Oxidative Stress Components Explored in Anoxic and Hypoxic Global Gene Expression Data

Olga B. Blokhina, Petri Törönen, and Kurt V. Fagerstedt

Abstract Global gene expression data were analyzed to search for the genes related to oxidative stress response, to examine the differences between hypoxia and anoxia, and to reveal new components of oxygen deprivation response escaped from the previous analyses. Gene Set Z-score (GSZ) was used to report gene ontology (GO) classes that showed significant regulation and also partial up- and downregulation in Arabidopsis anoxic and hypoxic microarray data sets. Under both anoxia and hypoxia significant upregulation was reported for anaerobic respiration, response to low oxygen levels, and response to hypoxia. Comparable high GSZ scores were shown for several oxidative stress-related GO classes and for functional groups of biological processes known to involve oxygen radical formation such as: cellular respiration, wounding, and response to high light and UV-B. Availability of oxygen in hypoxic experimental sets was marked by upregulation of several oxygenases, including ACC-oxidase responsible for ethylene synthesis. Consistent strong induction of several Fe-dependent ketoglutarate oxygenases (FeKGO) in the majority of hypoxic conditions analyzed suggests an important and yet unidentified function for these enzymes. Based on metabolic and gene expression studies we suggest that FeKGO may function in a bypass route for part of the TCA cycle (citrate-isocitrate) inhibited under hypoxia. This would incorporate 2-ketoglutarate supplied by activated GABA shunt and form succinate, a TCA cycle and mitochondrial electron transport chain substrate. FeKGO turnover is sustained by the putative route coupled to ascorbate–monodehydroascorbate cycling and hemoglobin-dependent NO elimination. The analysis strongly supports earlier findings that formation of activated oxygen and oxidative stress is an integral part of the response to oxygen deprivation. Several novel functional gene groups

O.B. Blokhina (✉) • K.V. Fagerstedt
Department of Biosciences, Division of Plant Biology, University of Helsinki, Helsinki, Finland
e-mail: olga.blokhina@helsinki.fi

P. Törönen
Institute of Biotechnology, University of Helsinki, Helsinki, Finland

J.T. van Dongen and F. Licausi (eds.), *Low-Oxygen Stress in Plants*, Plant Cell Monographs 21, DOI 10.1007/978-3-7091-1254-0_2, © Springer-Verlag Wien 2014

were highlighted by the analysis: upregulation of cysteamine dioxygenase activity and FeKGO and downregulation of circadian rhythm-related genes.

1 Introduction

During the past 2 decades reactive oxygen species (ROS) have developed in our minds from damaging stress molecules to stress-signaling agents important for the development of stress responses in practically all stresses that plants have to endure (Bailey-Serres and Chang 2005; Van Breusegem et al. 2008; Jaspers and Kangasjärvi 2010; Petrov and Van Breusegem 2012). The presence of ROS under oxygen deprivation stress is somewhat a paradox and indeed some oxygen is needed for ROS production under oxygen deprivation stress, and hence it is preferable to talk about severe hypoxia in most cases, and of a reoxygenation period when atmospheric oxygen conditions are again introduced (Branco-Price et al. 2008). The biochemistry behind ROS production and antioxidative defense as well as the damage produced under oxygen deprivation is well documented (Yan et al. 1996; Biemelt et al. 1998, 2000; Blokhina et al. 2000, 2001, 2003; Fukao and Bailey-Serres 2004; Santosa et al. 2007). The evidence for the regulatory role of ROS under oxygen deprivation in the control of gene expression (Pucciariello et al. 2012), the negative feedback regulation of H_2O_2 levels by Rop–RopGAP4 interaction (Baxter-Burrell et al. 2002), and the activation of MAPK kinases in response to mitochondrial ROS resulting in better survival under hypoxia (Chang et al. 2012), all point to a complex relationship between hypoxic metabolic rearrangements, ROS levels, and their cellular localization and affect the physiological outcome of oxidative stress. Naturally, the most important processes from the plant's point of view are the adaptations preserving the adenylate energy charge (Greenway and Gibbs 2003; Bailey-Serres and Voesenek 2008; Lee et al. 2011). During the past few years multiple routes have been elucidated for the production and consumption of ATP such as the pyrophosphate-dependent glycolysis (Huang et al. 2008), nitrate-dependent ATP synthesis under oxygen deprivation (Stoimenova et al. 2007), and ATP hydrolysis in animal mitochondria under anaerobic conditions (St-Pierre et al. 2000). On the other hand, bulky plant organs seem to avoid total anoxia in the tissues by metabolic control of respiration (Borisjuk et al. 2007; Zabalza et al. 2009).

It has been reassuring to note, as we show in this chapter, that bioinformatics analysis of oxygen deprivation arrays picked up multiple classes related to oxidative stress and ROS. We have also noticed that many different stresses such as high light and wounding are leading to the upregulation of oxidative stress-related genes also shared by oxygen deprivation array data.

In addition to ROS, during the recent years a vast amount of data has accumulated in favor of reactive nitrogen species (RNS) in plant tissues and their regulative role in adjusting metabolic events (Qiao and Fan 2008; Igamberdiev et al. 2010; Gupta et al. 2011a, b; Hebelstrup et al. 2012). Plant non-symbiotic hemoglobins are

emerging as major players in the regulation of NO levels under oxygen deprivation and therefore controlling the multiple functions exerted by NO, one of the most important being interaction with hormone signaling (Hebelstrup et al. 2007, 2012; Igamberdiev et al. 2010; Hill 2012). In addition, the inhibitory effect of NO on heme- and Fe–S cluster-containing enzymes such as aconitase, cytochrome *c* oxidase, and catalases directly affects cell energy status and defense against ROS (Navarre et al. 2000; Ederli et al. 2006; Blokhina and Fagerstedt 2010b). Furthermore, the accumulation of citrate due to NO-induced aconitase inhibition has been shown to result in activation of the alternative oxidase (Gupta et al. 2012), which controls superoxide formation and NO degradation in the mitochondrial electron transport chain under over-reduced conditions such as under the lack of oxygen (Gupta et al. 2009; Wulff et al. 2009; Blokhina and Fagerstedt 2010b).

When the response to oxidative stress associated with oxygen deprivation is studied on the whole genome level, the complexity of the outcome reflects several strategies used by the organism to overcome the stress without disturbing ROS signaling. We can presume that the same would be applicable also to NO signaling under hypoxic conditions. In plant cells and tissues the most straightforward approach would be a balanced increase in the expression of ROS producers and scavengers. At the onset of the oxidative stress, i.e., when ROS production goes out of control, the upregulation of defense and repair systems and coordinated expression of the corresponding signaling pathways would be beneficial (Luhua et al. 2008; Licausi et al. 2010). And, finally, metabolic alterations caused by oxygen deprivation can indirectly control internal oxygen concentration, preventing the onset of complete anoxia and, therefore control ROS and NO formation (Gupta et al. 2009; Zabalza et al. 2009; Blokhina and Fagerstedt 2010a).

Using novel bioinformatics approaches we have aimed to explore oxidative stress components on global gene expression level in oxygen deprivation arrays by comparing publicly available and in-house array data. We wished to dissect anoxic and hypoxic responses and to elucidate any new components shared by oxidative stress and oxygen deprivation stress responses.

2 Setup for Analyses of Multiple Data Sets on Global Gene Expression Under Oxygen Deprivation: Affymetrix and Agilent Platforms

Recently cross-species analyses of global gene expression and metabolic alterations under oxygen deprivation have been published (Narsai et al. 2011) along with the analysis on ROS-driven transcription under oxygen deprivation (Pucciariello et al. 2012). The latter work is based on the cross-comparison of global gene expression data under oxygen deprivation and ROS stresses (Pucciariello et al. 2012). In the present study we have undertaken a different approach: We used the enrichment analysis to monitor gene ontology (GO) classes that showed

significant regulation in anoxic and hypoxic microarray data sets (Table 1). We used two types of gene expression datasets in our analysis: Affymetrix and Agilent platform-based arrays. One of them was our in-house data generated with Agilent microarrays (Blokhina et al. unpublished) and the other was a collection of published gene expression datasets generated with Affymetrix microarrays. Due to the differences in the Affymetrix and Agilent layouts and to experimental conditions (darkness/dim light in Affymetrix and light in Agilent experiments), the data were analyzed separately. Agilent chips were used with three dyes (HyPer5, Cy3, Alexa488) and quantitated using Gene Pix Pro 6.1. After this the data was read into R, where we used background correction (Ritchie et al. 2007) and ComBat normalization (Johnson et al. 2007) to correct the various noise signals. Finally the data was processed using LIMMA package (Smyth 2005) from Bioconductor (Gentleman et al. 2004). From significantly up- and downregulated GO classes in Affymetrix and Agilent-based arrays returned by the analysis, only classes related to oxidative stress and related metabolic pathways were chosen and further discussed. The experiments where the analysis of global gene expression was performed under anoxia or under hypoxia were compared to assess the differences between the two treatments in terms of ROS production/defense. The GO annotations were downloaded from TAIR (http://www.arabidopsis.org) linked to a single locus name. We used an in-house developed enrichment method, Gene Set Z-score (GSZ) (Törönen et al. 2009), for the analysis. The GSZ analysis looks for the GO classes that show strong upregulation or downregulation. The strength of this method is that it can also monitor classes that show partial up- and partial downregulation and detect biological processes which were missed by other analyses (Törönen et al. 2009). GSZ has similarities to other published methods Gene Set Analysis (Efron and Tibshirani 2007) and Allez (Newton et al. 2007), and therefore we tested the enrichment also in a similar manner to these methods. Evaluation of results was done using 120 permutations of GO classes. Permutations were used to generate empirical *p*-values and also to scale the scores using the estimates for mean and standard deviation obtained from the permutations.

