

# Chapter 2

## Measurement of Transcripts Associated with Photorespiration and Related Redox Signaling

Amna Mhamdi, Pavel I. Kerchev, Patrick Willems, Graham Noctor, and Frank Van Breusegem

### Abstract

To study photorespiration and to characterize related components, gene expression analysis is a central approach. An overview of the experimental setup, protocols, and methods we use to investigate photorespiration-associated gene expression is presented. Within this chapter, we describe simple procedures to experimentally alter the photorespiratory flux and provide protocols for transcriptomic analysis with a focus on genes encoding photorespiratory proteins as well as those induced by photorespiratory hydrogen peroxide ( $H_2O_2$ ). Examples of typical results are presented and their significance to understanding redox signaling is discussed.

**Key words** Photorespiration,  $CO_2$ , Catalase,  $H_2O_2$ , Redox signaling, Transcriptomics

---

### 1 Introduction

Photorespiratory carbon and nitrogen recycling is a complex metabolic route that involves several intracellular compartments, impacting metabolite pools and redox/energy status at these locations [1, 2]. Classical mutagenesis screens starting in the 1970s established that the photorespiratory recycling pathway and related processes involve specific genes [3, 4]. These genes are characterized by (1) high expression in photosynthetic tissues and (2) a “photosynthetic”-type diurnal rhythm, in which transcripts peak at the end of the night period.

As the isolation of gene-specific mutants has shown, loss of function of many of the proteins involved in photorespiration leads to a “photorespiratory phenotype” characterized by a conditional inability to grow well under conditions favoring substantial rates of photorespiration. Examples of such gene-specific isoforms are GLUTAMINE SYNTHETASE2 (GS2), SERINE HYDROXY-METHYLTRANSFERASE1 (SHM1), and CATALASE2 (CAT2)

[5–7]. Genetic redundancy probably explains why no photorespiratory mutants for certain steps were identified using classical screens. For example, it now seems that the *GLYCOLATE OXIDASE1* (*GOX1*) and *GOX2* both have roles in converting glycolate to glyoxylate with the concomitant production of hydrogen peroxide ( $H_2O_2$ ) [8].

Genes involved in essential photorespiratory reactions in *Arabidopsis thaliana* are summarized in Table 1. Most of them are highly and relatively constitutively expressed [1, 2], especially compared to the marked fluctuations that one can observe in photorespiratory metabolites both in the short and long term. This is probably because (1) photorespiratory flux is mainly controlled by the initiating reaction catalyzed by ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) and (2) this reaction can occur at very high rates compared to most other plant metabolic pathways, and thus proteins must be in place to transform and transport the metabolites that are produced. Despite this view of photorespiration as a “housekeeping” pathway, evidence continues to emerge suggesting that at least some elements associated with photorespiration play a role in tuning plant responses to the environment [1, 10]. One notable aspect here is the fast  $H_2O_2$  production occurring in photorespiration [11] which can potentially contribute to processes such as plant responses to pathogens [12, 13].

**Table 1**  
**List of photorespiration-related genes in *Arabidopsis* and their expression levels in air and high  $CO_2$**

Localization	Enzyme name	ID	Transcript level		
			$hCO_2$	2d air	4d air
Chloroplast	Phosphoglycolate phosphatase				
	(PGLP1)	At5g36700	4393	4356	4657
Peroxisome	Glycolate oxidase				
	GOX1/GOX2	At3g14420/ At3g14415	6321	6943	7150*
Peroxisome	Catalase				
	CAT2	At4g35090	2721	2762	2898
Peroxisome	Glutamate:glyoxylate aminotransferase				
	GGT1	At1g23310	5989	4617*	5453
Peroxisome	Serine:glyoxylate aminotransferase				
	SGAT1	At2g13360	7703	11105*	9972*

(continued)

