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Salma Balazadeh, Nils Jaspert, Muhammad Arif, Bernd Mueller-Roeber and Veronica Graciela Maurino

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Expression of ROS-responsive genes and transcription factors after metabolic formation of H_2O_2 in chloroplasts

Salma Balazadeh[1§], Nils Jaspert[2§], Muhammad Arif[1], Bernd Mueller-Roeber[1] and Veronica G. Maurino[2*]

[1]Institute of Biochemistry and Biology, University of Potsdam, Potsdam, Germany.
[2]Plant Molecular Physiology and Biotechnology, Center of Excellence on Plant Sciences (CEPLAS), Heinrich-Heine-University, Düsseldorf, Germany.

[§]Both authors contributed equally to the work

[*]Corresponding author
Dr. Veronica G Maurino
Entwicklungs- und Molekularbiologie der Pflanzen
Heinrich-Heine-Universität
Universitätsstraße 1
40225 Düsseldorf, Germany
E-mail: veronica.maurino@uni-duesseldorf.de

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Abstract

Glycolate oxidase (GO) catalyses the oxidation of glycolate to glyoxylate, thereby consuming O_2 and producing H_2O_2. In this work, *Arabidopsis thaliana* plants expressing GO in the chloroplasts (GO plants) were used to assess the expressional behaviour of reactive oxygen species (ROS)-responsive genes and transcription factors (TFs) after metabolic induction of H_2O_2 formation in chloroplasts. In this organelle, GO uses the glycolate derived from the oxygenase activity of RubisCO. Here, to identify genes responding to an abrupt production of H_2O_2 in chloroplasts we used quantitative real-time PCR (qRT-PCR) to test the expression of 187 ROS-responsive genes and 1,880 TFs after transferring GO and wild-type plants grown at high CO_2 levels to ambient CO_2 concentration. Our data revealed coordinated expression changes of genes of specific functional networks 0.5 h after metabolic induction of H_2O_2 production in GO plants, including the induction of indole glucosinolate and camalexin biosynthesis genes. Comparative analysis using available microarray data suggests that signals for the induction of these genes through H_2O_2 may originate in the chloroplast. The TF profiling indicated an upregulation in GO plants of a group of genes involved in the regulation of proanthocyanidin and anthocyanin biosynthesis. Moreover, the upregulation of expression of TF and TF-interacting proteins affecting development (e.g., cell division, stem branching, flowering time, flower development) would impact growth and reproductive capacity, resulting in altered development under conditions that promote the formation of H_2O_2.

Keywords

Glycolate oxidase, H_2O_2, ROS-responsive genes, transcription factors

i. Introduction

Photosynthetic organisms are confronted with reactive oxygen species (ROS), such as singlet oxygen ($^1O_2$), the superoxide anion radical ($O_2^{-\cdot}$), the hydroxyl radical ($OH\cdot$), and hydrogen peroxide (H_2O_2), which may cause oxidative stress and damage to important biological molecules (Apel and Hirt, 2004; Møller et al., 2007). Plants in their natural environments are often exposed to sudden increases in light intensity, which results in the absorption of excitation energy in excess of that required for metabolism. In chloroplasts, when absorbed energy is in excess at photosystem II (PSII), $O_2^{-\cdot}$ is produced during the Mehler reaction by Fd-NADPH

oxidase at PSI and is dismutated by superoxide dismutase (SOD) to H_2O_2 (Ort and Baker, 2002; Asada, 2006). The photorespiratory pathway consumes photosynthetic reducing energy and produces H_2O_2 in the peroxisomes through the action of catalase (Maurino and Peterhansel, 2010). H_2O_2 is also produced during a variety of different reactions under stress conditions, often through the detoxification of $^1O_2$ and $O_2^{\cdot-}$. The generated H_2O_2 is scavenged by different antioxidant/enzyme reactions: the ascorbate and glutathione cycles, ascorbate peroxidase (APX), catalase, and peroxiredoxin (PRX) (Tripathi et al., 2009).

ROS generated in the chloroplast have been implicated as triggers of signaling pathways that influence expression of nuclear-encoded genes, which may initiate responses such as cell death or acclimation depending on the degree of the stress (Karpinski et al., 1999; Fryer et al., 2003; Op den Camp et al., 2003; Danon et al., 2005). H_2O_2 can take part in signaling acting as messenger either directly (e.g., by reversibly modifying critical thiol groups in target proteins; Neill et al., 2002) or by using an oxidized product as a secondary messenger (Møller et al., 2007). The H_2O_2-scavenging enzymes APX and dehydroascorbate reductase (DHAR) may act as highly efficient initiators of oxidative signaling by generating transient bursts of reduced glutathione. This in consequence triggers glutaredoxin-mediated protein oxidation (Neill et al., 2002). Cross talk between redox pools of different cellular compartments, possibly transmitted by a redox shift in cellular components, has also been suggested to be important for control of the expression of nuclear genes (Baier and Dietz, 2005; Leister, 2005). A generalized model of H_2O_2 signal transduction pathways suggests that H_2O_2 may also directly oxidize transcription factors (TFs) in either the cytosol or the nucleus. Alternatively, H_2O_2-mediated activation of a signaling protein such as a protein kinase may activate TFs (Mittler et al., 2004; Miao et al., 2007). TFs would interact with cognate H_2O_2-response elements in target gene promoters thereby modulating gene expression (Foyer and Noctor, 2005). Recently, Møller and Sweetlove (2010) put forward the hypothesis that H_2O_2 itself is unlikely to be the signaling molecule that selectively regulates nuclear-encoded chloroplastic genes but rather that oxidized peptides deriving from proteolysis of oxidized proteins would act as second messengers during retrograde ROS signaling. On the other hand, using spin trapping EPR spectroscopy in addition to chemical assays (employing Amplex Red reagent), Mubarakshina et al. (2010) showed that 5% of the H_2O_2 produced inside chloroplasts at high light intensities can actually be detected outside the organelles. This process

may involve the pass of H_2O_2 through aquaporins (Bienert et al., 2007) and might be sufficient to trigger signaling processes outside the chloroplasts.

Desikan et al. (2001) showed that approximately 1% of the transcriptome was altered in H_2O_2-treated *Arabidopsis thaliana* (*A. thaliana*) cell cultures. Although H_2O_2-responsive promoters have been identified (Desikan et al., 2001), specific H_2O_2-regulatory DNA sequences and their cognate TFs have not been isolated and characterized. In more recent studies genes involved in H_2O_2 signal transduction have been identified or proposed, including mitogen-activated protein kinases (MAPKs), various TFs of e.g. the NAC, ZAT and WRKY families, miRNAs and others (Van Breusegem et al., 2008; Li et al., 2011; Petrov and Van Breusegem, 2012). Moreover, using genome-wide analysis of catalase deficient *A. thaliana*, H_2O_2 was inferred to regulate the expression of genes encoding specific small heat shock proteins, several TFs and candidate regulatory proteins (Vandenabeele et al., 2004; Vanderauwera et al., 2005).