3 GO Classes Upregulated Under Anoxia and Hypoxia

3.1 Anoxia

As expected, in anoxic data represented by five Affymetrix experiments, the analysis returned a set of GO classes directly related to oxygen deprivation (Table 2). In five out of five arrays analyzed the following GO classes BP: 000906: anaerobic respiration; BP: 0036293: response to decreased oxygen levels; BP: 0070482: response to oxygen levels; and BP: 0001666: response to hypoxia, were significantly upregulated. Several ROS-related GO classed with similarly high scores were reported in at least two out of five conditions analyzed: BP: 0006979: response to oxidative stress (632 gene products); BP: 0000302: response to ROS

Table 1 Microarray experiments used for the analyses

Treatment	*Arabidopsis* ecotype	Experimental conditions	Reference	Abbreviation used
Anoxia 6 h	Col-0 seedlings, 4 d	Petri dishes, MS liquid medium, dark	Banti et al. (2010)	BA ax 6h
Anoxia 12 h	Landsberg *erecta*, 7 d	Vertical plates, MS solid medium, dim light. Total RNA[a]	Branco-Price et al. (2005)	BP ax 12h t
Submergence, anoxia 7 h	Col-0, shoot, ten-leaf rosette stage	Pot, soil, dark	Lee et al. (2011)	Lee Sub 7h
Submergence, anoxia 24 h	Col-0, shoot, ten-leaf rosette stage	Pot, soil, dark	Lee et al. (2011)	Lee Sub 24h
Anoxia 6 h	Col glabra, seedlings	Petri dishes, MS liquid medium, dark	Loreti et al. (2005)	LO ax 6h
Hypoxia 4 h	Col-0 seedlings, 7 d	Petri dishes, MS liquid medium, 1 % O_2, dark	Licausi et al. (2010)	LI Hy 4h
Hypoxia 0.5 h, 2 h, 48 h	Col2 roots, 10 d	Vertical plates, MS solid medium, 1 %, 4 % and 8 % O_2, dark	van Dongen et al. (2009)	vD 1% 0.5h, vD 1% 2h, vD 1% 48h, vD 4% 0.5h, vD 4% 2h, vD 4% 48h, vD 8% 0.5h, vD 8% 2h, vD 8% 48h
Hypoxia 9 h	Col-0 seedlings, *35S*: *His_6FLAG-RPL18B*, 7 d	Vertical plates, MS solid medium, dim light. Total RNA[a]	Branco-Price et al. (2008)	BP Hy 9h
Hypoxia 2 h, 24 h	Landsberg *erecta*, shoots, 16 d	Vertical plates, MS solid medium, light. Agilent	Blokhina et al. (unpublished)	BL Hy 2h/0h, BL Hy 24h/0h, BL Hy 2h/c2h, BL 24h/c24h

Data concerning treatments of wild-type plants only were extracted

[a]From these experiments only variants where total RNA was used for hybridisations were analyzed

Table 2 GO classes upregulated under anoxic conditions in Affymetrix arrays

GO class	BA ax 6h	BP ax 12h t	LEE Sub 24h	LEE Sub 7h	LO ax 6h
BP: 0009408 : response to heat	22,92	3,44	0,39	0,43	10,61
BP: 0042542 : response to hydrogen peroxide	**17,21**	**1,66**	**0,50**	**0,71**	**7,19**
BP: 0000302 : response to reactive oxygen species	**16,37**	**1,25**	**-0,06**	**0,02**	**6,41**
BP: 0009644 : response to high light intensity	**16,29**	**1,44**	**-0,10**	**0,20**	**7,52**
BP: 0009061 : anaerobic respiration	15,88	4,52	5,93	9,21	11,85
BP: 0009642 : response to light intensity	14,18	1,59	0,60	1,28	5,92
BP: 0009266 : response to temperature stimulus	14,11	2,75	-1,51	-1,49	6,90
BP: 0006950 : response to stress	12,01	1,25	7,10	5,41	5,98
BP: 0006979 : response to oxidative stress	**11,39**	**2,96**	**2,54**	**1,32**	**3,56**
BP: 0010200 : response to chitin	10,92	2,49	6,08	6,13	1,21
MF: 0001071 : nucleic acid binding transcription factor activity	10,60	3,28	2,86	2,88	4,30
MF: 0003700 : sequence-specific DNA binding transcription factor activity	10,60	3,28	2,86	2,88	4,30
BP: 0009628 : response to abiotic stimulus	9,16	3,28	-0,50	-0,07	6,08
BP: 0045333 : cellular respiration	8,95	1,59	2,34	4,30	7,20
BP: 0010286 : heat acclimation	8,86	1,56	0,65	-0,63	2,93
BP: 0015980 : energy derivation by oxidation of organic compounds	**8,61**	**1,79**	**2,00**	**4,00**	**6,98**
MF: 0009916 : alternative oxidase activity	**8,28**	**1,28**	**-0,36**	**2,50**	**1,50**
BP: 0042221 : response to chemical stimulus	8,23	1,75	2,87	7,04	2,21
BP: 2001141 : regulation of RNA biosynthetic process	7,90	1,91	1,66	1,51	3,34
BP: 0009743 : response to carbohydrate stimulus	7,88	2,15	3,83	5,07	0,15
BP: 0006355 : regulation of transcription, DNA-dependent	7,87	2,00	1,76	1,56	3,39
BP: 0051252 : regulation of RNA metabolic process	7,81	1,89	1,62	1,52	3,31
BP: 0010556 : regulation of macromolecule biosynthetic process	7,43	1,59	1,43	1,50	3,15
BP: 2000112 : regulation of cellular macromolecule biosynthetic process	7,43	1,59	1,43	1,50	3,15
BP: 0019219 : regulation of nucleobase-containing compound metabolic process	7,12	1,54	1,11	1,24	2,92
BP: 0009889 : regulation of biosynthetic process	7,03	1,37	1,14	1,23	2,88
BP: 0036293 : response to decreased oxygen levels	6,96	4,65	9,05	10,89	12,59
BP: 0070482 : response to oxygen levels	6,96	4,65	9,05	10,89	12,59
BP: 0051171 : regulation of nitrogen compound metabolic process	6,93	1,38	1,02	1,13	2,88
BP: 0010035 : response to inorganic substance	6,11	-2,79	-0,69	0,51	3,68
BP: 0001666 : response to hypoxia	5,76	4,50	9,11	10,70	11,02
BP: 0009415 : response to water stimulus	5,53	0,73	-0,94	-1,11	0,20
BP: 0009414 : response to water deprivation	5,49	0,89	-1,18	-1,10	0,16
BP: 0010224 : response to UV-B	**5,42**	**0,64**	**0,84**	**-0,27**	**5,21**
BP: 0009416 : response to light stimulus	5,15	2,20	-2,29	-2,13	3,91
BP: 0006091 : generation of precursor metabolites and energy	**5,11**	**-0,35**	**-0,88**	**0,70**	**3,85**
BP: 0010033 : response to organic substance	5,08	2,48	2,07	5,45	-0,98
BP: 0009411 : response to UV	**4,99**	**1,86**	**0,12**	**-0,97**	**4,93**
BP: 0009611 : response to wounding	**4,96**	**1,99**	**3,07**	**5,16**	**0,36**
BP: 0036294 : cellular response to decreased oxygen levels	4,92	2,84	8,74	10,02	7,75
BP: 0071453 : cellular response to oxygen levels	4,92	2,84	8,74	10,02	7,75
BP: 0071456 : cellular response to hypoxia	4,92	2,84	8,74	10,02	7,75
BP: 0009314 : response to radiation	4,90	2,06	-2,32	-2,29	3,98
BP: 0006952 : defense response	**4,89**	**-0,16**	**7,74**	**6,01**	**2,17**
BP: 0000160 : two-component signal transduction system (phosphorelay)	4,88	1,85	1,37	4,78	1,33
MF: 0016682 : oxidoreductase activity, acting on diphenols and related substances as donors, oxygen as acceptor	4,75	1,47	-0,09	2,74	1,97
BP: 0070301 : cellular response to hydrogen peroxide	4,75	0,30	1,39	-0,15	1,56

The first 50 significantly ($p < 0.01$) upregulated GO classes with the highest score are highlighted. GO classes are arranged according to the score in the first column. Bold font: GO classes related to ROS and oxidative stress. GO class was discussed as regulated if significant changes were observed in at least two anoxic conditions. See Table 1 for the column names

(384 gene products); BP: 0042542: response to hydrogen peroxide (263 gene products); BP: 0006091: generation of precursor metabolites and energy (664 gene products); BP: 0045333: cellular respiration (130 gene products); BP: 0009644: response to high light intensity (221 gene products); BP: 0010224: response to UV-B (104 gene products); and BP: 0009611: response to wounding (336 gene products). The set of significantly upregulated anoxic GO classes revealed not only the induction of genes involved in response to anoxia, but also reflected activation of oxygen and concurrent defense responses, engaging mitochondrial metabolism and respiratory chain in the response to the lack of oxygen and/or ROS.

Cross talk between different stresses such as high light intensity, wounding, and responses to UV light reflected the universal signaling role for ROS under diverse stress situations (Mittler et al. 2004; Van Breusegem et al. 2008; Potters et al. 2009; Petrov and Van Breusegem 2012). Therefore, many of the reported functional gene groups share similar members, heat shock-related genes being one of the examples along with a large group of cytochromes P450. The components of heat shock response have been extensively studied under oxygen deprivation and many are known to be activated under both anoxia and oxidative stress (Li et al. 2005; Loreti et al. 2005; Banti et al. 2010; Inzé et al. 2012).