**Table 1**  
**(continued)**

Localization	Enzyme name	ID	Transcript level		
			hCO <sub>2</sub>	2d air	4d air
Mitochondria	Glycine decarboxylase				
	GDCH1	At2g35370	6090	7933*	7273
	GDCH2	At2g35120	308	279	266
	GLDP1	At4g33010	8242	7610	8983
	GLDP2	At2g26080	1859	1532*	1776
	GDCT	At1g11860	5944	6051	6414
Mitochondria	Serine hydroxymethyl transferase				
	SHM1	At4g37930	9552	7150*	9386
Peroxisome	Hydroxypyruvate reductase				
	HPR1	At1g68010	6002	5689	6566
	HPR2	At1g79870	953	956	966
Chloroplast	Glycerate kinase				
	GLYK	At1g80380	1582	1632	1828
Chloroplast	Glutamine synthetase 2				
	GS2	At5g35630	9351	8773	9800
Chloroplast	Glutamate synthase (GOGAT)				
	GLU1	At5g04140	3204	2022*	3007
Chloroplast	Chloroplast envelope transporters				
	DIT1	At5g12860	5679	5653	5842
	DiT2.1	At5g64290	1659	1736	1773
	DiT2.2	At5g64280	475	611*	573*

Transcript levels in high CO<sub>2</sub> and air are taken from [9]. Data are means of three biological replicates obtained by ATH1 microarray analysis of plants growing at 3000  $\mu$ L/L CO<sub>2</sub> in short days (high CO<sub>2</sub>) for 5 weeks before transfer to 400  $\mu$ L/L CO<sub>2</sub> at the same day length for 2 or 4 days (2d air, 4d air).

\*Indicates significant difference at  $P < 0.05$  (Student's  $t$  test). Note that *GOX1* and *GOX2* are detected by the same probe set

In this chapter, we describe basic procedures to quantify transcripts in plant tissues by using transcriptomic and targeted methods, first with reference to the photorespiratory pathway in general and, second, with a specific focus on transcripts that can be used to assess stress responses induced by photorespiratory H<sub>2</sub>O<sub>2</sub>. We also describe protocols that can be applied to alter the photorespiration, including a simple and convenient protocol to promote photorespiratory flux in order to assess the response of plants to this condition.

---

## 2 Materials

### 2.1 Plant Material

1. The *Arabidopsis thaliana* (L.) Heynh. (accession Columbia-0 [Col-0]) seeds.
2. *Catalase2* (*cat2*) mutant seeds.

### 2.2 Growth Media and Solutions

1. Murashige and Skoog (MS) plant growth medium: For 1 L, weigh 4.3 g MS basal salt mixture, 0.5 g 2-(*N*-morpholino) ethanesulfonic acid (MES), 0.1 g *myo*-inositol, and 10 g sucrose. Add approximately 900 mL deionized water and adjust pH to 5.8 with 1 M KOH. Top up with water to 1 L and add 8 g tissue culture-grade agar. Autoclave at 120 °C, for 15 min. After cooling to 60 °C, pour 100 mL medium in each Petri dish.
2. Carbon dioxide gas: CO<sub>2</sub> (UN1013).
3. TRIzol reagent.
4. Chloroform.
5. Isopropanol.
6. 75% (v/v) ethanol.
7. Diethylpyrocarbonate (DEPC) water.
8. DNase.

### 2.3 Equipment

1. Plant growth chamber with CO<sub>2</sub> control (e.g., Vötsch or Snijders).
2. Surgical tape (3 M™ Micropore™).
3. Parafilm M®.
4. RNase-free tubes and filter tips.
5. cDNA synthesis kit (such as SuperScript II reverse transcriptase from ThermoFisher Scientific).
6. Petri dishes (diameter 15 cm).
7. Potting mixture (such as Jiffy).
8. Spectrophotometer (Nanodrop or equivalent).

---

## 3 Methods

Here we describe approaches that we have used in *Arabidopsis* to manipulate photorespiration and to quantify transcripts in these conditions. The methods can be adapted to other plants, although the small stature of *Arabidopsis* makes it particularly well suited to some of the procedures.

### **3.1 Experimental Settings to Conditionally Increase the Metabolic Flux Through the Photorespiratory Pathway**

The rate of photorespiratory flux is mainly controlled by the rate of RuBisCO oxygenation. Two factors that can be easily manipulated to vary this rate are (1) the CO<sub>2</sub> concentration surrounding leaves and (2) irradiance. Many studies of photorespiratory mutants have compared responses in air and high CO<sub>2</sub> at typical irradiance found in laboratory growth rooms (100–200 μmol/m<sup>2</sup>/s). In such studies, CO<sub>2</sub> levels of 3000 μL/L (7.5-fold current air levels) are sufficient to decrease photorespiration to negligible levels [7]. Other studies seek to supra-stimulate photorespiration. This can be achieved by increasing irradiance or by decreasing CO<sub>2</sub> availability to below typical air levels [14, 15].