To date, it is not known to which extent the chemical specificity of the ROS species and the cellular compartment of their release may contribute to the multiplicity of responses that occur in plants. A major challenge is to dissect the genetic networks that control ROS signaling and to assess specific and common responses towards different types of ROS signals. To this end, the molecular, biochemical and physiological responses of *A. thaliana* to elevated *in planta* levels of H_2O_2 were and are being investigated in various types of model systems including mutants altered in the ROS scavenging machinery (Maurino and Flügge, 2008). However, the analysis of dynamic physiological processes using (knock-out) mutants may not always be straightforward, especially when compensatory cellular mechanisms are induced. With respect to ROS-related mutants, changing the balance of scavenging enzymes may induce compensatory mechanisms such that signaling and oxidative damage effects may not be easily separated. Moreover, invasive experimental setups like the application of oxidative stress-causing agents may induce a non-specific oxidative stress that acts throughout the cell and triggers additional responses that may complicate the analysis of ROS signal transduction pathways (Maurino and Flügge, 2008). We have recently developed a tool to functionally dissect the action of plastid-generated H_2O_2, using plants overexpressing glycolate oxidase (GO) in plastids (GO plants; Fahnenstich et al., 2008). During photosynthesis, the oxygenase activity of ribulose 1,5-bisphosphate carboxylase/oxygenase (RubisCO) produces glycolate 2-phosphate within the chloroplasts,

which is then dephosphorylated to glycolate by phosphoglycolate phosphatase (Maurino and Peterhansel, 2010). In GO plants, glycolate is oxidized to glyoxylate by the plastidic GO, with the parallel production of H_2O_2. When growing under moderate photon fluxes and ambient CO_2 concentration (photorespiratory conditions) the GO plants remain smaller than the wild type, presenting a reduced rosette diameter and yellowish leaves due to H_2O_2 accumulation (Fahnenstich et al., 2008). In contrast, in non-photorespiratory conditions (e.g., at high CO_2 concentration) the oxygenase activity of RubisCO is abolished and thus, the metabolic flux through GO is suppressed, allowing GO plants to grow like wild type (Fahnenstich et al., 2008). Transferring GO plants from high to ambient CO_2 concentration specifically induces H_2O_2 formation in the chloroplasts (Fahnenstich et al., 2008). These properties permit the modulation of plastidic produced H_2O_2 levels by changing light intensity and/or CO_2 levels (Maurino and Flügge, 2008). Moreover, H_2O_2 is specifically generated without a concomitant accumulation of superoxide or singlet oxygen, which are common precursors of H_2O_2 during ROS generation in chloroplasts. A similar experimental set-up was employed in previous studies using catalase-deficient plants in which the production of peroxisomal H_2O_2 is induced by changing the conditions of plant growth from non-photorespiratory to photorespiratory (e.g., high light intensity) (Dat et al., 2001; Vandenabeele et al., 2004; Vanderauwera et al., 2005). The metabolic production of H_2O_2 may avoid the pleiotropic effects discussed above but it cannot be ruled out that ROS-unrelated reactions may still occur in both approaches due to abrupt changes in CO_2 level or light intensity.

In this work we attempted to identify genes strongly responding to an abrupt production of H_2O_2 in chloroplasts of *A. thaliana*. To this end we tested the expressional changes of 187 nuclear-encoded ROS-responsive genes and 1,880 TFs, using quantitative real-time (qRT)-PCR (Czechowski et al., 2004; Balazadeh et al., 2008; Wu et al., 2012) upon transfer of high CO_2-grown GO and wild-type plants to ambient CO_2 concentration. Our data revealed a rapid and coordinated expression response of ROS-affected genes of specific functional networks in GO including an early induction of indole glucosinolate and camalexin biosynthesis genes and an upregulation of a group of genes involved in the regulation of proanthocyanidin and anthocyanin biosynthesis. Moreover, the upregulation of expression of TF and TF-interacting proteins affecting development (e.g., cell division, stem branching, flowering time, flower development)

would impact growth and reproductive capacity, resulting in altered development under conditions that promote the formation of H_2O_2.

ii. Material and methods

Plant material

Arabidopsis thaliana (L.) Heynh. ecotype Columbia-0 (Col-0, wild-type) constitutively expressing glycolate oxidase (GO, At3g14420) in the plastids (GO plants) under the control of the Cauliflower Mosaic Virus 35S promoter were generated in our previous work (Fahnenstich et al., 2008). In these plants, to direct GO protein to chloroplasts, the stromal targeting presequence from *A. thaliana* phosphoglucomutase (At5g51820) was used (Fahnenstich et al., 2008). Wild-type and GO transgenic plants were grown in pots containing 3 parts of soil (Gebr. Patzer KG, Sinntal-Jossa, Germany) and one part of vermiculite (Basalt Feuerfest, Linz, Austria) under a 16h-light/8h-dark regime at photosynthetically active photon flux densities (PPFD) of 75 µmol quanta m^{-2} s^{-1} at 22°C day/18°C night temperatures and a CO_2 concentration of 3,000 ppm. After three weeks of growth plants were transferred to ambient CO_2 concentration (380 ppm) and the same PPFD. Whole rosettes were harvested at different time points after transfer, immediately frozen in liquid nitrogen and stored at -80°C until use for RNA isolation and H_2O_2 measurements.

Isolation of RNA and real-time PCR analysis

For the large-scale qRT-PCR analysis, total RNA was extracted from 100 mg leaves (fresh weight) using RNeasy Plant Mini kit (Qiagen, Valencia, USA) according to the manufacturer's protocol. DNAse I digestion was performed on 40 to 60 µg of total RNA using TURBO DNase Kit (Ambion, Cambridgeshire, UK) according to manufacturer's instructions. RNA integrity was checked on 1% (w/v) agarose gels and concentration measured with a Nanodrop ND-1000 spectrophotometer before and after DNAse treatment. Absence of genomic DNA was confirmed subsequently by quantitative PCR using primers that amplify an intron sequence of the gene At5g65080 (forward 5′-TTTTTTGCCCCCTTCGAATC-3′ and reverse 5′-ATCTTCCGCCACCACATTGTAC-3′). First-strand cDNA was synthesized from 8 - 10 µg of total RNA using RevertAid™ First Strand cDNA Synthesis Kit (Fermentas, St. Leon-Rot,

Germany) following the manufacturer's protocol. The efficiency of cDNA synthesis was estimated by qRT-PCR using two different primer sets annealing to the 5' and 3' ends, respectively, of a control gene (At3g26650, *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase). Primer sequences were as follows: for GAPDH3', forward 5'-TTGGTGACAACAGGTCAAGCA-3' and reverse 5'-AAACTTGTCGCTCAATGCAATC-3'; for GAPDH5', forward 5'-TCTCGATCTCAATTTCGCAAAA-3' and reverse 5'-CGAAACCGTTGATTCCGATTC-3'. Transcript levels of each gene were normalized to *ACTIN2* (At3g18780) transcript abundance (forward 5'-TCCCTCAGCACATTCCAGCAGAT-3' and reverse 5'-AACGATTCCTGGACCTGCCTCATC-3'). A total of 187 ROS-responsive genes (Wu et al., 2012) and 1,880 TFs (Czechowski et al., 2004; Balazadeh et al., 2008) were analysed by qRT-PCR as previously described (Caldana et al., 2007; Balazadeh et al., 2008). PCR reactions were run on an ABI PRISM 7900HT sequence detection system (Applied Biosystems, Darmstadt, Germany), and amplification products were visualized using SYBR Green (Applied Biosystems).