A large and diverse gene superfamily of cytochromes P450 is a third largest family in Arabidopsis (245 genes) after F-box proteins and receptor-like kinases (Nelson and Werck-Reichhart 2011). The P450 enzymes are heme-containing monooxygenases which produce superoxide anion during their catalytic action and are considered important for the activation of oxygen in signaling events (Lewis 2002). They fulfill multiple functions in plants: biosynthesis of secondary compounds such as flavonoids, isoflavonoids, phytoalexins, and carotenoids, which are known to protect against oxidative stress. The biosynthetic route for the plant hormones brassinosteroids and gibberellins also involve P450, as well as the synthesis of the signaling molecules salicylic and jasmonic acids (Dasgupta et al. 2011). The requirement for molecular oxygen and external electron donor (e.g., NADH) is not absolute for atypical CYP74 class of P450 enzymes, which may be of importance under oxygen deprivation. Members of CYP74 class are responsible for fatty acid hydroperoxide metabolism and participate in oxylipin biosynthesis (Hughes et al. 2009). However, close inspection of gene expression data revealed that two CYP74 genes that are present in oxygen deprivation arrays (hydroperoxidelyase AT4G15440 and allene oxide synthase AT5G42650) are not significantly regulated in most experiments and downregulated only in two conditions out of 23 analyzed. In the study on transcriptomic response of rice (*Oryza sativa*) coleoptiles to anoxia, similar down-regulation of P450 transcripts has been detected and discussed as an energy-saving strategy (Lasanthi-Kudahettige et al. 2007).

While assessing the results of the analysis one should also bear in mind that GO classes are hierarchically organized: e.g., the "response to oxidative stress" includes the response to ROS and the response to hydrogen peroxide. GO class "cellular respiration" contains the members coding for ATP synthesis, TCA cycle enzymes

and mitochondrial electron transport chain, and also anaerobic respiration class (GO: 0009061). This class is represented by nine uncharacterized gene products, which have been reported to be induced under anoxia (Gonzali et al. 2005; Mustroph et al. 2010) and almost half of them (AT3G10020, AT2G36220, AT1G05575, AT5G15120, AT5G10040) have been also implicated in response to oxidative stress and hydrogen peroxide (Baxter et al. 2007; Luhua et al. 2008; Inzé et al. 2012). In GO class "response to hydrogen peroxide" the most prominent and universal induction exhibited respiratory burst oxidase RbohD (At5g47910). Plant NADPH oxidases are key components of ROS-mediated signaling under diverse abiotic stresses, in plant–pathogen interactions, and during normal growth and development. It has been shown recently that nitrosylation of a critical cysteine residue negatively regulates RbohD, providing evidence for the cross talk between ROS and NO signaling via this negative feedback loop (Yun et al. 2011). However, other class representatives directly connected to ROS metabolism, such as ascorbate peroxidase 2 (AT3G09640) and monodehydroascorbate reductase (At3g09940), were shown to be moderately upregulated under hypoxic and anoxic conditions.

3.2 Hypoxia

Oxygenases under oxygen deprivation. The hypoxic set of the arrays presented by both Affymetrix and Agilent platforms (analyzed separately) shared with anoxic arrays several significantly upregulated oxygen deprivation-related classes and oxidative stress-related classes: BP: 0015980: energy derivation by oxidation of organic compound, BP: 0045333: cellular respiration, BP: 0009611: response to wounding, BP: 0010224: response to UV-B, BP: 0042542: response to hydrogen peroxide, and BP: 0000302: response to ROS (Tables 3 and 4). The availability of oxygen in hypoxic experiments was signified by a set of GO classes different from anoxia showing the upregulation of oxygenases, the enzymes which incorporate oxygen into organic substrates: GO:0016702:oxidoreductase activity, acting on single donors with incorporation of molecular oxygen, incorporation of two oxygen atoms, GO: 0016701: oxidoreductase activity, acting on single donors with incorporation of molecular oxygen, MF: 0051213: dioxygenase activity, and MF: 0047800: cysteamine dioxygenase activity.

Many microarray studies reveal the intrinsic connection between oxygen deprivation stress and the increased expression of the genes coding for oxygenases, (e.g., Fe-dependent ketoglutarate oxygenases, ACC-oxidase, desaturases, alternative oxidase, etc.). Fe-dependent ketoglutarate oxygenases (FeKGO) showed consistent upregulation over many hypoxic sets analyzed. It is a large gene superfamily which requires Fe^{2+} as a cofactor, and some of the class enzymes utilize ascorbate as an electron donor. Oxidation of a substrate is coupled to decarboxylation of 2-ketoglutarate to yield succinate and CO_2 (Loenarz and Schofield 2008). In mammalian tissues these enzymes are involved in histone and DNA demethylation

Table 3 GO classes upregulated under hypoxic conditions in Affymetrix arrays

GO class	LI Hy 4h	vD 1.% 0.5h	vD 1% 2h	vD1% 48h	vD 4% 0.5h	vD 4% 2h	vD 4% 48h	vD 8% 0.5h	vD 8% 2h	vD 8% 48h	BP Hy 9h
BP: 0009061 : anaerobic respiration	15,96	30,15	31,40	23,09	26,40	42,90	22,38	29,38	29,18	9,24	27,78
BP: 0036293 : response to decreased oxygen levels	14,82	9,27	19,33	11,39	8,17	14,51	9,84	8,69	10,75	5,30	11,61
BP: 0070482 : response to oxygen levels	14,82	9,27	19,33	11,39	8,17	14,51	9,84	8,69	10,75	5,30	11,61
BP: 0036294 : cellular response to decreased oxygen levels	13,75	2,25	13,80	2,52	1,06	4,47	1,13	0,74	2,20	-0,94	5,21
BP: 0071453 : cellular response to oxygen levels	13,75	2,25	13,80	2,52	1,06	4,47	1,13	0,74	2,20	-0,94	5,21
BP: 0071456 : cellular response to hypoxia	13,75	2,25	13,80	2,52	1,06	4,47	1,13	0,74	2,20	-0,94	5,21
BP: 0001666 : response to hypoxia	12,86	7,97	16,90	9,92	6,93	12,79	8,53	8,10	9,78	5,27	10,34
MF: 0043168 : anion binding	8,16	8,22	7,30	8,39	8,25	8,22	10,46	15,73	9,83	5,33	10,90
BP: 0010200 : response to chitin	8,16	4,56	4,44	0,41	-0,39	0,74	-3,38	-1,50	1,59	-2,19	12,70
MF: 0016702 : oxidoreductase activity, acting on single donors with incorporation of molecular oxygen, incorporation of two atoms of oxygen	**6,15**	**5,74**	**8,20**	**7,39**	**6,90**	**8,58**	**9,40**	**6,55**	**9,24**	**7,57**	**5,51**
BP: 0009743 : response to carbohydrate stimulus	5,43	3,71	4,61	0,31	0,52	0,92	-2,52	-0,68	1,83	-2,60	9,94
MF: 0051213 : dioxygenase activity	**5,28**	**3,18**	**5,85**	**3,87**	**4,53**	**5,33**	**4,06**	**4,72**	**6,04**	**3,57**	**3,53**
BP: 0045333 : cellular respiration	**5,18**	**14,74**	**12,36**	**10,69**	**13,48**	**15,14**	**9,98**	**17,67**	**18,59**	**6,21**	**13,73**
MF: 0016701 : oxidoreductase activity, acting on single donors with incorporation of molecular oxygen	**5,17**	**4,44**	**6,24**	**5,64**	**5,17**	**5,63**	**7,36**	**5,26**	**5,87**	**6,85**	**5,51**
MF: 0047800 : cysteamine dioxygenase activity	**5,16**	**14,39**	**12,98**	**12,77**	**15,51**	**21,90**	**16,23**	**19,73**	**22,15**	**10,14**	**8,84**
MF: 0001071 : nucleic acid binding transcription factor activity	5,12	2,57	2,09	-6,21	-1,74	1,13	-6,92	0,63	0,95	-5,67	3,06
MF: 0003700 : sequence-specific DNA binding transcription factor activity	5,12	2,57	2,09	-6,21	-1,74	1,13	-6,92	0,63	0,95	-5,67	3,06
BP: 0015980 : energy derivation by oxidation of organic compounds	5,08	15,55	11,84	10,41	13,91	15,12	9,78	18,51	18,22	6,21	13,53
BP: 0080167 : response to karrikin	4,88	7,33	3,45	4,10	6,12	3,36	5,20	4,74	6,48	3,00	6,34
MF: 0003950 : NAD+ ADP-ribosyltransferase activity	4,53	11,33	8,17	2,75	10,13	8,43	3,74	7,45	4,91	1,40	5,86
BP: 0009408 : response to heat	4,35	-0,18	-0,46	7,89	0,80	0,50	0,31	1,00	0,08	0,65	18,86
MF: 0016157 : sucrose synthase activity	4,28	7,33	10,18	11,49	10,03	17,56	10,28	13,23	12,65	8,01	3,81
BP: 0006950 : response to stress	4,26	1,80	5,79	4,53	3,64	4,24	1,85	2,96	4,38	-1,21	17,40
BP: 0006355 : regulation of transcription, DNA-dependent	4,26	3,13	1,58	-5,62	-1,23	1,86	-7,53	2,49	0,67	-5,07	2,66
BP: 2001141 : regulation of RNA biosynthetic process	4,21	3,20	1,54	-5,62	-1,18	1,86	-7,42	2,48	0,77	-5,04	2,57
BP: 0051252 : regulation of RNA metabolic process	4,18	3,21	1,55	-5,50	-1,09	1,84	-7,38	2,46	0,74	-5,03	2,62
BP: 0009611 : response to wounding	**4,15**	**3,91**	**3,72**	**2,94**	**4,03**	**4,92**	**0,59**	**3,72**	**5,43**	**0,00**	**5,70**
BP: 0010193 : response to ozone	**4,01**	**-0,23**	**0,25**	**3,40**	**-0,48**	**-0,76**	**-2,40**	**-0,09**	**-1,28**	**-2,00**	**0,96**
MF: 0000287 : magnesium ion binding	3,84	2,46	1,93	2,26	2,10	4,31	2,27	3,54	3,08	1,59	2,42
BP: 0009628 : response to abiotic stimulus	3,79	2,69	5,93	5,85	3,95	2,78	2,93	4,55	2,47	-0,76	11,88
BP: 0019219 : regulation of nucleobase-containing compound metabolic process	3,68	2,91	1,37	-5,46	-1,37	1,76	-7,22	2,49	0,61	-4,96	2,35