#### **3.1.1 In Vitro Photorespiration-Promoting Conditions**

The procedure below can be used to promote the rate of photorespiration by placing plants in conditions that promote exhaustion of CO<sub>2</sub> and generation of O<sub>2</sub>, processes that can be reinforced by the use of continuous illumination. This protocol has been used to assess the importance of mutations or chemical treatments in wild-type and catalase-deficient Arabidopsis [8, 15].

1. Sow surface-sterilized seeds in Petri dishes containing 100 mL solid MS medium.
2. Evenly space 16 Col-0 seeds in one part of the dish and 16 *cat2* seeds in the other part.
3. Seal the plates with one-layer surgical tape.
4. Place the plates for 3–4 days at 4 °C in the dark to facilitate even germination.
5. Transfer the plates to a controlled growth environment (16-h/8-h light/dark, light intensity, approximately 50 μmol/m<sup>2</sup>/s, 21 °C).
6. After 3 weeks, remove the surgical tape and seal the plates with several layers of Parafilm M<sup>®</sup> to prevent the influx of fresh air.
7. Transfer the plates to a controlled growth environment with continuous light (light intensity approximately 50 μmol/m<sup>2</sup>/s, 21 °C) (*see Note 1*).

#### **3.1.2 Transfer from CO<sub>2</sub>-Enriched Atmosphere to Ambient Air at Moderate or High Irradiance**

1. Germinate cold-treated seeds on a potting mixture in a growth chamber with CO<sub>2</sub> control.
2. Maintain CO<sub>2</sub>-enriched atmosphere and controlled environmental conditions (*see Note 2*).
3. After 3–5 weeks, stop the CO<sub>2</sub> supply and allow the CO<sub>2</sub> levels to equalize between the growth chamber and the ambient air.
4. To compare the effect of CO<sub>2</sub> only, maintain all other conditions identical to those at high CO<sub>2</sub>.

5. To drive photorespiration at particularly high rates, increase the irradiance simultaneously with the transfer of the plants to air, if necessary by transfer to another growth chamber with a higher light intensity (*see Note 3*).

### 3.2 Analyses of Photorespiration-Related Transcripts

Besides genes encoding RuBisCO subunits, there are about 20 genes involved in the core photorespiratory pathway (Table 1). Expression levels of these genes can be monitored during transcriptomic analyses with tools, such as the Affymetrix ATH1 microarray or, more frequently now, RNA sequencing. For more targeted specific analyses, quantitative polymerase chain reaction (qPCR) is more convenient. If desired, both can be performed on the same RNA preparation.

In addition to transcripts directly involved in photorespiration, analysis of H<sub>2</sub>O<sub>2</sub>-sensitive marker genes has been widely used to probe oxidative stress and related signaling processes in model plants deficient in catalase. The photorespiratory *Arabidopsis cat2* mutant allows cellular redox state and gene expression to be manipulated in an easy and predictable manner [16]. Its use has significantly furthered our understanding of interactions in oxidative stress and allowed H<sub>2</sub>O<sub>2</sub>-sensitive genes to be defined [17–19].

#### 3.2.1 RNA Extraction

For transcriptomic or more targeted studies, use at least three biological replicates.

1. Harvest 50–100 mg leaf material in RNase-free tube and grind tissue under liquid nitrogen to a fine powder.
2. Add 1 mL TRIzol reagent and vortex to dissolve and homogenize the sample.
3. Incubate for 5 min at room temperature.
4. Add 200  $\mu$ L chloroform, shake by hand for 15 s, and incubate at room temperature for 15 min.
5. Centrifuge for 15 min at  $11,000 \times g$  at 4 °C.
6. Precipitate RNA by adding 500  $\mu$ L of isopropanol to the aqueous phase in a fresh RNase-free tube.
7. Incubate at room temperature for 10 min.
8. Centrifuge for 10 min at  $11,000 \times g$  at 4 °C.
9. Wash the RNA pellet with 1 mL of 75% (v/v) ethanol.
10. Centrifuge for 5 min at room temperature.
11. Dry the pellet for 10 min at room temperature.
12. Redissolve the RNA in 40  $\mu$ L DEPC water and incubate at 65 °C for 10 min.
13. Leave the sample on ice overnight (*see Note 4*).
14. Centrifuge the sample for 10 min at  $11,000 \times g$  and 4 °C and transfer the supernatant to a new RNase-free tube.