H_2O_2 measurements

Levels of H_2O_2 were determined using the Amplex® Red Technology (Life Technologies, Darmstadt, Germany) following the manufacturer´s instructions. Amplex Red (N-acetyl-3,7-dihydroxyphenoxazine) reacts with H_2O_2 in the presence of horseradish peroxidase and forms the fluorescent product resorufin. For the determinations, 100 mg leaves (fresh weight) were ground in liquid nitrogen into a fine powder and resuspended with 0.15 mL extraction buffer prepared as indicated by the manufacturer. This suspension was centrifuged at 4°C at 13,000 rpm for 15 min. Five µL of the supernatant, 45 µL distilled water and 50 µL of Amplex® Red solution were added to a microtitre plate. After 30 min incubation in the dark fluorescence was measured by excitation at 560 nm and emission reads at 590 nm. A calibration curve was established with known H_2O_2 concentrations.

Gene expression network analysis

The two genes that were most strongly induced under photorespiratory conditions in GO plants at the 0.5 and 6 h time points (At3g02840 and At1g17180, respectively) were used as baits to identify globally coexpressed genes using the ATTED-II database (http://atted.jp), which allows

evaluating genes that are coexpressed under five experimental conditions (tissue, abiotic stress, biotic stress, hormones and light conditions) (Obayashi et al., 2009).

iii. Results and discussion

Induction of H_2O_2 formation in GO plants

The production of H_2O_2 in leaves of plants overexpressing glycolate oxidase (GO) in the plastids (Fahnenstich et al., 2008) was analysed after activation of photorespiration by transferring high CO_2-grown plants to ambient-CO_2 conditions. As shown in **Table 1**, higher levels of H_2O_2 were determined in GO than in wild-type plants at 0.5 and 4 h after transfer while GO and wild-type plants maintained under non-photorespiratory conditions (3,000 ppm CO_2) showed similar H_2O_2 levels at both time points (**Table 1**). Note, that as the measurements were performed using whole-leaf extracts the expected differences in chloroplastic H_2O_2 levels between GO and wild-type plants under photorespiratory condition may be actually higher than determined here.

Expression profiling of ROS marker genes in GO and wild-type plants after the induction of H_2O_2 formation in chloroplasts

To study the impact of an abrupt production of H_2O_2 in chloroplasts on nuclear gene expression, we analysed transcript level changes of 187 ROS-responsive genes using a previously established qRT-PCR platform (detailed in Wu et al., 2012). The genes included in the platform were chosen from published reports and our own experiments and represent four different groups that were already shown to be rapidly induced by (i) superoxide radical ($O_2^{\cdot-}$; 18 genes), (ii) singlet oxygen ($^1O_2$; 22 genes), (iii) H_2O_2 (53 genes), or (iv) different types of ROS (general ROS-responsive genes; 94 in total).

Gene expression was analysed in whole rosettes of three-week-old wild-type and GO plants at 0.5, 4, 6 and 12 h after shifting high-CO_2-grown plants (non-photorespiratory condition) to ambient CO_2 concentration (photorespiratory condition). Expression profiling was performed in two biological replicates and log-fold change (log2 FC) ratios of expression changes were calculated for GO and wild-type plants by comparing gene expression levels before and after the CO_2 concentration shift. A total of 131 genes were expressed in all examined samples (**Table A1**

in Appendix). The remaining 56 genes did not yield detectable PCR amplicons, indicating no or marginal expression under our experimental conditions.

Considering a 3-fold expression difference cut-off, 120 genes displayed differential expression in GO and/or wild-type plants upon transfer from high to ambient CO_2 concentration; the vast majority of the affected genes (116 in total) were up-regulated, and only four genes were down-regulated (**Figure 1**, **Table A1** in Appendix). Most noticeably, expression of 58 genes was induced in GO plants already within 0.5 h after the transfer to ambient CO_2 condition, whilst only a single gene was induced in the wild type at the same time point (**Figure 1**). Importantly, however, many genes showed also high expression in the wild type at later time points after the CO_2 concentration shift but the expressional changes were in most cases more pronounced in GO than wild-type plants (**Figure 1**, and following chapter). Thus, our data indicate that similar sets of ROS-responsive genes responded to the CO_2 shift in GO and wild-type plants; however, the dynamics of the transcriptional responses were clearly different in the two types of plants, being faster and more prominent in the GO plants.

Early induction of indole glucosinolate and camalexin biosynthesis genes in GO plants

To identify transcripts responsive to metabolically produced H_2O_2 we focused our analysis on the 0.5- and 6-h time points. Genes were considered differentially expressed when the fold change was more than 3-fold ($\log_2 \geq 1.56$).

At 0.5 h after shifting plants to ambient CO_2 concentration, 58 of the 131 expressed genes were induced in GO plants by more than 3-fold, whilst in the wild type the expression change was less than 3-fold, suggesting that these genes participate in early signaling steps triggered by the production of H_2O_2 under photorespiratory conditions (**Table 2**). After 6 h, seven of these genes showed wild-type levels of expression (below 3-fold), while 29 were further overexpressed only in GO (**Table 2**). Although at 6 h after transfer to ambient CO_2 the expression fold-change (FC) of the remaining 22 genes was higher than 3 in both, GO and wild-type (WT) plants, the expression change between GO and wild-type (FC_{GO}/FC_{WT}) was higher than 2 for 16 of these genes (**Table 2**), indicating that their higher expression in GO plants was triggered by the elevated levels of H_2O_2.

Later responding genes, which were affected only after 6 h under photorespiratory conditions, were also identified. From the 23 genes that showed an expression change of above 3-fold in GO, 13 were only induced in GO, while 10 genes were induced in both, GO and WT. The FC ratio in GO and WT (FC_{GO}/FC_{WT}) was above 2 for the 10 genes (**Table 3**), indicating that their expression in GO plants is controlled by the higher levels of H_2O_2, similar to the early-responsive genes.

The most highly up-regulated gene in GO plants at 0.5 h after induction of H_2O_2 production was At3g02840 (encoding a putative U-box-type E3 ubiquitin ligase, known to respond immediately-early to fungal elicitation) (**Table 2**). We used the ATTED-II database (http://atted.jp; Obayashi et al., 2009) to discover genes coexpressed with At3g02840 and observed that 45 of the 58 genes induced at 0.5 h after induction of H_2O_2 production cluster together (**Table 2**), indicating that metabolically produced H_2O_2 in GO plants induces the coordinate expression of functionally related genes. A similar analysis using the most highly expressed gene at 6 h after induction of H_2O_2 production (At1g17180, encoding glutathione S-transferase Tau 25) indicated that another group of eight genes are coordinately expressed in GO plants at this later time point (**Table 3**).