The first 50 significantly ($p < 0.01$) upregulated GO classes with the highest score are highlighted. GO classes are arranged according to the score in the first column. Bold font: GO classes related to ROS and oxidative stress. GO class was discussed as regulated when significant score was observed in at least three hypoxic conditions. See Table 1 for the column names

and in other similar hydroxylation and desaturation reactions (Loenarz and Schofield 2008). FeKGO named Prolyl-4-hydroxylases have been implicated in oxygen sensing in humans via hydroxylation of critical prolyl residues in the hypoxia-inducible factor (HIF) transcription factor (Berra et al. 2006). Intriguingly, in plants over-expression of the prolyl-hydroxylase AtP4H1 (AT2G43080) resulted in "hypoxia-in-normoxia" phenotype and concomitant upregulation of growth-, development-, and hypoxia-related genes (Asif et al. 2009). However, in the hypoxic and anoxic arrays analyzed here, the expression pattern for AtP4H1was insignificant.

In the context of oxygen deprivation stress, one of the most important members of the FeKGO family is ACC-oxidase (1-aminocyclopropane-1-carbohylate oxidase, AT2G19590, AT1G03400, AT1G62380, AT2G25450, AT5G43440, AT5G43450), an oxygenase which is involved in the synthesis of ethylene. ACC-oxidase is one of the examples of ascorbate-dependent reaction of FeKGO:

1-aminocyclopropane-1-carboxylate + ascorbate + O_2 → ethylene + cyanide + dehydroascorbate + CO_2 + $2H_2O$

Hence, ascorbate availability and turnover under hypoxic conditions accompanied by oxidative stress can affect not only H_2O_2 detoxification via the ascorbate–glutathione cycle, but also control ethylene biosynthesis. Ethylene is an important

Table 4 GO classes upregulated under hypoxic conditions in Agilent arrays

GO class	BL Hy 2h/0h	BL Hy 24h/0h	BL Hy 2h/c2h	BL Hy 24h/c24h
BP: 0009061 : anaerobic respiration	19,84	18,24	21,37	18,65
BP: 0009266 : response to temperature stimulus	7,81	18,19	13,02	19,92
BP: 0009408 : response to heat	4,40	16,96	14,35	24,10
MF: 0030976 : thiamine pyrophosphate binding	20,48	16,14	18,43	15,97
MF: 0043168 : anion binding	20,69	15,14	18,75	15,06
MF: 0004737 : pyruvate decarboxylase activity	20,72	14,60	20,09	14,62
BP: 0009628 : response to abiotic stimulus	12,28	14,03	18,48	15,17
BP: 0015980 : energy derivation by oxidation of organic compounds	11,02	13,84	10,73	14,29
BP: 0045333 : cellular respiration	**8,91**	**12,21**	**11,37**	**14,09**
BP: 0009642 : response to light intensity	2,97	10,03	9,22	13,98
BP: 0009644 : response to high light intensity	**1,27**	**9,95**	**8,90**	**14,09**
CC: 0043229 : intracellular organelle	10,34	9,88	10,78	10,67
CC: 0043226 : organelle	10,25	9,86	10,74	10,63
BP: 0006091 : generation of precursor metabolites and energy	10,05	9,54	9,85	9,37
BP: 0036293 : response to decreased oxygen levels	10,13	9,48	7,76	7,45
BP: 0070482 : response to oxygen levels	10,13	9,48	7,76	7,45
MF: 0047800 : cysteamine dioxygenase activity	**13,21**	**9,31**	**8,62**	**7,54**
CC: 0043227 : membrane-bounded organelle	11,33	9,14	10,82	8,86
BP: 0042221 : response to chemical stimulus	10,23	9,04	12,70	9,97
BP: 0005983 : starch catabolic process	8,45	8,90	-1,18	2,15
BP: 0044247 : cellular polysaccharide catabolic process	8,45	8,90	-1,18	2,15
BP: 0009251 : glucan catabolic process	8,45	8,90	-1,18	2,15
BP: 0042542 : response to hydrogen peroxide	**2,32**	**8,89**	**7,47**	**11,81**
BP: 0009409 : response to cold	4,58	8,78	3,93	6,19
CC: 0044429 : mitochondrial part	1,87	8,30	3,10	8,65
BP: 0001666 : response to hypoxia	9,36	8,29	6,96	6,21
CC: 0070013 : intracellular organelle lumen	1,50	8,28	7,46	13,67
CC: 0043233 : organelle lumen	1,50	8,28	7,46	13,67
BP: 0000302 : response to reactive oxygen species	**2,37**	**8,28**	**6,74**	**11,21**
CC: 0005739 : mitochondrion	**3,23**	**8,21**	**4,83**	**7,34**
MF: 0005515 : protein binding	6,31	8,09	5,62	7,59
CC: 0005761 : mitochondrial ribosome	0,79	7,82	0,25	8,67
BP: 0010286 : heat acclimation	2,38	7,81	0,81	7,41
BP: 0009631 : cold acclimation	2,88	7,75	-1,38	3,56
CC: 0005634 : nucleus	1,24	7,69	4,45	11,34
CC: 0044428 : nuclear part	1,66	7,64	7,14	13,48
BP: 0044275 : cellular carbohydrate catabolic process	7,36	7,51	-0,66	1,50
BP: 0009416 : response to light stimulus	5,51	7,49	12,69	9,15
CC: 0044446 : intracellular organelle part	5,90	7,32	9,09	9,54
BP: 0010224 : response to UV-B	**5,71**	**7,32**	**13,69**	**12,47**
CC: 0044422 : organelle part	5,86	7,28	9,02	9,46
BP: 0010035 : response to inorganic substance	6,88	7,25	12,64	10,46
BP: 0009314 : response to radiation	5,21	7,23	12,08	8,80
CC: 0030964 : NADH dehydrogenase complex	1,93	7,19	-0,26	6,40
CC: 0045271 : respiratory chain complex I	1,93	7,19	-0,26	6,40
BP: 0006397 : mRNA processing	3,59	7,11	2,58	7,36

The first 50 significantly ($p < 0.01$) upregulated GO classes with the highest score are highlighted. GO classes are arranged according to the score in the first column. Bold font: GO classes related to ROS and oxidative stress. GO class was discussed as regulated when significant score was observed in at least two hypoxic conditions. See Table 1 for the column names

signaling molecule under the lack of oxygen-coordinating morphological adaptations (stem elongation, aerenchyma, and adventitious root formation) and metabolic rearrangements (Fukao and Bailey-Serres 2008; Sairam et al. 2008; Licausi 2011). It has been shown that manipulation of hypoxia-induced Hb1 expression enhanced ethylene levels and ACC-oxidase activity in Hb-downregulated maize lines, possibly via modulation of NO levels by Hb1 (Manac'h-Little et al. 2005). NO can also control ethylene biosynthesis via S-nitrosylation of methionine adenosyl transferase leading to reversible inhibition of the ethylene precursor S-adenosyl methionine synthesis (Lindermayr et al. 2006). Ascorbate and NO levels are not the only cross points between ethylene and oxidative metabolism. An investigation into tocopherol biosynthesis, the main lipid-soluble antioxidant, in *Arabidopsis* mutants defective in ethylene perception and signaling, showed 30 % reduction in tocopherol levels under stress and suggested a regulatory role for ethylene in tocopherol biosynthesis (Cela et al. 2009).

Other examples of Fe2KG oxygenases, equally important under hypoxia as judged by their expression pattern, may be several other members of the oxygenase family: AT4G33910, AT1G20270, and AT3G28480. All of them showed quite strong upregulation in the majority of the conditions analyzed. The specific role for these oxygenases under oxygen deprivation is not clear. Taking into account the transcriptional and metabolic changes occurring under hypoxia, a metabolic association between the TCA cycle, Hbs, and NO under hypoxia can be suggested (Fig. 1). This is supported by strong and ubiquitous upregulation of non-symbiotic Hbs, activation of GABA shunt, moderate induction of ascorbate peroxidase (AT3G09640) and monodehydroascorbate reductase (AT3G09940), the physiological evidence on MDHAR- and ascorbate-sustained NO scavenging by hemoglobin (Igamberdiev et al. 2006), the rearrangements in the TCA cycle and succinate accumulation observed under oxygen deprivation(Rocha et al. 2010), and the dependence of FeKGO on ascorbate in the conversion of 2-ketoglutarate to succinate in Fe2KGO catalyzed reaction (Loenarz and Schofield 2008).