### 3.2.2 Transcriptomic Analysis

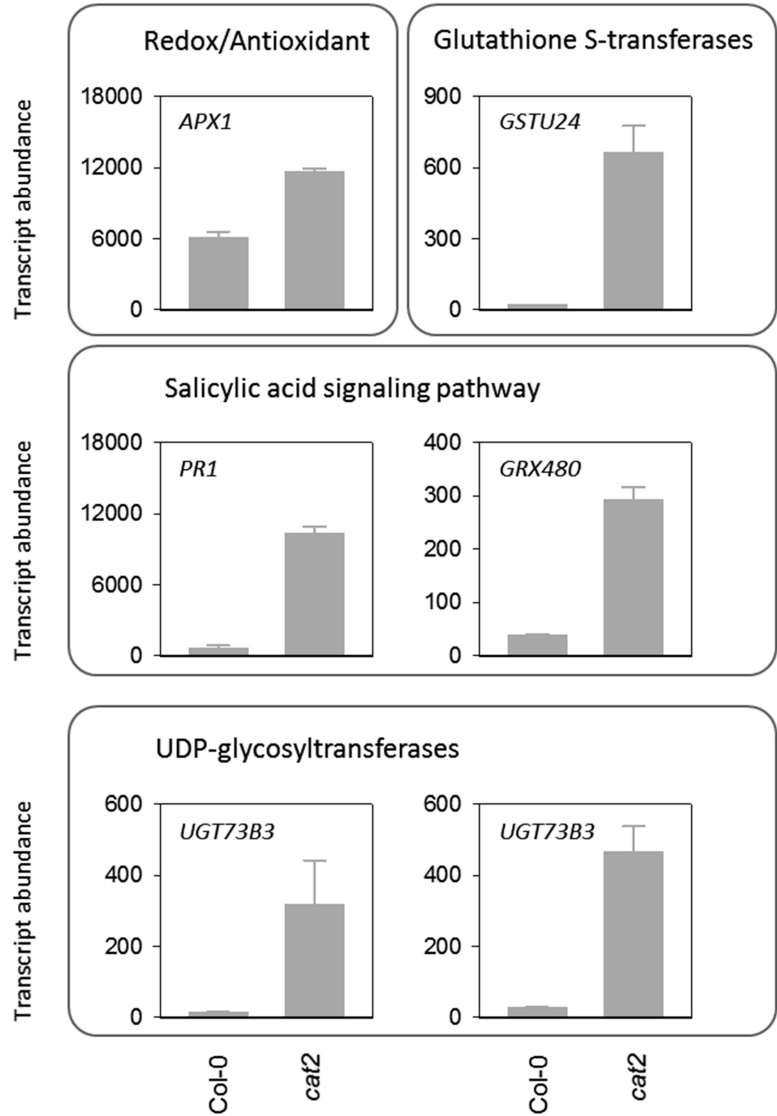
Transcriptomic analysis is typically performed by specialized platforms. Until recently, hybridization techniques that cover a large part of the transcriptome, such as microarray chips, have been used most widely. Data processing of Affymetrix microarrays is relatively standardized, with the preferred method being Robust Multi-chip Average for normalization [20, 21] followed by differential expression analysis with R-packages such as limma [22].

Presently, high-throughput RNA sequencing (RNA-seq) is the method of choice. Compared to microarrays, RNA-Seq is more efficient for detecting low-abundance transcripts, separating different splice forms, identifying genetic variants, and offering a broad dynamic range. However, a good experimental design is a crucial prerequisite for an informative RNA-seq analysis and dependent on the aim of the study. In previous RNA-seq studies of photorespiration in *Arabidopsis*, we sequenced 20–30 million 75-bp single-end reads per sample with three replicates per condition [8]. Compared to microarray data processing, RNA-seq analysis requires more bioinformatics expertise and resources. A simplified workflow to determine differential gene expression by RNA-seq is described below.