Recently, Inzé et al. (2012) listed the 85 most strongly H_2O_2-responsive genes in catalase loss-of-function mutants shifted from low- to high-light conditions, where H_2O_2 is produced in peroxisomes by the action of photorespiratory GOs. Interestingly, 23 of the 81 genes, which changed their expression in the GO plants were also differentially expressed in catalase loss-of-function mutants (**Tables 2** and **3**), indicating that they respond to enhanced levels of H_2O_2 independent of the site of its generation; the remaining genes may then represent candidates preferentially responsive to H_2O_2 produced in chloroplasts. Many of the genes upregulated in GO plants encode proteins or TFs of currently unknown specific functions. Interestingly, however, several of the early-responsive genes are involved in tryptophan-derived biosynthesis of the phytoanticipins camalexin and indole glucosinolates, i.e., secondary metabolites that have antifungal and insect-deterring functions (Kliebenstein et al., 2001; Bednarek et al., 2009). These genes encode (i) the transcription factor WRKY33 (At2g38470), which is involved in controlling camalexin biosynthesis (Birkenbihl et al., 2012); (ii) the Myb-type transcription factor

HIG1/MYB51 (At1g18570) involved in the positive regulation of indole glucosinolate biosynthesis by activating several target genes (Gigolashvili et al., 2007); (iii) the O-methyltransferases IGMT1 (At1g21100) and IGMT2 (At1g21120), which catalyze the transfer of a methyl group to the hydroxy indole glucosinolate hydroxyindol-3-ylmethylglucosinolate (4 and 1OH-I3M, respectively) to form methoxyindol-3-ylmethylglucosinolate (4 and 1MO-I3M, respectively) (Pfalz et al., 2011); and (iv) cytochrome P450 monooxygenase CYP81F2 (At5g57220), that is essential for the pathogen-induced accumulation of 4-methoxyindol-3-ylmethylglucosinolate (4MI3G) (Bednarek et al., 2009). Our data thus show the early induction of indole glucosinolate and camalexin biosynthesis genes in GO plants after metabolic formation of H_2O_2 through the activation of genes encoding enzymes involved in intermediate metabolite conversions and of TFs that act on several target genes of these biosynthetic pathways.

Transcription factor profiling

To understand the potential effects of overexpression of GO in chloroplasts on the nuclear transcriptional program, we next broadened our analysis by testing the expression of 1,880 TFs using a highly sensitive quantitative real-time PCR (qRT-PCR) platform (Czechowski et al., 2004; Balazadeh et al., 2008). Considering the data obtained from the profiling of the ROS-responsive genes, we analysed the expression at 0.5 h after induction of H_2O_2 production to capture the early-responsive TFs. Expression profiling was performed in two biological replicates and log-fold change (log2 FC) ratios of expression changes were calculated for GO and wild-type plants by comparing gene expression levels before and after the transfer of plants grown at high CO_2 to ambient CO_2.

TFs most strongly responding to H_2O_2 were identified by comparing their expression fold-change in GO and wild-type plants. A TF was considered differentially expressed when the FC in GO was more than 3-fold ($\log_2 \geq 1.56$) and less than 2-fold in the wild type ($\log_2 \geq 1.0$) (**Table 4**). Analysis of transcript profiles revealed that the expression of 1,449 genes, representing 77% of all TF genes tested, could be detected (**Table A2** in Appendix). The remaining 23% (431 of the 1,880 TFs) did not yield detectable PCR amplicons, indicating no or very weak expression in the tested material.

335 At 0.5 h after shifting plants to ambient CO_2 concentration, 78 of the 1,449 genes were induced
336 by more than 3-fold in GO plants, whereas in wild-type plants the expression changes of the
337 same genes were less than 2-fold (**Table 4**). Using published data, the involvement/participation
338 of the TFs in specific biological processes (**Table 4**) could be assessed, which allowed the
339 classification of the TFs into 5 functional groups (FG) enriched with specific gene ontology
340 categories (**Figure 2**). FG1 contains TFs involved in the regulation of proanthocyanidin and
341 anthocyanin biosynthesis (**Table 4** and **Figure 2**). The TFs TT8 and MYB75 affecting the gene
342 expression of dihydroflavonol 4-reductase (Debeaujon et al., 2003) are included in this FG. FG2
343 contains TFs affecting developmental processes like lateral root formation (GATA23), flowering
344 (FD1, ANAC089, TEM2 and SNZ), shoot branching (MYB2 and BRC2), senescence
345 (ANAC092/ORE1) and cell division (ANAC068 and HAT4) (**Table 4** and **Figure 2**). The
346 activation of these TFs in GO plants would result in altered growth and flowering (see below and
347 Fahnenstich et al., 2008). FG3 includes TFs and TF-interacting proteins negatively regulating
348 jasmonate (JA) signalling (*JAZ7*, *JAZ8*, *JAZ9*, *JAZ10*, *WRKY50* and *WRKY51*; Staswick, 2009;
349 Chico et al., 2008; Gao et al., 2011) (**Table 4** and **Figure 2**). JAZ proteins bind directly to the
350 key transcription factor MYC2 and thereby prevent JA-dependent gene transcription (Chini et
351 al., 2007; Pauwels et al., 2010). At the same time *JAZ* genes are rapidly induced by JA and some
352 are MYC2-regulated. This feedback loop regulation would provide a rapid on and off switch of
353 the pathway involving JA. Transcriptional activation of *JAZ* genes was found to occur in
354 response to several biotic and abiotic challenges (Yan et al., 2007). JAZ proteins would also
355 exert their effects on post-wound inhibition of vegetative growth in *A. thaliana* (Yan et al., 2007)
356 and as repressors of necrosis and/or programmed cell death during development in tobacco (Oh
357 et al., 2012). In GO plants, the action of *JAZ* genes together with those of FG2 would impact
358 growth and reproductive capacity, resulting in altered development under conditions that
359 promote the formation of H_2O_2. FG4 includes TFs with diverse functions in plant defense and
360 signaling, e.g., activators of tryptophan-derived biosynthesis of camalexin (JUB1/ANAC042)
361 and indole glucosinolates (MYB122), as well as regulators of photomorphogenesis (STH2)
362 (**Table 4** and **Figure 2**). The early activation of camalexin and indole glucosinolate biosynthesis
363 was also observed in the analysis performed with the ROS-responsive gene platform (**Table 1**).
364 Finally, FG5 includes TFs with currently unknown functions (**Table 4** and **Figure 2**).
365

366 The analysis of the transcript profiles at 0.5 h after induction of H_2O_2 production in GO plants
367 (**Table A2** in Appendix) also revealed a group of 13 genes that are downregulated in GO relative
368 to wild-type plants (**Table 5**). The function of five of these genes is currently unknown, but
369 interestingly, the remaining eight genes positively control developmental processes. The down-
370 regulation of expression of these TFs in GO plants together with the upregulation of expression
371 of TFs negatively affecting development (see FG2, **Table 3**) would act in concert to arrest
372 growth and especially to delay the transition from the vegetative to the reproductive phase.
373 Consistently, our previous results showed that GO plants growing under photorespiratory
374 conditions are smaller than wild-type plants, presenting a reduced rosette diameter and a delay in
375 flowering time (Fahnenstich et al., 2008).
376
377 **iv. Concluding remarks**
378 The metabolic induction of H_2O_2 formation in chloroplasts of GO plants under photorespiratory
379 conditions triggered a faster and more prominent transcriptional response of ROS-responsive
380 genes in these plants than in wild type. The changes of the transcriptional activities observed in
381 GO plants early after induction of H_2O_2 production in chloroplasts suggest the establishment of
382 responses that resemble those occurring at early times after wounding or herbivore attack, where
383 H_2O_2 is also produced (Orozco-Cárdenas and Ryan, 1999). These responses include (i) the
384 retardation of development, which in part would be linked to JA signaling, and (ii) the
385 production of the phytoanticipins indole glucosinolates and camalexin. As in the case of
386 herbivore attack, the retardation of development such as reductions in growth and reproduction
387 observed in GO plants could be regarded as a strategy to allow more resource allocation to
388 support defense and tolerance responses (Zavala and Baldwin, 2006). The data also suggest that
389 signals for the early induction of indole glucosinolate and camalexin biosynthesis genes in GO
390 plants through H_2O_2 may originate in chloroplasts as these genes showed no modified expression
391 in catalase loss-of-function mutants (Inzé et al., 2012).
392
393 **Conflict of interest statement**
394 The authors declare that the research was conducted in the absence of any commercial or
395 financial relationships that could be construed as a potential conflict of interest.
396

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Figures

Figure 1. Venn diagram of the number of ROS-responsive genes differentially expressed in wild-type and GO plants at different time points (0.5, 4, 6 and 12 h) after the transfer of plants grown at high CO_2 concentration (3,000 ppm) to ambient CO_2 concentration (380 ppm).

