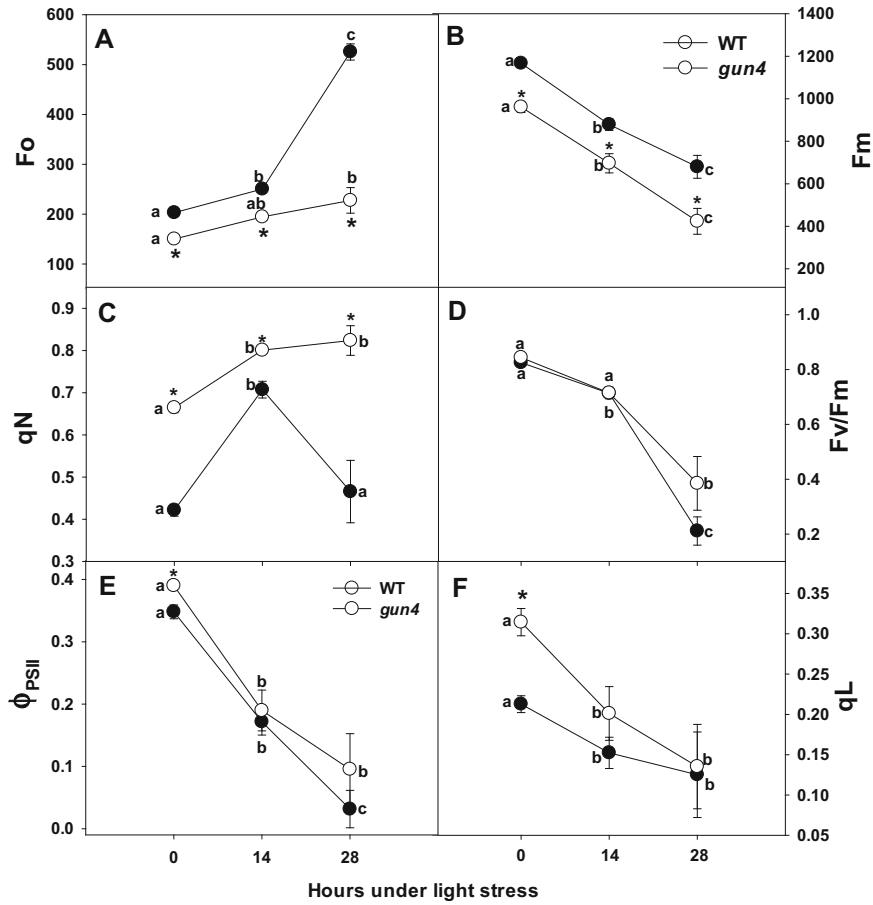

Fig. 3 Mean values and standard error of minimal (Fo) (**a**) and maximal (Fm) (**b**) fluorescence of dark-adapted leaves, non-photochemical quenching (qN) (**c**), potential quantum yield of the PSII (Fv/Fm) (**d**), effective quantum yield of the PSII (Φ_{PSII}) (**e**), and photochemical quenching (qL) (**f**) in leaves of wild type (WT) and *gun4* mutant under different light stress condition. Values followed by the *same letter* in each genotype and different light stress conditions are not statistically different by Dunnet test ($P < 0.05$). *Asterisks* indicate statistic difference between genotypes in the same stress condition by Student *t* test ($P < 0.05$) (n = 5)

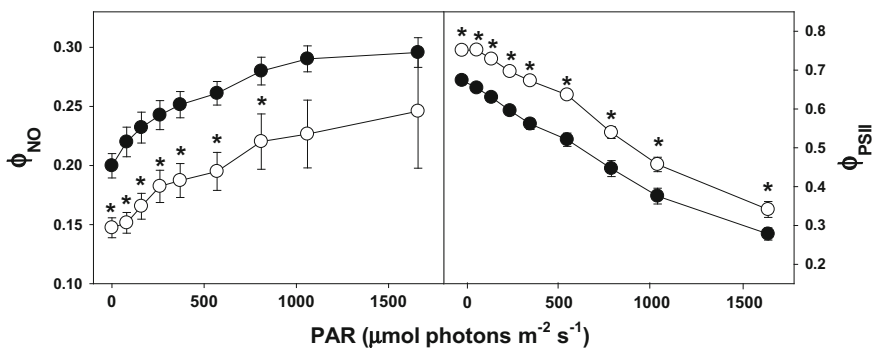

Fig. 4 Mean values and standard error of unregulated energy dissipation of photosystem II (Φ_{NO}) and photochemical quantum yield of PSII (Φ_{PSII}) (**b**) in leaves of wild type (WT) and *gun4* mutant under different light intensities. *Asterisks* indicate statistic difference between genotypes in the same light condition by Student *t* test ($P < 0.05$) (n = 6)

different between WT and *gun4* mutant (Table 2). No differences were found in the percentage of ^{14}C flux redistribution to basic amino acids, sugars, starch, and cell wall. However, mild reduction in the percentage of ^{14}C to organic acids was observed in *gun4* mutant (Table 2).