1. Obtain raw reads and perform quality controls, using tools such as FastQC (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) and NGSQC [23].
2. Trim adaptor sequences and remove low-quality reads or bases using tools as Trimmomatic [24].
3. Align preprocessed reads to a genome using spliced aligners such as TopHat [25] or STAR [26].
4. Quantify on gene level using tools such as htseq-count [27] or featureCounts [28].
5. Perform differential expression analysis R-packages such as EdgeR [29] or DESeq2 [30].
6. Define differentially expressed genes (DEGs) by a fold change and significance threshold.
7. Inspect lists of DEGs manually or by bioinformatics analyses, such as gene set enrichment analysis and motif discovery.

Analyses using the ATH1 microarray chip revealed that most of the photorespiratory transcripts are not very sensitive to transfer from high CO<sub>2</sub> to air, at least within the 4-day timescale of our experiment (Table 1). Nevertheless, enzyme and protein activities may respond to altered photorespiratory rates at other levels. For example, extractable activities of catalase increase about twofold within 4 days after transfer to air, an effect that is dependent on expression of the photorespiratory CAT2 isoform [7].

Figure 1 shows an example of microarray data of selected marker genes for which the expression is driven by H<sub>2</sub>O<sub>2</sub> in the



**Fig. 1** Example of  $H_2O_2$ -inducible genes. Transcript abundance in Col-0 and *cat2* plants grown from seeds for 3 weeks under long-day air conditions. Data are means  $\pm$  SE of three biological replicates extracted from microarray data. Gene-specific primers for qPCR analyses are given in Table 2

*cat2* mutant. These include genes that respond rapidly (within hours) to transfer from nonphotorespiratory conditions to air (specific glutathione *S*-transferases (GSTs) and UDP-glucuronosyl-transferases (UGTs)) as well as ASCORBATE PEROXIDASE1 APX1, encoding a cytosolic ascorbate peroxidase that is important in oxidative stress responses [19, 31]. Under some circumstances, catalase-deficient plants growing in photorespiratory conditions



strongly activate pathogenesis-related (PR) responses, including marked accumulation of salicylic acid and induction of related genes [12]. *PR1* expression is an excellent marker of this response; although this gene is induced much more slowly by photorespiratory  $\text{H}_2\text{O}_2$  than the others shown in Fig. 1, typically several days are required in *cat2* growing at moderate irradiance. While catalase-deficient plants are useful systems to dissect the role of oxidative stress in plant responses to the environment, the precise significance of photorespiration in plant responses to pathogens in catalase-depleted plants is yet to be established. Interestingly, many of the genes shown in Fig. 1 can also be significantly induced by growing wild-type plants at high  $\text{CO}_2$ , where photorespiration is repressed [32].

### 3.2.3 qPCR Analysis of Transcripts That Are Strongly Induced by Photorespiratory $\text{H}_2\text{O}_2$

The *cat2* photorespiratory mutant is one of several excellent model systems that have been used to identify reliable markers for oxidative stress [14, 33, 34]. This is crucial given the difficulties surrounding direct measurement of reactive oxygen species (ROS; [35]). Quantification of ROS-responsive genes is an essential part of the armory available to evaluate oxidative stress responses. With this objective, at least two of the genes shown in Fig. 1 can be analyzed by targeted qPCR using the primer sequences given in Table 2, according to the following protocol.

1. Grow *Arabidopsis* Col-0, *cat2*, and related lines in parallel in conditions that drive photorespiration. In our hands, growth of plants in air from seeds in short-day or long-day conditions at a light intensity of  $200 \mu\text{mol}/\text{m}^2/\text{s}$  is sufficient to activate  $\text{H}_2\text{O}_2$  signaling in *cat2* without stressing the wild type (*see* **Note 5**).
2. Harvest 50–100 mg of leaf material rapidly by plunging it into liquid nitrogen and store at  $-80^\circ\text{C}$  for a maximum of 6 months. Use three to four biological replicates (three or four independent extractions using different samples).
3. Extract RNA as above (*see* Subheading 3.2.1).
4. Measure the RNA concentration with a spectrophotometer (*see* **Note 6**). Reads at optical density (OD) 260 and 280 are required.
5. Remove DNA contamination by treating 1  $\mu\text{g}$  of the RNA sample with DNase.
6. Prepare cDNA from DNase-treated samples using one the cDNA synthesis kits commercially available and according to the manufacturer's instructions.
7. Perform the qPCR to quantify  $\text{H}_2\text{O}_2$ -inducible transcripts using two or more of the gene-specific primer pairs (Table 2) (*see* **Note 7**).