Figure 2. Pie chart representation of the five functional groups (FG) of early H_2O_2-responsive TFs in GO plants. FG5 includes genes for which a distinct biological function has not been reported yet.

547 **Table 1.** Levels of H_2O_2 measured in whole rosettes (µmol /g FW) after shifting high CO_2-grown
548 wild-type and GO plants to ambient CO_2 concentration for 0.5 and 4 h. Samples from control
549 plants maintained in high CO_2 were processed in parallel. Values indicate the mean ± SE of three
550 independent samples and those set in bold face indicate significant differences to the
551 corresponding wild-type value calculated by Student´s *t* test (P<0.05). WT: wild type.
552

	0.5 h		4 h	
	high CO_2	ambient CO_2	high CO_2	ambient CO_2
WT	2.4 ± 0.2	2.3 ± 0.2	2.5 ± 0.4	2.7 ± 0.1
GO	2.5 ± 0.3	3.0 ± 0.3	2.6 ± 0.2	**3.4 ± 0.0**

553

554 **Table 2.** ROS-responsive genes (58 in total) the expression of which was enhanced by more than
555 3-fold in GO plants 0.5 h after shifting plants grown at high CO_2 concentration (3,000 ppm) to
556 ambient CO_2 concentration (380 ppm). Genes are listed according to the difference of the
557 expression change between GO and wild-type (WT) plants (FC_{GO}/FC_{WT}) at 0.5 h. FC_{GO}/FC_{WT}
558 values higher than 2 are shown in bold face. AGI: gene identification number given by the
559 Arabidopsis Genome Initiative. Genes also induced in catalase loss-of function mutants are
560 highlighted with an asterisk (*) (Inzé et al., 2012). Genes included in the same gene coexpression
561 network of At3g02840 (putative U-box-type E3 ubiquitin ligase) are highlighted in bold face
562 (http://atted.jp; Obayashi et al., 2009). The gene annotation was retrieved from TAIR
563 (http://arabidopsis.org/index.jsp).
564

	0.5 h			6 h			
AGI	FC_{WT}	FC_{GO}	FC_{GO}/FC_{WT}	FC_{WT}	FC_{GO}	FC_{GO}/FC_{WT}	Annotation
Upregulated in GO at 0.5 h							
At1g69890	1.3	15.9	**12.0**	1.2	2.2	1.8	Protein of unknown function
At2g40000*	0.9	10.2	**11.2**	1.6	2.1	1.3	Ortholog of sugar beet HS1 PRO-1 2 (HSPRO2)
At2g18210	1.0	8.4	**8.2**	1.1	2.9	**2.6**	Protein of unknown function
At1g18570	1.2	6.8	**5.7**	0.7	2.5	**3.6**	Myb-type transcription factor (HIG1/MYB51)
At1g21100	1.6	6.9	**4.2**	0.7	1.5	**2.2**	Indole glucosinolate O-methyltransferase (IGMT1)
At5g64310	1.2	3.8	**3.1**	3.1	1.1	0.4	Arabinogalactan protein (AGP1C) of unknown function
At5g28630	1.4	4.0	**2.9**	0.3	0.4	1.5	Protein of unknown function
Upregulated in GO at 0.5 h and 6 h							
Change in gene expression in WT at 6 h < 3							
At3g02840*	1.1	79.3	**71.2**	2.9	65.4	**22.8**	Putative U-box-type E3 ubiquitin ligase
At2g37430*	0.9	53.3	**62.7**	1.9	190.8	**100.9**	C2H2 and C2HC zinc finger superfamily protein (ZAT11)
At1g05575*	1.5	45.6	**29.9**	0.6	4.3	**6.8**	Protein of unknown function
At2g38470	1.4	31.5	**22.3**	1.8	7.5	**4.3**	WRKY-type transcription factor (WRKY33)
At4g17490	1.5	21.5	**14.7**	1.3	6.9	**5.2**	Ethylene-responsive element binding factor (ERF6)
At5g47230	1.3	17.4	**13.8**	1.5	4.4	**3.0**	Ethylene-responsive element binding factor (ERF5)
At1g66060	1.3	17.5	**13.2**	2.7	5.1	1.9	Protein of unknown function
At2g32030	1.3	16.4	**12.2**	1.9	24.5	**12.9**	Putative GNAT-type N-acetyltransferase
At2g26530*	1.2	13.3	**10.7**	2.1	5.4	**2.5**	Protein of unknown function; AR781
At1g21120	1.0	9.9	**10.3**	0.7	15.9	**22.1**	Indole glucosinolate O-methyltransferase (IGMT2)
At1g35210	1.1	11.2	**10.2**	2.9	14.4	**4.9**	Protein of unknown function
At3g55980*	1.4	14.3	**10.1**	1.2	4.0	**3.3**	Salt-inducible zinc finger 1, SZF1 (C3H47)
At2g33710	1.0	7.5	**7.7**	0.8	12.6	**15.0**	Putative ERF-type transcription factor