Induction of non-symbiotic class1 hemoglobin under hypoxic conditions is well documented (Dordas et al. 2003; van Dongen et al. 2009; Dordas 2009; Lee et al. 2011), but this upregulation is not specific to the lack of oxygen. Expression patterns of both Hb1 and Hb2 vary in different tissues as well as in response to different types of stress. *Hb1* genes have been reported to be upregulated by abiotic stresses (Trevaskis et al. 1997; Shimoda et al. 2005; Zhao et al. 2008, 2009), treatments with nitrogen compounds including NO, plant hormones, sucrose, and H_2O_2 (Trevaskis et al. 1997; Wang et al. 2000; Sakamoto et al. 2004; Shimoda et al. 2005; Qu et al. 2006). The main physiological function of non-symbiotic hemoglobins under oxygen deprivation relies on NO-binding properties of oxyhemoglobin. The functioning of Hb-NO cycle under hypoxia which controls elevated NO levels and utilizes NADH accumulating under hypoxia has been suggested (Igamberdiev et al. 2005, 2010) and further linked to hypoxically functioning mitochondria (Igamberdiev and Hill 2009). Due to the interference with NO signaling, changes and/or manipulation of Hb expression affects many transcriptional and metabolic events which are under NO control (Lee et al. 2011; Hill

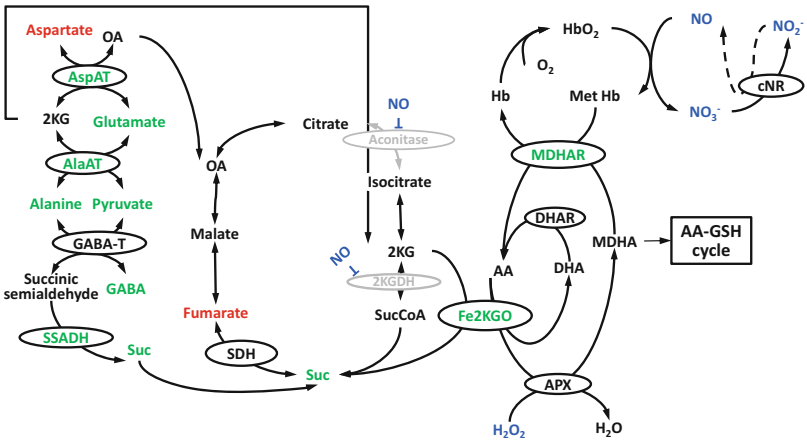

Fig. 1 Hypoxia-induced TCA cycle modification in Arabidopsis shoots: the involvement of Fe^{2+}-dependent ketoglutarate oxidase, GABA shunt, non-symbiotic hemoglobins and NO. NO accumulating under hypoxia is controlled by hypoxically induced non-symbiotic Hbs in an NADH-coupled reaction. To complete the cycle MetHb has to be regenerated by a MetHb reductase. MDHAR supports Hb turnover under hypoxia acting as MetHb reductase (Igamberdiev et al. 2006). In turn, the product of MDHAR reaction, ascorbate, can be oxidized either via the ascorbate–glutathione cycle (operational in the cytosol and mitochondria), or by a novel route suggested by metabolomics and microarray studies under oxygen deprivation. Fe-dependent 2-ketoglutarate oxygenase utilizes 2KG and ascorbate in the presence of oxygen to form a TCA cycle and ETC metabolite, succinate. 2KG needed for the reaction is supplied through the reactions of GABA shunt, which spans the cytosol and mitochondria. These supplementary enzymatic reactions are engaged to feed succinate and oxaloacetate into the TCA cycle bypassing inactivated TCA cycle components, and thus enhance/modify TCA cycle. TCA cycle enzymes are omitted for clarity. *AA* ascorbic acid, *AspAT* aspartate aminotransferase, *AlaAT* alanine aminotransferase, *DHA* dehydroascorbic acid, *GABA-T* GABA transaminase, *OA* oxaloacetate, *Fe2KGO* Fe^{2+}-dependent 2-ketoglutarate oxygenase, *2KG* 2-ketoglutarate, *cNR* cytosolic nitrate reductase, *SSADH* succinic semialdehyde dehydrogenase, *2KGDH* 2-ketoglutarate dehydrogenase, *MetHb* methemoglobin, *MDHAR* monodehydroascorbate reductase. *Green font*—metabolites accumulated and enzymes induced, *red font*—metabolites depleted in Arabidopsis shoots under hypoxia, *gray font*—enzymes inhibited by NO

2012). Hb-sustained NO elimination may have physiological significance also under non-hypoxic conditions via controlling the NO influx into signaling and metabolic events. Such control will affect major stress-related parameters: e.g., the redox state of the cell (NAD(P)H/$NAD(P)^+$, ascorbate/dehydroascorbate, and GSH/GSSG ratios); rate of mitochondrial respiration via direct COX inhibition, and interference with Fe–S TCA cycle enzymes, and may constitute the physiological mechanism for Hb-associated improvement of cellular energy status. Under oxygen deprivation stress Hbs improve the energy status of the cell when MetHb regeneration to Hb is coupled to a cascade of metabolic reactions which result in modification/enhancement of the TCA cycle and incorporates hypoxic metabolites in this adaptive response involving GABA shunt (Fig. 1).

Activation of GABA shunt enzymes and their regulation by hypoxic metabolites is a well-characterized phenomenon during periods with lack of oxygen (Branco-Price et al. 2008; Rocha et al. 2010). In the current study, strong significant upregulation of SSADH (AT1G79440) in hypoxic but not in anoxic arrays was detected. Further evidence for the importance of GABA metabolism controlling oxidative species came from the study of *Arabidopsis* T-DNA knockout mutants of succinic semialdehyde dehydrogenase (SSADH), the mitochondrial enzyme which converts succinic semialdehyde to succinate in the GABA shunt (Bouché et al. 2003). Arabidopsis *ssdh* mutants exhibited a dwarf phenotype, were sensitive to environmental stresses (light, UV-B, heat), and showed enhanced H_2O_2 accumulation (Bouché et al. 2003). The physiological mechanism for GABA shunt-dependent alleviation of oxidative stress can rely on the support of the TCA cycle via succinate and/or NADH and, therefore, decreasing the likelihood of mitochondrial ROS formation (Fait et al. 2005, 2008).

3.3 BP: 0045333: cellular respiration. Relationship between the TCA cycle, mitochondrial electron transport chain, and oxidative stress under hypoxia

The plasticity of the TCA cycle and adjacent metabolic reactions, i.e., the rearrangement of the cycle to a non-cyclic mode in response to stresses including hypoxia, has been recently suggested (Branco-Price et al. 2008; Rocha et al. 2010; Sweetlove et al. 2010). In the latter work hypoxia-induced metabolic changes and inhibition of critical TCA cycle enzymes resulted in adaptive modifications which led to incorporation of hypoxic metabolites into the TCA cycle and production of extra ATP (Rocha et al. 2010). In the study on the rearrangement of mitochondrial protein complexes under oxidative stress, several key metabolites of central carbon metabolism have been reported to associate or dissociate from the protein complexes (Obata et al. 2011). Some of the affected enzymes have been reported also to respond to oxygen deprivation: monodehydroascorbate reductase and alanine aminotransferase (Baxter et al. 2007). Hence, under stress conditions mitochondria as complex dynamic systems respond to the changing environment by metabolic and structural rearrangements. The investigation of expression pattern of TCA cycle and related enzymes in microarray studies revealed either downregulation of the cycle enzymes, or non-significant changes, with significant inhibition of citrate synthase (At2g44350) and isocitrate dehydrogenase (At5g03290). Hence, most of the regulation might occur on post-transcriptional level. Indeed, translational repression of mRNA encoding TCA cycle enzymes has been shown under hypoxia (Branco-Price et al. 2008). Prominent upregulation of the other cellular respiration GO class members, NADH dehydrogenases, was revealed by GSZ analysis and examination of their expression pattern in microarrays. The induction of NADH dehydrogenases occurred irrespective of oxygen levels in the experiment. Induced

NADH dehydrogenases were coded both by nuclear (At2g07711) and mitochondrial genome (Atmg00650, Atmg00060), although the expression of mitochondrial genes cannot be reliably determined with whole genome arrays. Interestingly, the upregulation of At2g07711 and Atmg00060 was strictly attributed to oxygen deprivation, whereas at the onset of reoxygenation these NADH dehydrogenases were downregulated (Branco-Price et al. 2008). The mitochondrial electron transport chain is also an important source of ROS during strongly reduced conditions, such as during oxygen deprivation (Rhoads et al. 2006; Navrot et al. 2007; Hoffman et al. 2007; Blokhina and Fagerstedt 2010b). Several mechanisms can lead to the formation of ROS under unfavorable conditions and, in turn, mitochondrial ROS control NO levels via peroxynitrite (Planchet et al. 2005; Borisjuk et al. 2007; Benamar et al. 2008; Gupta et al. 2011a). The expression of genes and subsequent metabolic changes which affect electron transport, reducing equivalents and substrate supply in mitochondria, will control the rate of ROS/NO formation due to the electron leakage from the electron transport chain, and ultimately the ATP synthesis.