**Table 2**

**Primer sequences for qPCR analyses of transcripts for enzymes involved in photorespiratory  $H_2O_2$  metabolism and genes induced by increased photorespiratory  $H_2O_2$**

Transcript	Transcript ID	Oligonucleotide name <sup>a</sup>	Sequence
<i>Photorespiratory enzymes</i>			
CATALASE			
CAT2	At4g35090	qCAT2for	5'-TGCTGGAAACTACCCTGAATGG-3'
		qCAT2rev	5'-TCAACACCATACGTCCAACAGG-3'
GLYCOLATE OXIDASE			
GOX1	At3g14420	qGO1for	5'-TCTCATTGGCAGCTGAAGGA-3'
		qGO1rev	5'-GAGTGTCCCATTTCGGTGGTA-3'
GOX2	At3g14415	qGO2for	5'-TTTGCACTAGCTGCTGAAGGA-3'
		qGO2rev	5'-ATAACCTGGGCAAATGGCGT-3'
<i>Photorespiratory H<sub>2</sub>O<sub>2</sub>-inducible genes</i>			
ASCORBATE PEROXIDASE 1			
APX1	At1g07890	qAPX1for	5'-GCACTATTGGACGACCCTGT-3'
		qAPX1rev	5'-GCAAACCCAAGCTCAGAAAG-3'
PATHOGENESIS-RELATED 1			
PR1	At2g14610	qPR1for	5'-AGGCTAACTACAACCTACGCTGCG-3'
		qPR1rev	5'-GCTTCTCGTTCACATAATTCCCAC-3'
GLUTAREDOXIN CC-TYPE			
GRX480	At1g28480	qGRX480for	5'-GCGGTCCTTGAGATTGATG-3'
		qGRX480rev	5'-AAACCGCCGGTAACTTCAC-3'
GLUTATHIONE S-TRANSFERASE U 24			
GSTU24	At1g17170	qGSTU24for	5'-GGCGAGTATGTTTGGGATG-3'
		qGSTU24rev	5'-TTCATCTCGAGGAGCAAGG-3'
UDP-GLYCOSYL TRANSFERASE 74E2			
UGT74E2	At1g05680	qUGT74E2for	5'-GAATCGTCCTCATACCCGAAT-3'
		qUGT74E2rev	5'-GCTTTGGACCCATTTCAACA-3'
UGT73B3	At4g34131	qUGT73B3for	5'-CCTCACCACACCTCTCAACTC-3'
		qUGT73B3rev	5'-TCTGGTAACCCGAGATCCAC-3'

<sup>a</sup>for forward, rev reverse

8. Run the amplification in duplicate or triplicate technical repeats.
9. To analyze the data, produce a single mean value for each extract by averaging the technical repeats, if they show acceptable levels of reproducibility. From the single values generated for each extract, obtain the final means ( $n = 3$  or  $4$ ) for each genotype or condition and compare the statistical significance using  $P < 0.05$  (see **Note 8**).