At2g25735*	1.8	10.8	**5.9**	0.6	3.2	**5.7**	Protein of unknown function
At5g54490	1.5	7.8	**5.3**	1.5	4.1	**2.8**	PBP1, Pinoid Binding Protein 1
At1g19020*	1.8	9.3	**5.1**	1.9	27.7	**14.5**	Protein of unknown function
At5g51190	1.4	6.8	**4.9**	1.8	5.0	**2.7**	Putative ERF-type transcription factor
At3g02800*	1.1	5.0	**4.5**	1.2	4.1	**3.4**	Tyrosine phosphatase (ATPFA-DSP3)
At5g64905	1.4	6.0	**4.4**	2.1	33.0	**16.0**	Putative peptide elicitor Pep3 precursor protein (ProPep3)
At1g76600*	1.4	5.8	**4.3**	1.7	11.0	**6.3**	Protein of unknown function
At3g23230	2.2	9.4	**4.2**	2.3	17.1	**7.5**	Putative ERF-type transcription factor (ERF98)
At1g59590	1.3	5.2	**4.1**	1.4	5.2	**3.8**	Zinc finger protein (ZCF37) of unknown function
At4g18880*	1.6	6.5	**4.0**	1.9	4.6	**2.4**	Heat stress-type transcription factor (HsfA4a/HSF21)
At2g41640	1.1	4.0	**3.7**	2.2	6.0	**2.7**	Protein of unknown function
At1g28190*	1.1	3.9	**3.5**	2.4	5.6	**2.3**	Protein of unknown function
At5g57220	2.8	9.1	**3.3**	1.3	6.9	**5.2**	Cytochrome P450 monooxygenase (CYP81F2)
At1g26380*	1.2	3.4	**3.0**	2.1	39.0	**18.9**	UDP-N-acetylmuramate dehydrogenase of unknown function
At2g31945	1.3	3.5	**2.7**	2.3	5.1	**2.2**	Protein of unknown function
At4g11280	1.5	3.0	**2.0**	1.4	5.9	**4.3**	1-Aminocyclopropane-1-carboxylate synthase (ACS6)
Change in gene expression in WT at 6 h > 3							
At1g80840	1.1	38.0	**35.4**	5.5	56.4	**10.3**	WRKY-type transcription factor (WRKY40)
At5g04340*	0.8	27.5	**32.6**	7.3	8.6	**1.2**	C2H2-zinc-finger-TF (C1-2iD-04) of unknown function
At2g22880	1.7	50.8	**29.4**	6.2	28.1	**4.5**	Protein of unknown function
At1g27730	1.1	30.1	**26.8**	6.0	28.0	**4.7**	C2H2-zinc-finger-TF (ZAT10/STZ)
At5g27420	1.2	32.7	**26.3**	3.0	15.0	**5.0**	Putative ubiquitin ligase, ATL subfamily (ATL31)
At1g61340	1.5	24.3	**16.3**	6.6	10.5	1.6	ATFBS1. F-Box stress induced 1 of unknown function
At5g59820	1.0	14.3	**14.1**	4.6	61.7	**13.5**	C2H2-zinc-finger-TF (ZAT12)
At5g24110*	1.0	13.3	**13.8**	3.3	91.2	**28.0**	WRKY-type transcription factor (WRKY30)
At4g24570	1.4	18.8	**13.6**	3.6	3.3	0.9	Dicarboxylate carrier (DIC2)
At3g10930	1.7	19.0	**11.5**	8.5	38.8	**4.6**	Protein of unknown function
At3g25250*	1.5	17.3	**11.4**	11.1	153.6	**13.8**	Putative protein kinase (AGC2/OXI1)
At4g39670*	1.3	14.3	**10.7**	31.7	184.9	**5.8**	Sphingosine transfer protein; accelerated death 11 (ACD11)
At1g77450*	0.8	7.3	**9.5**	5.6	6.9	1.2	NAC-type transcription factor (ANAC032)
At1g72520	1.7	15.7	**9.3**	4.8	9.7	**2.0**	Lipoxygenase (LOX4)
At3g48650	1.9	14.8	**7.6**	6.2	7.4	1.2	14a-related protein of unknown function
At4g21390	1.8	13.0	**7.0**	6.5	28.9	**4.4**	Putative S-domain-type receptor protein kinase
At5g63790*	0.8	5.4	**7.0**	3.1	11.1	**3.6**	NAC-type transcription factor (ANAC102)
At4g37370	1.4	7.0	**5.0**	2.8	67.5	**24.1**	Cytochrome P450 monooxygenase (CYP81D8)
At1g63720	1.5	5.0	**3.2**	3.1	6.1	1.9	Hydroxyproline-rich glycoprotein family protein
At2g18690	1.5	4.4	**3.0**	6.6	24.7	**3.7**	Protein of unknown function
At1g05340*	1.2	3.4	**2.7**	4.3	7.9	1.8	Protein of unknown function
At1g57630	1.4	3.4	**2.4**	20.2	36.0	1.8	Protein of unknown function

565

Table 3. ROS-responsive genes (23 in total) the expression of which was enhanced more than 3-fold in GO plants 6 h after shifting plants grown at high CO_2 concentration to ambient CO_2 concentration. Genes are listed according to the difference of the expression change between GO and wild-type (WT) plants (FC_{GO}/FC_{WT}) at 6 h. FC_{GO}/FC_{WT} values higher than 2 are shown in bold face. AGI: gene identification number given by the Arabidopsis Genome Initiative. Genes also induced in catalase loss-of function mutants are highlighted with an asterisk (*) (Inzé et al., 2012). Genes included in the same gene coexpression network of At1g17180 (*GSTU25*) are highlighted in bold face (http://atted.jp; Obayashi et al., 2009). The gene annotation was retrieved from TAIR (http://arabidopsis.org/index.jsp).

	0.5 h			6 h			
AGI	FC_{WT}	FC_{GO}	FC_{GO}/FC_{WT}	FC_{WT}	FC_{GO}	FC_{GO}/FC_{WT}	Annotation
Upregulated in GO at 6 h							
Change in gene expression in WT<3							
At1g26420	1.5	1.6	1.1	2.4	17.6	**7.3**	Putative reticuline dehydrogenase
At2g15480	1.0	2.8	**2.8**	1.2	7.2	**6.0**	UDP-dependent glycosyl transferase (UGT73B5)
At1g10040	1.2	1.8	1.5	2.1	10.4	**5.0**	Putative hydrolase
At2g29490	0.7	1.9	**2.8**	2.3	10.4	**4.5**	Tau glutathione S-transferase (GSTU1)
At5g46080	1.1	1.9	1.8	1.2	3.7	**3.1**	Putative protein kinase
At1g80820	1.2	1.5	1.3	2.5	7.8	**3.1**	Cinnamoyl CoA-reductase, involved in lignin biosynthesis
At3g09410	1.2	0.8	0.7	1.2	3.2	**2.7**	Putative pectin acetylesterase
At2g29500*	1.0	1.1	1.2	1.5	3.8	**2.5**	HSP20-type protein (HSP17.6B-CI); unknown function
At4g22530*	1.3	0.8	0.7	2.4	5.9	**2.4**	Putative methyltransferase
At4g15975	1.7	1.3	0.7	1.6	3.7	**2.4**	Putative ubiquitin ligase (RRE4/ATL17)
At2g38340	1.0	0.7	0.7	2.7	6.1	**2.2**	Putative AP2-type transcription factor (DREB2E)
At3g13790	1.3	1.3	1.1	2.5	5.0	**2.0**	Putative cell wall invertase (CwINV1)
At1g76070	1.2	2.6	**2.2**	1.6	3.3	**2.0**	Protein of unknown function
Change in gene expression in WT>3							
At1g17180	0.6	0.9	1.4	7.5	104.0	**13.8**	Tau glutathione S-transferase (GSTU25)
At1g15520	1.2	0.8	0.7	12.4	111.9	**9.0**	ABC transporter (ABCG40/PDR12)
At1g17170	1.0	1.3	1.4	5.7	40.4	**7.0**	Tau glutathione S-transferase (GSTU24)
At1g74360	1.0	2.2	**2.2**	4.2	14.2	**3.4**	Putative LRR-type receptor protein kinase
At2g38250*	1.2	1.7	1.4	4.4	13.7	**3.1**	Putative trihelix-type transcription factor
At5g51060	1.3	1.0	0.7	14.4	44.0	**3.1**	Respiratory burst oxidase homolog (AtRBOHC/RHD2)
At5g20230	1.5	2.8	1.8	9.9	28.3	**2.9**	Senescence associated gene (BCB/SAG14)
At2g41380	1.1	1.2	1.1	9.6	21.2	**2.2**	Putative S-adenosyl-L-methionine-dependent methyltransferase

At1g13340	1.0	2.1	**2.0**	3.4	6.8	**2.0**	Protein of unknown function
At5g48850	1.1	0.8	0.7	3.3	7.4	**2.2**	Protein of unknown function (ATSDI1)

575

Table 4. Transcription factors the expression of which was enhanced by more than 3-fold in GO plants, but less than 2-fold in wild-type plants 0.5 h after shifting plants grown at high CO_2 concentration to ambient CO_2 concentration. Genes are listed according to the difference of the expression change between GO and wild-type (WT) plants (FC_{GO}/FC_{WT}). AGI: gene identification number given by the Arabidopsis Genome Initiative. A function was described for a gene when its involvement in a biological process/function was experimentally backed up as described in PubMed (www.ncbi.nlm.nih.gov/pubmed) or TAIR (http://arabidopsis.org/index.jsp). FG: functional group.