3.4 Cysteamine dioxygenase activity

One of the oxygenase classes picked up by analysis in both Affymetrix and Agilent arrays was MF: 0047800: cysteamine dioxygenase activity. The class members catalyze the reaction cysteamine + O_2 = H^+ + hypotaurine and are represented by five uncharacterised genes of *Arabidopsis*: AT1G18490, AT2G42670, AT3G58670, AT5G15120, and AT5G39890. The latter two have been associated with the hypoxic response (Mustroph et al. 2010; Branco-Price et al. 2005), regulation of hydrogen peroxide metabolism, salicylic acid signaling, and xylem development. The molecular functions of these proteins in plants are currently unknown. Interestingly, the upregulation of the genes belonging to the GO class "cysteamine dioxygenase activity" was specific for oxygen deprivation. Upon reoxygenation (Branco-Price et al. 2008) this GO class was downregulated and showed the lowest significant score in the analysis (data not shown). In animal tissues hypotaurine is a precursor in taurine biosynthesis (2-aminoethansulfonic acid). Taurine, a sulfur containing amino acid, accumulates to a high level in animal tissues and executes a number of important functions: it can act as antioxidant, as an intracellular osmoregulator, as a neurotransmitter, and can stabilize the membranes and regulate Ca^{2+} entry into the cell (Brosnan and Brosnan 2006). Taurine and hypotaurine metabolism is also closely associated with the enzymatic pathways involving pyruvate and alanine, ketoglutarate and glutamate. However, in plants where taurine content is extremely low, in the range of nmol/gFW (the highest content was found in *Opuntia*, lentil, and red algae) (Kataoka and Ohnishi 1986; Huxtable 1992), it is difficult to predict the physiological role for this metabolite and to ascribe a specific function under oxygen deprivation.

4 Downregulated GO classes under anoxia and hypoxia

The analysis of downregulated GO classes revealed a number of biological processes already known to be inhibited under the lack of oxygen, such as cell wall- and photosynthesis-related classes. None of the GO classes related to formation, processing, and detoxification of ROS were reported as downregulated. In five out of five anoxic conditions in the Affymetrix set the strongest downregulation was detected in the endomembrane system (CC: 0012505), followed by the response to auxin stimulus (BP: 0009733) and the response to hormone stimulus (BP: 0009725). The physiological role for auxin biochemistry depression and particularly auxin binding under anoxia is not completely understood, but the addition of exogenous sucrose has been shown to alleviate anoxia-imposed inhibition on the auxin-related genes (Loreti et al. 2005). It has also been shown that multiple components of auxin-signaling pathway are inhibited by apoplastic ROS (exemplified by O_3 treatment) (Blomster et al. 2011) and that hypoxic stress is accompanied by intensive ROS formation in the apoplastic space (Blokhina et al. 2001). Biological processes downregulated in the hypoxic set (11 conditions in Affymetrix and 4 in Agilent sets) were much more diverse and shared some similarities with anoxic response (CC: 0012505: endomembrane system, BP: 0009733: response to auxin stimulus, BP: 0042430: indole-containing compound metabolic process). The latter class includes genes responsible for, e.g., defense against bacteria and fungi (camalexin biosynthesis), and are represented by a diverse cytochrome P450 group, MYB transcription factors that regulate, e.g., metabolism of aromatic amino acids, and mitogen-activated protein kinases MAPK shown to regulate ethylene signaling pathway downstream the ethylene receptor (Yoo and Sheen 2008). Cytochromes P450 are oxygenases which require molecular oxygen for their functioning and are known to have complicated expression pattern during oxygen deprivation (Lasanthi-Kudahettige et al. 2007). The Affymetrix set of hypoxic experiments also included a number of downregulated GO classes related to biotic stress response and hypoxic metabolism-associated GO classes (also reported as significantly up- or downregulated by GSZ analysis, e.g., BP: 0036294: cellular response to decreased oxygen levels, BP: 0071453: cellular response to oxygen levels, BP: 0071456: cellular response to hypoxia). This fact reflects both the power of statistical approaches to discriminate between induced and repressed genes in the same functional group, and the complexity of response to the lack of oxygen. In the Agilent data set, where hypoxia was performed under light conditions, strong inhibition of photosynthesis was confirmed: the first 20 most downregulated GO classes were related to chloroplast and thylakoid functioning, followed by nucleosome and DNA assembly and organization. Interestingly, there were only eight significantly downregulated GO classes common for Affymetrix and Agilent hypoxic sets: BP: 0009725: response to hormone stimulus; BP: 0009737: response to abscisic acid stimulus; BP: 0009719: response to endogenous stimulus; BP: 0010033: response to organic substance; BP: 0042221:

response to chemical stimulus; BP: 0009628: response to abiotic stimulus; BP: 0007623: circadian rhythm; and BP: 0048511: rhythmic process.

5 Concluding Remarks

In this chapter we have successfully implemented new bioinformatics approaches (Törönen et al. 2009) for the analysis of large sets of global expression data to reveal oxidative stress components in *Arabidopsis* under anoxia and hypoxia on the whole genome level. Response to oxygen deprivation stress-associated ROS and NO formation in terms of gene expression is not straightforward and incorporates the upregulation of some ROS-producing components and antioxidative defense components and relies largely on metabolic rearrangements caused by the lack of oxygen. Judging by the analysis of significantly regulated GO classes, these metabolic alterations include modification of the TCA cycle, incorporation of hypoxic metabolites in efficient energy production, and regulation of mitochondrial electron transport chain as a potent ROS and NO producer.

Interestingly, the analysis returned several significantly regulated GO classes some of which, to our knowledge, have not been discussed previously in connection with oxygen deprivation, for example the downregulated BP: 0007623: circadian rhythm and strongly upregulated MF: 0047800: cysteamine dioxygenase activity. Interestingly, the circadian rhythm classes were reported to be affected in the hypoxic arrays performed under different conditions: most Affymetrix experiments were performed in the dark, whereas Agilent under light conditions. Therefore, it is tempting to speculate that regulation of circadian genes can be part of oxygen deprivation response. The enrichment of a hypoxic set with FeKGO oxygenases suggests a specific role for these enzymes and distinguishes hypoxic and anoxic responses. According to our hypothesis the important role for FeKGO oxygenases lies in the incorporation of hypoxic metabolites into energy production via connection with NO detoxification, MetHb turnover, and ascorbate metabolism. Therefore, this upregulated enzymatic system feeds TCA cycle intermediates succinate and oxaloacetate into the cycle bypassing inactivated TCA cycle components, and thus improves energy production under these harsh conditions.

Future research is needed in the dissection of redundant metabolic adjustments which involve the TCA cycle, GABA shunt, glycolysis, FeKGO oxygenases, and ROS/NO chemistry. These responses seem to vary between plant species and depend on the degree of oxygen deprivation. The plasticity of the TCA cycle can be one of the adaptation mechanisms.

None of the GO classes related to formation, processing, and detoxification of ROS were reported as downregulated, emphasizing the fact that oxidative stress is an integral part of oxygen deprivation response. Accumulation of ROS, especially in the apoplastic space, may underlie the downregulation of auxin signaling under the lack of oxygen and provide further evidence for the signaling roles of ROS. In this chapter we have shown that ROS play an essential role in the formation of

hypoxic and anoxic response incorporating gene regulation and oxidative stress as integral parts of this response.

Acknowledgments We wish to thank Julia Bailey-Serres, Joost van Dongen, Pierdomenico Perata, and Chiara Pucciariello for supplying the microarray datasets for our analysis. This work was supported by the Academy of Finland grant no. 123826.

References

Asif M, Trivedi P, Misra P, Nath P (2009) Prolyl-4-hydroxylase (AtP4H1) mediates and mimics low oxygen response in Arabidopsis thaliana. Funct Integr Genomics 9:525–535

Bailey-Serres J, Chang R (2005) Sensing and signalling in response to oxygen deprivation in plants and other organisms. Ann Bot (Lond) 96:507–518

Bailey-Serres J, Voesenek LACJ (2008) Flooding stress: acclimations and genetic diversity. Annu Rev Plant Biol 59:313–339

Banti V, Mafessoni F, Loreti E, Alpi A, Perata P (2010) The heat-inducible transcription factor HsfA2 enhances anoxia tolerance in Arabidopsis. Plant Physiol 152:1471–1483

Baxter CJ, Redestig H, Schauer N, Repsilber D, Patil KR, Nielsen J, Selbig J, Liu J, Fernie AR, Sweetlove LJ (2007) The metabolic response of heterotrophic Arabidopsis cells to oxidative stress. Plant Physiol 143:312–325

Baxter-Burrell A, Yang Z, Springer PS, Bailey-Serres J (2002) RopGAP4-dependent Rop GTPase rheostat control of Arabidopsis oxygen deprivation tolerance. Science 296:2026–2028

Benamar A, Rolletschek H, Borisjuk L, Avelange-Macherel M, Curien G, Mostefai HA, Andriantsitohaina R, Macherel D (2008) Nitrite–nitric oxide control of mitochondrial respiration at the frontier of anoxia. Biochim Biophys Acta—Bioenergetics 1777:1268–1275

Berra E, Ginouves A, Pouyssegur J (2006) The hypoxia-inducible-factor hydroxylases bring fresh air into hypoxia signalling. EMBO Rep 7:41–45

Biemelt S, Keetman U, Albrecht G (1998) Re-aeration following hypoxia or anoxia leads to activation of the antioxidative defense system in roots of wheat seedlings. Plant Physiol 116:651–658

Biemelt S, Keetman U, Mock H, Grimm B (2000) Expression and activity of isoenzymes of superoxide dismutase in wheat roots in response to hypoxia and anoxia. Plant Cell Environ 23:135–144

Blokhina O, Fagerstedt KV (2010a) Oxidative metabolism, ROS and NO under oxygen deprivation. Plant Physiol Biochem 48:359–373

Blokhina O, Fagerstedt KV (2010b) Reactive oxygen species and nitric oxide in plant mitochondria: origin and redundant regulatory systems. Physiol Plant 138:447–462

Blokhina OB, Virolainen E, Fagerstedt KV, Hoikkala A, Wähälä K, Chirkova TV (2000) Antioxidant status of anoxia-tolerant and -intolerant plant species under anoxia and reaeration. Physiol Plant 109:396–403

Blokhina OB, Chirkova TV, Fagerstedt KV (2001) Anoxic stress leads to hydrogen peroxide formation in plant cells. J Exp Bot 52:1179–1190