---

## 4 Notes

1. To ensure the efficiency of the RGCL assay, inspect the integrity of the parafilm every day and, if cracked, add new layers.
2. For *Arabidopsis* plants, 3000  $\mu\text{L/L}$   $\text{CO}_2$  is sufficient to largely abolish photorespiration. Growth conditions could be adjusted to day length of choice, light intensity of approximately 120–200  $\mu\text{mol/m}^2/\text{s}$ , and 65% relative humidity.
3. Note that photorespiration saturates with similar kinetics to photosynthesis, so that an irradiance of 600  $\mu\text{mol/m}^2/\text{s}$  may be sufficient to achieve maximum rates of photorespiration in *Arabidopsis*.
4. Samples should be incubated for at least for 2 h.
5. As detailed in Subheading 3.1, other strategies can be used to promote photorespiration.
6. RNA concentrations could be measured with 1–2  $\mu\text{L}$  of the sample, in case a Nanodrop is used, but for other spectrophotometers, larger amounts might be needed.
7. We use *ACTIN2* as a reference gene. If necessary, a second reference gene can be used (such as *PYROPHOSPHATASE2* or *UBIQUITIN*), but in our conditions, *ACTIN2* transcript signals do not show significant variability and similar results are obtained with different reference genes.
8. qPCR is often better able to detect differences but effects should be qualitatively similar to those observed in microarray analyses.

---

## Acknowledgments

We thank Martine De Cock for help in preparing the revised version of the manuscript. This work was supported by the Agence Nationale de la Recherche project CYNTHIOL (no. ANR12-BSV6-0011) to G.N. and by grants from Ghent University Multidisciplinary Research Partnership “Sustainable BioEconomy”

(project 01MRB510W), the Interuniversity Attraction Poles Program (IUAP P7/29), and the Research Foundation-Flanders (grant no. G0D7914N) to F.V.B., and also by the Scientific Exchange program Flanders-France (grant Tournesol T.2008.21) to G.N. and F.V.B.P.K. is a recipient of Omics@VIB Marie Curie COFUND fellowship.

## References

1. Foyer CH, Bloom AJ, Queval G et al (2009) Photorespiratory metabolism: genes, mutants, energetics, and redox signaling. *Annu Rev Plant Biol* 60:455–484
2. Obata T, Florian A, Timm S, Bauwe H, Fernie AR (2016) On the metabolic interactions of (photo)respiration. *J Exp Bot* 67:3003–3014
3. Somerville CR (1986) Analysis of photosynthesis with mutants of higher plants and algae. *Annu Rev Plant Physiol* 37:467–507
4. Leegood RC, Lea PJ, Adcock MD et al (1995) The regulation and control of photorespiration. *J Exp Bot* 46:1397–1414
5. Blackwell RD, Murray AJS, Lea PJ (1987) Inhibition of photosynthesis in barley with decreased levels of chloroplastic glutamine synthetase. *J Exp Bot* 38:1799–1809
6. Engel N, Ewald R, Gupta KJ, Zrenner R, Hagemann M, Bauwe H (2011) The presequence of Arabidopsis serine hydroxymethyltransferase SHM2 selectively prevents import into mesophyll mitochondria. *Plant Physiol* 157:1711–1720
7. Queval G, Issakidis-Bourguet E, Hoeberichts FA et al (2007) Conditional oxidative stress responses in the Arabidopsis photorespiratory mutant *cat2* demonstrate that redox state is a key modulator of daylength-dependent gene expression and define photoperiod as a crucial factor in the regulation of H<sub>2</sub>O<sub>2</sub>-induced cell death. *Plant J* 52:640–657
8. Kerchev P, Waszczak C, Lewandowska A et al (2016) Lack of GLYCOLATE OXIDASE1, but not GLYCOLATE OXIDASE2, attenuates the photorespiratory phenotype of CATALASE2-deficient Arabidopsis. *Plant Physiol* 171:1704–1719
9. Queval G, Neukermans J, Vanderauwera S et al (2012) Day length is a key regulator of transcriptomic responses to both CO<sub>2</sub> and H<sub>2</sub>O<sub>2</sub> in *Arabidopsis*. *Plant Cell Environ* 35:374–387
10. Kangasjärvi S, Neukermans J, Li S et al (2012) Photosynthesis, photorespiration, and light signalling in defence responses. *J Exp Bot* 63:1619–1636
11. Noctor G, Veljovic-Jovanovic S, Driscoll S et al (2002) Drought and oxidative load in the leaves of C<sub>3</sub> plants: a predominant role for photorespiration? *Ann Bot* 89:841–850
12. Chaouch S, Queval G, Vanderauwera S et al (2010) Peroxisomal hydrogen peroxide is coupled to biotic defense responses by ISOCHORISMATE SYNTHASE1 in a daylength-related manner. *Plant Physiol* 153:1692–1705
13. Rojas CM, Senthil-Kumar M, Wang K et al (2012) Glycolate oxidase modulates reactive oxygen species-mediated signal transduction during nonhost resistance in *Nicotiana benthamiana* and *Arabidopsis*. *Plant Cell* 24:336–352
14. Vanderauwera S, Zimmermann P, Rombauts S et al (2005) Genome-wide analysis of hydrogen peroxide-regulated gene expression in Arabidopsis reveals a high light-induced transcriptional cluster involved in anthocyanin biosynthesis. *Plant Physiol* 139:806–821
15. Kerchev P, Mühlenbock P, Denecker J et al (2015) Activation of auxin signalling counteracts photorespiratory H<sub>2</sub>O<sub>2</sub>-dependent cell death. *Plant Cell Environ* 38:253–265
16. Mhamdi A, Queval G, Chaouch S et al (2010) Catalase function in plants: a focus on *Arabidopsis* mutants as stress-mimic models. *J Exp Bot* 61:4197–4220
17. Mhamdi A, Hager J, Chaouch S et al (2010) Arabidopsis GLUTATHIONE REDUCTASE1 plays a crucial role in leaf responses to intracellular hydrogen peroxide and in ensuring appropriate gene expression through both salicylic acid and jasmonic acid signaling pathways. *Plant Physiol* 153:1144–1160
18. Tognetti VB, Van Aken O, Morreel K et al (2010) Perturbation of indole-3-butyric acid homeostasis by the UDP-glucosyltransferase *UGT74E2* modulates *Arabidopsis* architecture and water stress tolerance. *Plant Cell* 22:2660–2679
19. Vanderauwera S, Suzuki N, Miller G et al (2011) Extranuclear protection of