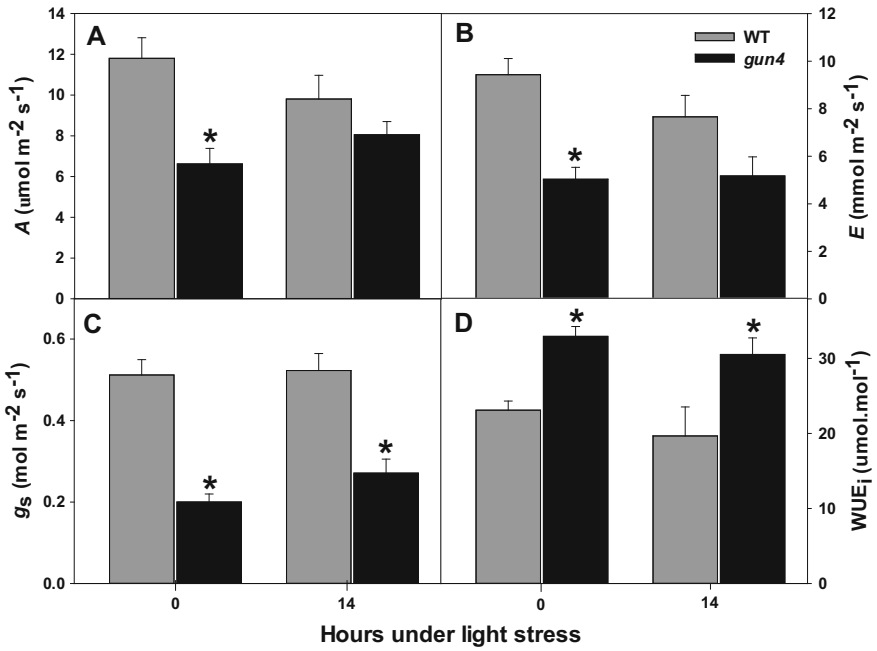

Fig. 5 Mean values and standard error of gas exchange parameters in leaves of wild type (WT) and *gun4* mutant submitted to 0 or 14 h of light stress. *A* net photosynthetic rate, g_s stomatal conductance, *E* transpiration rate, WUE_i intrinsic water use efficiency. *Asterisks* indicate statistic difference between genotypes in the same stress condition by Student *t* test ($P < 0.05$) (n = 5)

Table 2 Mean values and standard error of total absorption (Bq g^{-1}) and redistribution of ^{14}C (%) in leaves of wild type (WT) and *gun4* mutant

	WT	*gun4*
Total absorption (Bq g^{-1})	5.3676,1 ± 279	5.844,1 ± 293
Redistribution of ^{14}C (% of total)		
Starch and cell wall	36.27 ± 2.99	39.05 ± 2.84
Sugars	51.70 ± 2.35	50.50 ± 2.83
Basics amino acids	7.16 ± 0.34	6.93 ± 0.54
Organic acids	4.88 ± 0.48*	3.52 ± 0.38
Error	0.23	0.22

Asterisks indicate statistic difference between the genotypes by Student *t* test ($P < 0.05$) (n = 5)

4 Discussion

4.1 *gun4* has greater light energy transfer efficiency in PSII despite lower both chlorophyll and carotenoid contents

Given the role of GUN4 for chlorophyll biosynthesis, the mutation in this gene leads to reduced level of photosynthetic pigments in Arabidopsis leaves. In turn it may lead to small LHCII and reduced capacity of light energy absorption in *gun4* plants, evidenced by the lower F_o values compared to WT. There are evidences in plants and cyanobacteria that the mutation in genes of chlorophyll biosynthetic pathway, including GUN4, result in smaller LHCII due the less components of light harvesting complex and less subunits of PSII (Havaux et al. 2007; Sobotka et al. 2008; Hansson and Jensen 2009). In this context, *gun4* phenotype is similar to other mutants of chlorophyll biosynthetic pathway, such as *chl27* and *sg1*. CHL27 codifies the enzyme Mg-ProtoIX monomethyl ester ciclase (EC 1.14.13.81) (Fig. 1). The *chl27* mutant has less chlorophyll content and reduced both LHCI (30 % of reduction) and LHCII (50 % of reduction) content. However, the effective quantum yield of both PSI and PSII increase in *chl27* (Hansson and Jensen 2009). The photochemical efficiency of *chl27* mutant is greater than WT a despite of minor capacity of light absorption, similar to observed here in *gun4* mutant. Interestingly, *gun4* and *chl27* plants showed a flexibility of PSII to increase the capacity of energy transfer despite of fewer molecules for light absorption.