Blokhina O, Virolainen E, Fagerstedt KV (2003) Antioxidants, oxidative damage and oxygen deprivation stress: a review. Ann Bot (Lond) 91:179–194

Blomster T, Salojärvi J, Sipari N, Brosché M, Ahlfors R, Keinänen M, Overmyer K, Kangasjärvi J (2011) Apoplastic reactive oxygen species transiently decrease auxin signaling and cause stress-induced morphogenic response in Arabidopsis. Plant Physiol 157:1866–1883

Borisjuk L, Macherel D, Benamar A, Wobus U, Rolletschek H (2007) Low oxygen sensing and balancing in plant seeds: a role for nitric oxide. New Phytol 176:813–823

Bouché N, Fait A, Bouchez D, Møller SG, Fromm H (2003) Mitochondrial succinic-semialdehyde dehydrogenase of the γ-aminobutyrate shunt is required to restrict levels of reactive oxygen intermediates in plants. Proc Natl Acad Sci U S A 100:6843–6848

Branco-Price C, Kawaguchi R, Ferreira RB, Bailey-Serres J (2005) Genome-wide analysis of transcript abundance and translation in Arabidopsis seedlings subjected to oxygen deprivation. Ann Bot (Lond) 96:647–660

Branco-Price C, Kaiser KA, Jang CJH, Larive CK, Bailey-Serres J (2008) Selective mRNA translation coordinates energetic and metabolic adjustments to cellular oxygen deprivation and reoxygenation in *Arabidopsis thaliana*. Plant J 56:743–755

Brosnan JT, Brosnan ME (2006) The sulfur-containing amino acids: an overview. J Nutr 136:1636S–1640S

Cela J, Falk J, Munné-Bosch S (2009) Ethylene signaling may be involved in the regulation of tocopherol biosynthesis in Arabidopsis thaliana. FEBS Lett 583:992–996

Chang R, Jang C, Branco-Price C, Nghiem P, Bailey-Serres J (2012) Transient MPK6 activation in response to oxygen deprivation and reoxygenation is mediated by mitochondria and aids seedling survival in *Arabidopsis*. Plant Mol Biol 78:109–122

Dasgupta K, Ganesan S, Manivasagam S, Ayre B (2011) A cytochrome P450 monooxygenase commonly used for negative selection in transgenic plants causes growth anomalies by disrupting brassinosteroid signaling. BMC Plant Biol 11:67

Dordas C (2009) Nonsymbiotic hemoglobins and stress tolerance in plants. Plant Sci 176:433–440

Dordas C, Rivoal J, Hill RD (2003) Plant haemoglobins, nitric oxide and hypoxic stress. Ann Bot (Lond) 91:173–178

Ederli L, Morettini R, Borgogni A, Wasternack C, Miersch O, Reale L, Ferranti F, Tosti N, Pasqualini S (2006) Interaction between nitric oxide and ethylene in the induction of alternative oxidase in ozone-treated tobacco plants. Plant Physiol 142:595–608

Efron B, Tibshirani R (2007) On testing the significance of sets of genes. Ann Appl Stat 1:107–129

Fait A, Yellin A, Fromm H (2005) GABA shunt deficiencies and accumulation of reactive oxygen intermediates: insight from Arabidopsis mutants. FEBS Lett 579:415–420

Fait A, Fromm H, Walter D, Galili G, Fernie AR (2008) Highway or byway: the metabolic role of the GABA shunt in plants. Trends Plant Sci 13:14–19

Fukao T, Bailey-Serres J (2004) Plant responses to hypoxia—is survival a balancing act? Trends Plant Sci 9:449–456

Fukao T, Bailey-Serres J (2008) Ethylene—a key regulator of submergence responses in rice. Plant Sci 175:43–51

Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, Ellis B, Gautier L, Ge Y, Gentry J, Hornik K, Hothorn T, Huber W, Iacus S, Irizarry R, Leisch F, Li C, Maechler M, Rossini AJ, Sawitzki G, Smith C, Smyth G, Tierney L, Yang JY, Zhang J (2004) Bioconductor: open software development for computational biology and bioinformatics. Genome Biol 5:R80

Gonzali S, Loreti E, Novi G, Poggi A, Alpi A, Perata P (2005) The use of microarrays to study the anaerobic response in Arabidopsis. Ann Bot (Lond) 96:661–668

Greenway H, Gibbs J (2003) *Review*: mechanisms of anoxia tolerance in plants. II. Energy requirements for maintenance and energy distribution to essential processes. Funct Plant Biol 30:999–1036

Gupta KJ, Zabalza A, van Dongen JT (2009) Regulation of respiration when the oxygen availability changes. Physiol Plant 137:383–391

Gupta KJ, Igamberdiev AU, Manjunatha G, Segu S, Moran JF, Neelawarne B, Bauwe H, Kaiser WM (2011a) The emerging roles of nitric oxide (NO) in plant mitochondria. Plant Sci 181:520–526

Gupta KJ, Hebelstrup KH, Mur LAJ, Igamberdiev AU (2011b) Plant hemoglobins: important players at the crossroads between oxygen and nitric oxide. FEBS Lett 585:3843–3849

Gupta KJ, Shah JK, Brotman Y, Jahnke K, Willmitzer L, Kaiser WM, Bauwe H, Igamberdiev AU (2012) Inhibition of aconitase by nitric oxide leads to induction of the alternative oxidase and to a shift of metabolism towards biosynthesis of amino acids. J Exp Bot 63:1773–1784

Hebelstrup KH, Igamberdiev AU, Hill RD (2007) Metabolic effects of hemoglobin gene expression in plants. Gene 398:86–93

Hebelstrup KH, van Zanten M, Mandon J, Voesenek LACJ, Harren FJM, Cristescu SM, Møller IM, Mur LAJ (2012) Haemoglobin modulates NO emission and hyponasty under hypoxia-related stress in Arabidopsis thaliana. J Exp Bot 63:5581–5591

Hill RD (2012) Non-symbiotic haemoglobins—what's happening beyond nitric oxide scavenging? AoB Plants 2012:pls004

Hoffman DL, Salter JD, Brookes PS (2007) Response of mitochondrial reactive oxygen species generation to steady-state oxygen tension: implications for hypoxic cell signaling. Am J Physiol Heart Circ Physiol 292:H101–H108

Huang S, Colmer TD, Millar AH (2008) Does anoxia tolerance involve altering the energy currency towards PPi? Trends Plant Sci 13:221–227

Hughes RK, De Domenico S, Santino A (2009) Plant cytochrome CYP74 family: biochemical features, endocellular localisation, activation mechanism in plant defence and improvements for industrial applications. ChemBioChem 10:1122–1133

Huxtable RJ (1992) Physiological actions of taurine. Physiol Rev 72:101–163

Igamberdiev AU, Hill RD (2009) Plant mitochondrial function during anaerobiosis. Ann Bot (Lond) 103:259–268

Igamberdiev AU, Baron K, Manac'h-Little N, Stoimenova M, Hill RD (2005) The haemoglobin/nitric oxide cycle: involvement in flooding stress and effects on hormone signalling [Review]. Ann Bot (Lond) 96:557–564

Igamberdiev AU, Bykova NV, Hill RD (2006) Nitric oxide scavenging by barley hemoglobin is facilitated by a monodehydroascorbate reductase-mediated ascorbate reduction of methemoglobin. Planta 223:1033–1040

Igamberdiev AU, Bykova NV, Shah JK, Hill RD (2010) Anoxic nitric oxide cycling in plants: participating reactions and possible mechanisms. Physiol Plant 138:393–404

Inzé A, Vanderauwera S, Hoeberichts FA, vanDorpe M, van Gaever T, van Breusegem F (2012) A subcellular localization compendium of hydrogen peroxide-induced proteins. Plant Cell Environ 35:308–320

Jaspers P, Kangasjärvi J (2010) Reactive oxygen species in abiotic stress signaling. Physiol Plant 138(4):405–413

Johnson WE, Li C, Rabinovic A (2007) Adjusting batch effects in microarray expression data using empirical Bayes methods. Biostatistics 8:118–127

Kataoka H, Ohnishi N (1986) Occurrence of taurine in plants. Agric Biol Chem 50(7):1887–1888

Lasanthi-Kudahettige R, Magneschi L, Loreti E, Gonzali S, Licausi F, Novi G, Beretta O, Vitulli F, Alpi A, Perata P (2007) Transcript profiling of the anoxic rice coleoptile. Plant Physiol 144:218–231

Lee SC, Mustroph A, Sasidharan R, Vashisht D, Pedersen O, Oosumi T, Voesenek LACJ, Bailey-Serres J (2011) Molecular characterization of the submergence response of the Arabidopsis thaliana ecotype Columbia. New Phytol 190:457–471

Lewis DFV (2002) Oxidative stress: the role of cytochromes P450 in oxygen activation. J Chem Technol Biotechnol 77:1095–1100

Li C, Chen Q, Gao X, Qi B, Chen N, Xu S, Chen J, Wang X (2005) AtHsfA2 modulates expression of stress responsive genes and enhances tolerance to heat and oxidative stress in Arabidopsis. Sci China C Life Sci 48:540–550

Licausi F (2011) Regulation of the molecular response to oxygen limitations in plants. New Phytol 190:550–555

Licausi F, Van Dongen JT, Giuntoli B, Novi G, Santaniello A, Geigenberger P, Perata P (2010) HRE1 and HRE2, two hypoxia-inducible ethylene response factors, affect anaerobic responses in Arabidopsis thaliana. Plant J 62:302–315

Lindermayr C, Saalbach G, Bahnweg G, Durner J (2006) Differential inhibition of Arabidopsis methionine adenosyltransferases by protein S-nitrosylation. J Biol Chem 281:4285–4291