- chromosomal DNA from oxidative stress. *Proc Natl Acad Sci U S A* 108:1711–1716
20. Bolstad BM, Irizarry RA, Åstrand M et al (2003) A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. *Bioinformatics* 19:185–193
  21. Irizarry RA, Hobbs B, Collin F et al (2003) Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* 4:249–264
  22. Smyth GK (2004) Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. *Stat Appl Genet Mol Biol* 3:Article 3
  23. Dai M, Thompson RC, Maher C et al (2010) NGSQC: cross-platform quality analysis pipeline for deep sequencing data. *BMC Genomics* 11(Suppl 4):S7
  24. Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30:2114–2120
  25. Trapnell C, Pachter L, Salzberg SL (2009) TopHat: discovering splice junctions with RNA-Seq. *Bioinformatics* 25:1105–1111
  26. Dobin A, Davis CA, Schlesinger F et al (2013) STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* 29:15–21
  27. Anders S, Pyl PT, Huber W (2015) HTSeq—a Python framework to work with high-throughput sequencing data. *Bioinformatics* 31:166–169
  28. Liao Y, Smyth GK, Shi W (2014) featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* 30:923–930
  29. Robinson MD, McCarthy DJ, Smyth GK (2010) edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* 26:139–140
  30. Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 15:550
  31. Davletova S, Rizhsky L, Liang H et al (2005) Cytosolic ascorbate peroxidase 1 is a central component of the reactive oxygen gene network of Arabidopsis. *Plant Cell* 17:268–281
  32. Mhamdi A, Noctor G (2016) High CO<sub>2</sub> primes plant biotic stress defences through redox-linked pathways. *Plant Physiol* 172:929–942
  33. Gadjev I, Vanderauwera S, Gechev TS et al (2006) Transcriptomic footprints disclose specificity of reactive oxygen species signaling in Arabidopsis. *Plant Physiol* 141:436–445
  34. Willems P, Mhamdi A, Stael S et al (2016) The ROS wheel: refining ROS transcriptional footprints. *Plant Physiol* 171:1720–1733
  35. Noctor G, Mhamdi A, Foyer CH (2016) Oxidative stress and antioxidative systems: recipes for successful data collection and interpretation. *Plant Cell Environ* 39:1140–1160



Photorespiration

Methods and Protocols

Fernie, A.; Bauwe, H.; Weber, A. (Eds.)

2017, XIV, 274 p. 40 illus., 25 illus. in color., Hardcover

ISBN: 978-1-4939-7224-1

A product of Humana Press