	0.5 h after transfer to ambient CO_2						
AGI	**FC_{WT}**	**FC_{GO}**	**FC_{GO}/FC_{WT}**	**Gene family**	**Annotation**	**Function**	**FG**
At5g19790	0.2	26.9	176.5	AP2/EREBP	RAP2.11	Modulates response to low potassium	4
At5g56200	0.1	14.5	169.0	C2H2	C1-4iB-01	Unknown function	5
At5g32460	1.3	123.7	92.9	B3	B3	Unknown function	5
At4g09820	0.8	34.4	45.5	bHLH	TT8	Regulation of proanthocyanidin and anthocyanin biosynthesis; affects dihydroflavonol 4-reductase gene expression.	1
At2g37430	1.9	80.4	43.3	C2H2	ZAT11	Unknown function	5
At1g48150	0.1	3.6	38.9	MADS	AGL74	Unknown function	5
At2g34600	0.4	8.4	24.1	ZIM	JAZ7	Jasmonate signaling; cambium regulator	3
At3g07260	0.8	19.0	22.7	FHA		Unknown function	5
At1g66380	1.9	40.0	21.6	MYB	MYB114	Regulates later steps of anthocyanin biosynthesis	1
At1g27730	1.8	36.3	20.5	C2H2	ZAT10/STZ	Involved in plant defense responses	4
At1g56650	0.6	12.1	20.1	MYB	MYB75	Involved in anthocyanin metabolism; regulates dihydroflavonol reductase expression	1
At5g37415	0.5	8.8	17.6	MADS	AGL105	Unknown function	5
At3g53340	0.4	6.5	17.5	CCAAT-HAP3	NF-YB10	Unknown function	5
At4g00250	0.4	6.3	16.8	GeBP	-	Indirect regulation of cytokinin response genes	2
At5g26930	0.7	9.6	13.5	C2C2(Zn)GATA	GATA-23	Controls lateral root founder cell specification	2
At4g26930	0.4	4.6	13.0	MYB	MYB97	Unknown function	5
At1g48000	1.3	13.8	11.1	MYB	MYB112	Unknown function	5
At5g51190	1.9	18.5	9.9	AP2/EREBP	-	Unknown function	5
At5g43540	0.4	3.2	8.8	C2H2	C1-1iAf-03	Unknown function	5
At3g55980	1.9	15.7	8.4	C3H	SZF1	Regulates salt stress responses	4
At1g74080	0.5	4.0	8.3	MYB	MYB122	Activator of the indole glucosinolate biosynthesis	4
At1g68880	0.6	5.1	8.1	bZIP	bZIP8	Unknown function	5
At4g35900	1.0	7.5	8.0	bZIP	bZIP14/FD-1	Required for regulation of flowering	2
At1g30135	0.8	5.9	7.6	ZIM	JAZ8	Represses jasmonate-regulated growth and defense	3

						responses	
At4g01350	0.6	4.6	7.5	CHP-rich	-	Intracellular signal transduction, oxidation-reduction process, response to chitin	4
At1g43160	1.2	8.8	7.5	AP2/EREBP	RAP2.6	Regulation of development	2
At5g26170	0.8	6.3	7.5	WRKY	WRKY50	Repression of jasmonate-mediated signaling	3
At1g29280	0.8	5.5	7.2	WRKY	WRKY65	Unknown function	5
At1g75540	0.8	5.2	6.8	C2C2(Zn)CO	STH2	Positive regulation of photomorphogenesis	4
At2g33710	1.9	11.4	5.9	AP2/EREBP	ERF112	Unknown function	5
At3g01600	0.6	3.6	5.8	NAC	ANAC044	Unknown function	5
At5g27050	1.4	8.2	5.7	MADS	AGL101	Unknown function	5
At5g01380	0.9	5.3	5.7	Trihelix	-	Unknown function	5
At1g65130	1.2	6.4	5.5	C2H2	C2-1iB-03	Unknown function	5
At5g23260	1.0	5.4	5.4	MADS	AGL32/TT16	Regulates proanthocyanidin biosynthesis	1
At3g11580	0.9	4.6	5.4	ABI3/VP1	AP2/B3-like	Seed development	2
At3g56770	0.8	4.5	5.3	bHLH	-	Unknown function	5
At1g65110	0.6	3.2	5.1	C2H2	C2-1iB-01	Unknown function	5
At2g47190	1.2	6.0	4.9	MYB	MYB2	Inhibits cytokinin-mediated branching at late stages of development	2
At5g52260	1.0	4.7	4.8	MYB	MYB19	Unknown function	5
At5g39610	1.1	5.5	4.8	NAC	ANAC092/ORE1	Regulator of leaf senescence	2
At4g18880	1.6	7.4	4.6	HSF	HsfA4a/HSF21	Unknown function	5
At4g37610	1.1	4.8	4.3	TAZ	BTB5	Unknown function	5
At1g18960	1.3	5.4	4.3	MYB	-	Unknown function	5
At5g02470	0.8	3.2	4.0	E2F/DP	DPA	Endoreduplication control	2
At5g26880	1.0	3.8	3.9	MADS	AGL26	Unknown function	5
At1g68800	0.9	3.5	3.8	TCP	TCP12/BRC2	Prevents axillary bud development and outgrowth	2
At5g07500	1.9	7.0	3.7	C3H	C3H54	Required for heart-stage embryo formation	2
At4g01540	1.3	4.2	3.4	NAC	ANAC068	Mediates cytokinin signaling during cell division	2
At5g51780	1.6	5.4	3.3	bHLH	-	Unknown function	5
At2g42150	1.1	3.5	3.2	BD	-	Unknown function	5
At5g13220	1.1	3.4	3.2	ZIM	JAZ10/TIFY9	Jasmonate signaling repressor	3
At5g22290	1.2	3.8	3.1	NAC	ANAC089	Negative regulator of floral initiation	2
At2g13150	1.0	3.1	3.1	bZIP	bZIP31	Unknown function	5
At1g70700	1.1	3.3	3.0	ZIM	JAZ9	Jasmonate signaling repressor	3
At5g62320	1.2	3.5	3.0	MYB	MYB99	Unknown function	5
At4g39070	1.2	3.5	2.9	C2C2(Zn)CO	DBB2	Unknown function	5
At2g30250	1.6	4.4	2.8	WRKY	WRKY25	Involved in response to various abiotic stresses	4
At5g64810	1.7	4.8	2.8	WRKY	WRKY51	Repression of jasmonate-mediated signaling	3
At3g05800	1.9	5.3	2.7	bHLH	AIF1	Involved in brassinosteroid signaling	4
At3g01970	1.4	3.8	2.6	WRKY	WRKY45	Unknown function	5
At1g75490	1.7	4.4	2.7	AP2/EREBP	DREB2D	Unknown function	5
At1g68840	1.2	3.1	2.5	AP2/EREBP	RAV2/TEM2	Repressor of flowering	2
At1g79180	1.4	3.3	2.5	MYB	MYB63	Activates secondary wall biosynthesis	2