Loenarz C, Schofield CJ (2008) Expanding chemical biology of 2-oxoglutarate oxygenases. Nat Chem Biol 4:152–156

Loreti E, Poggi A, Novi G, Alpi A, Perata P (2005) A genome-wide analysis of the effects of sucrose on gene expression in Arabidopsis seedlings under anoxia. Plant Physiol 137:1130–1138

Luhua S, Ciftci-Yilmaz S, Harper J, Cushman J, Mittler R (2008) Enhanced tolerance to oxidative stress in transgenic Arabidopsis plants expressing proteins of unknown function. Plant Physiol 148:280–292

Manac'h-Little N, Igamberdiev AU, Hill RD (2005) Hemoglobin expression affects ethylene production in maize cell cultures. Plant Physiol Biochem 43:485–489

Mittler R, Vanderauwera S, Gollery M, Van Breusegem F (2004) Reactive oxygen gene network of plants. Trends Plant Sci 9:490–498

Mustroph A, Lee SC, Oosumi T, Zanetti ME, Yang H, Ma K, Yaghoubi-Masihi A, Fukao T, Bailey-Serres J (2010) Cross-kingdom comparison of transcriptomic adjustments to low-oxygen stress highlights conserved and plant-specific responses. Plant Physiol 152:1484–1500

Narsai R, Rocha M, Geigenberger P, Whelan J, van Dongen JT (2011) Comparative analysis between plant species of transcriptional and metabolic responses to hypoxia. New Phytol 190 (2):472–487

Navarre DA, Wendehenne D, Durner J, Noad R, Klessig DF (2000) Nitric oxide modulates the activity of tobacco aconitase. Plant Physiol 122:573–582

Navrot N, Rouhier N, Gelhaye E, Jacquot J (2007) Reactive oxygen species generation and antioxidant systems in plant mitochondria. Physiol Plant 129:185–195

Nelson D, Werck-Reichhart D (2011) A P450-centric view of plant evolution. Plant J 66:194–211

Newton MA, Quintana FA, den Boon JA, Sengupta S, Ahlquist P (2007) Random-set methods identify distinct aspects of the enrichment signal in gene-set analysis. Ann Appl Stat 1:85–106

Obata T, Matthes A, Koszior S, Lehmann M, Araújo WL, Bock R, Sweetlove LJ, Fernie AR (2011) Alteration of mitochondrial protein complexes in relation to metabolic regulation under short-term oxidative stress in Arabidopsis seedlings. Phytochemistry 72:1081–1091

Petrov VD, Van Breusegem F (2012) Hydrogen peroxide—a central hub for information flow in plant cells. AoB Plants 2012:pls014

Planchet E, Jagadis Gupta K, Sonoda M, Kaiser WM (2005) Nitric oxide emission from tobacco leaves and cell suspensions: rate limiting factors and evidence for the involvement of mitochondrial electron transport. Plant J 41:732–743

Potters G, Pasternak TP, Guisez Y, Jansen MAK (2009) Different stresses, similar morphogenic responses: integrating a plethora of pathways. Plant Cell Environ 32:158–169

Pucciariello C, Parlanti S, Banti V, Novi G, Perata P (2012) Reactive oxygen species-driven transcription in Arabidopsis under oxygen deprivation. Plant Physiol 159:184–196

Qiao W, Fan L (2008) Nitric oxide signaling in plant responses to abiotic stresses. J Integr Plant Biol 50:1238–1246

Qu Z, Zhong N, Wang H, Chen A, Jian G, Xia G (2006) Ectopic expression of the cotton non-symbiotic hemoglobin gene GhHbd1 triggers defense responses and increases disease tolerance in Arabidopsis. Plant Cell Physiol 47:1058–1068

Rhoads DM, Umbach AL, Subbaiah CC, Siedow JN (2006) Mitochondrial reactive oxygen species. Contribution to oxidative stress and interorganellar signaling. Plant Physiol 141:357–366

Ritchie ME, Silver J, Oshlack A, Holmes M, Diyagama D, Holloway A, Smyth GK (2007) A comparison of background correction methods for two-colour microarrays. Bioinformatics 23:2700–2707

Rocha M, Licausi F, Araujo WL, Nunes-Nesi A, Sodek L, Fernie AR, van Dongen JT (2010) Glycolysis and the tricarboxylic acid cycle are linked by alanine aminotransferase during hypoxia induced by waterlogging of Lotus japonicus. Plant Physiol 152:1501–1513

Sairam R, Kumutha D, Ezhilmathi K, Deshmukh P, Srivastava G (2008) Physiology and biochemistry of waterlogging tolerance in plants. Biol Plant 52:401–412

Sakamoto A, Sakurao S, Fukunaga K, Matsubara T, Ueda-Hashimoto M, Tsukamoto S, Takahashi M, Morikawa H (2004) Three distinct Arabidopsis hemoglobins exhibit peroxidase-like activity and differentially mediate nitrite-dependent protein nitration. FEBS Lett 572:27–32

Santosa I, Ram P, Boamfa E, Laarhoven L, Reuss J, Jackson M, Harren F (2007) Patterns of peroxidative ethane emission from submerged rice seedlings indicate that damage from reactive oxygen species takes place during submergence and is not necessarily a post-anoxic phenomenon. Planta 226:193–202

Shimoda Y, Nagata M, Suzuki A, Abe M, Sato S, Kato T, Tabata S, Higashi S, Uchiumi T (2005) Symbiotic rhizobium and nitric oxide induce gene expression of non-symbiotic hemoglobin in Lotus japonicus. Plant Cell Physiol 46:99–107

Smyth GK (2005) LIMMA: linear models for microarray data. In: Bioinformatics and Computational Biology Solutions Using R and Bioconductor; Gentleman R, Carey V, Huber W, Irizarry R, Dudoit S (eds) Springer, New York, pp 397–420

Stoimenova M, Igamberdiev AU, Gupta K, Hill RD (2007) Nitrite-driven anaerobic ATP synthesis in barley and rice root mitochondria. Planta 226:465–474

St-Pierre J, Brand MD, Boutilier RG (2000) Mitochondria as ATP consumers: cellular treason in anoxia. Proc Natl Acad Sci U S A 97:8670–8674

Sweetlove LJ, Beard KFM, Nunes-Nesi A, Fernie AR, Ratcliffe RG (2010) Not just a circle: flux modes in the plant TCA cycle. Trends Plant Sci 15:462–470

Törönen P, Ojala P, Marttinen P, Holm L (2009) Robust extraction of functional signals from gene set analysis using a generalized threshold free scoring function. BMC Bioinformatics 10:307

Trevaskis B, Watts RA, Andersson CR, Llewellyn DJ, Hargrove MS, Olson JS, Dennis ES, Peacock WJ (1997) Two hemoglobin genes in Arabidopsis thaliana: the evolutionary origins of leghemoglobins. Proc Natl Acad Sci U S A 94:12230–12234

Van Breusegem F, Bailey-Serres J, Mittler R (2008) Unraveling the tapestry of networks involving reactive oxygen species in plants. Plant Physiol 147:978–984

van Dongen JT, Frohlich A, Ramirez-Aguilar SJ, Schauer N, Fernie AR, Erban A, Kopka J, Clark J, Langer A, Geigenberger P (2009) Transcript and metabolite profiling of the adaptive response to mild decreases in oxygen concentration in the roots of Arabidopsis plants. Ann Bot (Lond) 103:269–280

Wang R, Guegler K, LaBrie ST, Crawford NM (2000) Genomic analysis of a nutrient response in Arabidopsis reveals diverse expression patterns and novel metabolic and potential regulatory genes induced by nitrate. Plant Cell Online 12:1491–1509

Wulff A, Oliveira HC, Saviani EE, Salgado I (2009) Nitrite reduction and superoxide-dependent nitric oxide degradation by Arabidopsis mitochondria: influence of external NAD(P)H dehydrogenases and alternative oxidase in the control of nitric oxide levels. Nitric Oxide 21:132–139

Yan B, Dai Q, Liu X, Huang S, Wang Z (1996) Flooding-induced membrane damage, lipid oxidation and activated oxygen generation in corn leaves. Plant Soil 179:261–268

Yoo S, Sheen J (2008) MAPK signaling in plant hormone ethylene signal transduction. Plant Signal Behav 3:848–849

Yun B, Feechan A, Yin M, Saidi NBB, Le Bihan T, Yu M, Moore JW, Kang J, Kwon E, Spoel SH, Pallas JA, Loake GJ (2011) S-nitrosylation of NADPH oxidase regulates cell death in plant immunity. Nature 478:264–268

Zabalza A, van Dongen JT, Froehlich A, Oliver SN, Faix B, Gupta KJ, Schmalzlin E, Igal M, Orcaray L, Royuela M, Geigenberger P (2009) Regulation of respiration and fermentation to control the plant internal oxygen concentration. Plant Physiol 149:1087–1098

Zhao L, Gu R, Gao P, Wang G (2008) A nonsymbiotic hemoglobin gene from maize, ZmHb, is involved in response to submergence, high-salt and osmotic stresses. Plant Cell Tissue Organ Cult 95:227–237

Zhao M, Chen L, Zhang L, Zhang W (2009) Nitric reductase-dependent nitric oxide production is involved in cold acclimation and freezing tolerance in Arabidopsis. Plant Physiol 151:755–767

http://www.springer.com/978-3-7091-1253-3

Low-Oxygen Stress in Plants
Oxygen Sensing and Adaptive Responses to Hypoxia
van Dongen, J.; Licausi, F. (Eds.)
2014, XI, 426 p. 49 illus., 33 illus. in color., Hardcover
ISBN: 978-3-7091-1253-3