Light stress has been reported as signal to reduce the expression of nuclear genes that codify light-harvesting chlorophyll a/b-binding proteins (LHCB) via a pathway that involve GUN4 and ABI4 transcription factor (*abscisic acid-insensitive 4*) (Larkin et al. 2003; Larkin and Ruckle 2008). Probably this is a photo-protection response in order to reduce light

excess absorption in PSII and minimize oxidative damages caused by excess of light in WT plants. Furthermore, *gun* mutants exhibited high-level expression of LHCB genes under stress (Susek et al. 1993; Mochizuki et al. 2001; Eberhard et al. 2008; Peter and Grimm 2009; Brzezowski et al. 2014). It suggests that the expression of GUN and LHCB genes are highly coordinated. Indeed, the analysis of GUN4 co-expression network showed that many genes related to photosynthesis and structure of photosystems are co-expressed with GUN4, including many genes of LHCB family (Table S1). Similarly, the single *sg1* mutant and the double *sg1 gun4* mutant presented low levels of chlorophyll but high levels of genes related to photosystem II (psbA, psbB) and carbon fixation (RbcS, RbcL) (Hu et al. 2014). It suggests that GUN4, like SG1, is important not only for chlorophyll biosynthesis but also for expression of genes related to the photosynthetic process; which was indeed showed in this work. The *up-regulation* of LHCB genes in *gun4* mutant under stress may optimize the light energy transfer from LHCII to reaction center of PSII, contributing to the greater light energy transfer efficiency in LHCII/PSII despite low chlorophyll and carotenoids content. Furthermore, in the absence of light stress, *gun4* plants showed lower values of Φ_{NO} than WT. This reduction in constitutive loss of energy can be a flexibility of *gun4* LHCII to maximize the energy transfer to reaction center of PSII, contributing for the greater photochemical capacity of *gun4* plants.

4.2 GUN4 mutation minimize the effects of light stress

The decrease in F_v/F_m and the increase in F_o in both genotypes after 28 h of light stress suggest the occurrence of photoinhibition on photochemical apparatus (Maxwell and Johnson 2000; Müller et al. 2001). The increases in F_o can be related to damages in the reaction center of PSII and/or in the LHCII (Demmig-Adams and Adams 1992; Bolhàr-Nordenkampf and Öquist 1993). Damages on PSII light stress-induced can also be associated with increases in unregulated energy dissipation of PSII (Φ_{NO}) (Klughammer and Schreiber 2008). Indeed it was observed increases in Φ_{NO} in both genotypes after 14 h of light stress (data not shown). It is important to highlight that although light stress was sufficient to induce damages on PSII of both WT and *gun4* mutant, the decreases observed in F_v/F_m and the increases in F_o after severe light stress were minor in *gun4* plants. Furthermore, WT plants were unable to keep a high qN value after 28 h of light stress as observed in *gun4* plants. Thus, considering that *gun4* mutant absorbs less light energy (lower F_o values) and has greater capacity to dissipate energy (higher qN values), it suggests that the oxidative damage on PSII light stress-induced is minor in *gun4* plants. However, the mechanism by which *gun4* plants dissipate more efficiently the excess of energy absorbed is still unknown.

4.3 Total $^{14}CO_2$ leaf absorption suggests a diffusive limitation for photosynthesis in *gun4* mutant

Although *gun4* plants showed lower values of stomatal conductance (g_s) and net photosynthetic rate (A) compared to WT in the absence of stress, no differences were detected between the genotypes in the total $^{14}CO_2$ fixation as well as in starch fraction after $^{14}CO_2$ fixation, a photosynthetic product accumulated in the light (Zeeman et al. 2007). It suggests that the lower capacity for CO_2 fixation observed in *gun4* plants in absence of stress can be related to the diffusional limitation of CO_2 rather than a biochemical limitation, once the feeding experiment was carried out under saturating levels of CO_2 where stomata present no barrier to CO_2 assimilation.

5 Conclusions

Taken together, the results of this work showed that GUN4 mutation have high impact on gas exchange through the leaves, photosynthetic pigment contents, and on light energy transfer capacity of the PSII. It suggests that this gene is important not only for the chlorophyll biosynthesis but also for process that regulates the transfer of energy from LHCII to reaction center of PSII. Furthermore, because of the phenotype displayed by *gun4* mutant differs from other *gun* mutants, it indicates that *gun4* phenotype cannot be explained solely by the reduction in chlorophyll content. The flexibility of *gun4* PSII to maximize light energy transfer reflects an acclimation of LHCII in order to compensate the reduced ability of light absorption. It highlights the importance of *gun4*

mutant to investigate the mechanisms that control LHCII plasticity, an important aspect to understand plant light stress tolerance.

Acknowledgments This research was supported by the CNPq (National Council for Technological and Scientific Development, Brazil) and FAPEMIG (Foundation for Research Assistance of the Minas Gerais State, Brazil). Scholarships granted by CNPq, CAPES and FAPEMIG are gratefully acknowledged. DMD was recipient of a scholarship from CAPES.

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