At4g09460	1.7	3.6	2.2	MYB	MYB8	Unknown function	5
At1g66600	1.4	3.0	2.1	WRKY	WRKY63	Involved in the regulation of responses to ABA and drought stress	4
At2g43500	1.5	3.1	2.1	NIN-like	-	Unknown function	5
At4g01520	1.8	3.7	2.0	NAC	ANAC067	Unknown function	5
At1g21000	1.6	3.2	2.0	PLATZ	-	Unknown function	5
At3g27810	1.7	3.4	2.0	MYB	MYB21	Petal and stamen development	2
At5g67300	1.6	3.1	1.9	MYB	MYB44	Regulates ethylene signaling	4
At2g39250	1.7	3.1	1.8	AP2/EREBP	SNZ	Represses flowering	2
At4g16780	1.7	3.1	1.8	HB	HB2/HAT4	Involved in cell expansion and cell proliferation	2
At4g24240	1.8	3.2	1.8	WRKY	WRKY7	Involved in plant defense responses	4
At4g01930	1.8	3.1	1.7	BPC/BRR	-	Unknown function	5
At5g62020	1.8	3.1	1.7	HSF	HsfB2a/HSF6	Unknown function	5
At2g43000	1.9	3.2	1.7	NAC	JUB1/ANAC042	Regulates camalexin biosynthesis and longevity	4
At4g17785	1.9	3.2	1.6	MYB	MYB39	Unknown function	5

585

585 **Table 5.** Transcription factors the expression of which was reduced by more than 3-fold in GO
586 plants 0.5 h after shifting plants grown at high CO_2 concentration to ambient CO_2 concentration.
587 Genes are listed according to the difference of the expression change between wild-type (WT)
588 and GO plants (FC_{WT}/FC_{GO}). A function was described for a gene when its involvement in a
589 biological process/function was experimentally backed up as described in PubMed
590 (www.ncbi.nlm.nih.gov/pubmed) or TAIR (http://arabidopsis.org/index.jsp).
591

	0.5 h after transfer to ambient CO_2					
AGI	FC_{WT}	FC_{GO}	FC_{WT}/FC_{GO}	Gene family	Annotation	Function
At3g02310	47.1	0.12	380.8	MADS	SEP2/AGL4	Flower and ovule development
At3g13850	2.0	0.02	129.5	AS2 (LOB) I	ASL30/LBD22	Unknown function
At4g00260	21.6	0.23	92.1	B3	MEE45	MATERNAL EFFECT EMBRYO ARREST 45
At4g27330	2.4	0.03	78.6	NZZ	NZZ/SPL	Controls stamen identity
At1g54760	11.6	0.31	37.6	MADS	AGL85	Unknown function
At3g60460	4.6	0.26	17.9	MYB	DUO1	Plays essential role in sperm cell specification
At2g45650	3.4	0.20	17.3	MADS	AGL6/RSB1	Involved in axillary bud formation; control of flowering time and lateral organ development
At5g26950	2.0	0.17	12.2	MADS	AGL93	Unknown function
At3g15170	1.9	0.16	11.9	NAC	ANAC054/CUC1	Shoot apical meristem formation and auxin-mediated lateral root formation; formation of organ boundary
At5g58280	0.8	0.15	5.3	B3	-	Unknown function
At5g15800	1.0	0.21	5.0	MADS	SEP1/AGL2	Involved in flower and ovule development
At3g56660	1.3	0.26	5.0	bZIP	bZIP49	Unknown function
At5g23000	0.6	0.18	3.3	MYB	MYB37/RAX1	Regulates axillary meristem formation; earliest spatial marker for future axillary meristems

592

Figure 1.JPEG

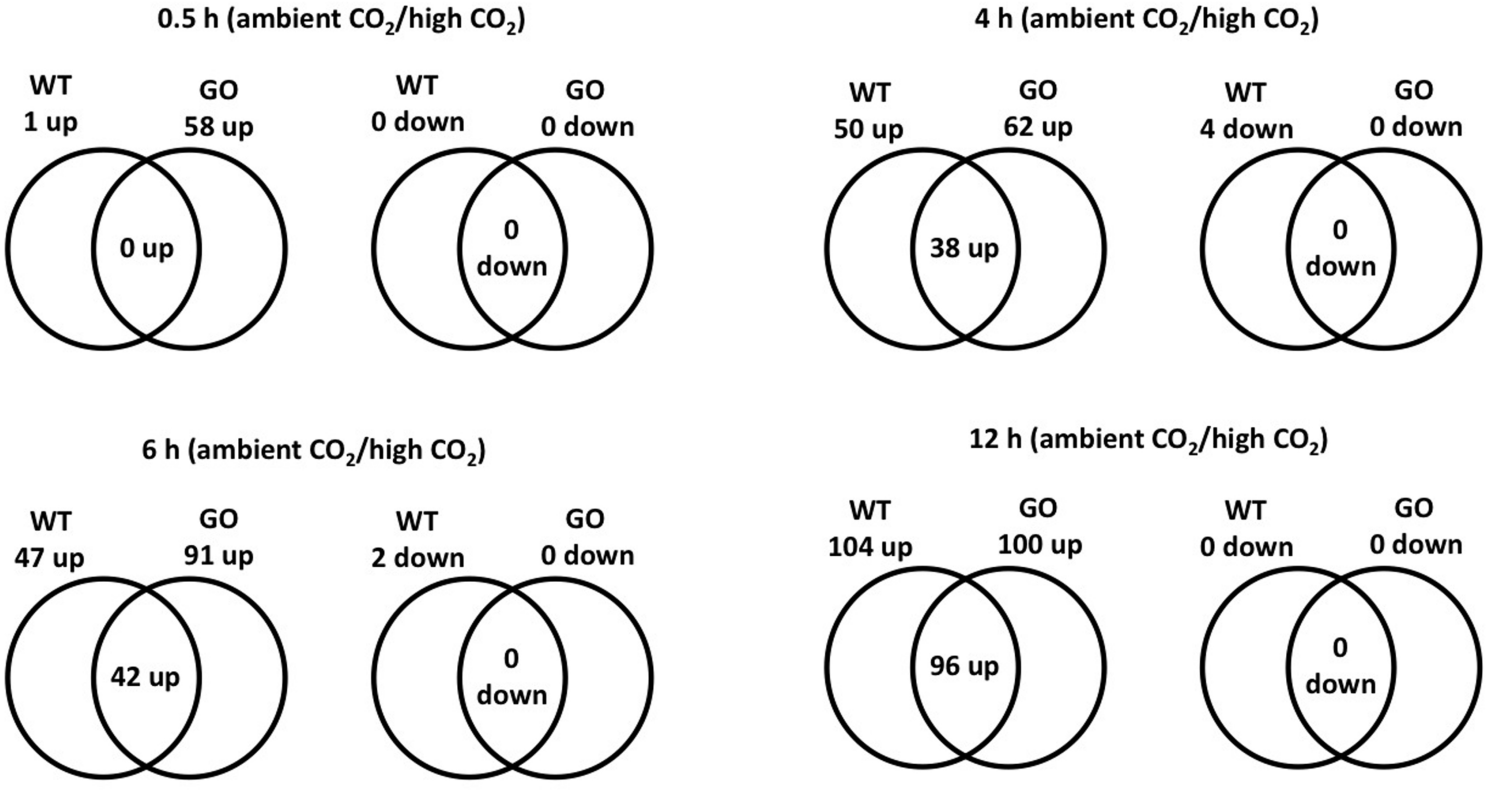

Figure 2.JPEG