Low Oxygen Signaling and Tolerance in Plants

FRANCESCO LICAUSI AND PIERDOMENICO PERATA

PlantLab, Scuola Superiore Sant’Anna, Piazza Martiri della Libertà 33, 56127 Pisa, Italy

I. Introduction: Plant Cells Dealing with Low Oxygen .......................... 140
II. Oxygen Sensing in Eukaryotes .................................................. 142
III. Oxygen Sensors in Plants ......................................................... 143
   A. Direct Sensing by Oxygen Binding ........................................ 144
   B. Indirect Oxygen Sensing ..................................................... 145
IV. Low-Oxygen Signal Transduction in Plants .................................. 147
   A. Transcriptional Regulation of Hypoxic Signal ......................... 148
   B. Other Elements Involved in Hypoxic Signaling ....................... 152
V. Low-Oxygen Related Stresses: Energy Deficits and Consequences ...... 154
   A. CoX, AoX, and Impaired Energy Production ............................ 155
   B. Drawbacks of Metabolic Adaptations to Hypoxia ...................... 156
   C. The Re-Oxygenation Stress .................................................. 157
VI. Metabolic Adaptation to Energy Crisis ...................................... 157
   A. Lactate Synthesis and Accumulation ..................................... 158
   B. Ethanol Production ............................................................. 159
   C. Other Products of the Anaerobic Metabolism ............................ 160
   D. Reserves Mobilization to Fuel the Glycolytic Flux .................. 161
   E. Mitochondrial Function Under Low-Oxygen Conditions .............. 164
VII. Dealing with Oxygen Shortages: Avoidance Strategies ................. 165
   A. Leaf Gas Films ................................................................. 166
   B. Fast Elongation ............................................................... 167
   C. Low Oxygen-Induced Adventitious Rooting ............................. 169
   D. Aerenchyma Formation ...................................................... 170
ABSTRACT

Plants often experience low-oxygen conditions, not only as an environmental stress condition but also as part of their normal developmental process. Oxygen deficiency signaling in the plant cell has been shown to involve reactive oxygen and nitrogen species, hormones, and calcium as secondary messengers, similarly to the low-oxygen signaling observed in other eukaryotic systems. However, in plants, evidences suggesting the existence on an oxygen sensor are scant. To date, research efforts have been aimed at understanding the strategies used by plants to low oxygen. Anatomical modifications, which encompass leaf elongation, adventitious rooting production and aerenchyma formation, can help the plants to avoid the stress consequent to low oxygen availability. On the other hand, metabolic adaptations enable the plant tissue to survive while experiencing oxygen deficiency, mainly coupling structural maintenance with energy saving. Application of the knowledge already available to crops may provide solutions for both agricultural and industrial processes involving low-oxygen conditions.

I. INTRODUCTION: PLANT CELLS DEALING WITH LOW OXYGEN

The Earth is the only planet in the solar system with an oxygen rich atmosphere. Evolving biological organisms have developed mechanisms that link efficient energy production with oxygen availability. This has made life on earth strictly dependent on oxygen not only for energy production but also for a number of different biochemical reactions. However, oxygen limitations are a normal part of the developmental process, especially in multicellular organisms which, as a consequence, have developed different solutions for oxygen transport.

Despite the fact that plants are able to produce oxygen in their photosynthetic tissues, non-green organs are extremely sensitive to low-oxygen concentrations. Although a well developed aerenchyma can be effective in conducting oxygen in some organs, plants, unlike animals, lack efficient oxygen distribution systems (Van Dongen et al., 2008).

While anoxia is usually described as a condition whereby Cytochrome c oxidase (COX) is impeded to donate electrons to oxygen, given its extremely low concentration, hypoxia has been defined as an expression of inadequate
oxygen availability that hinders other oxidases, such as the Alternative oxidase (AOX) while COX retain a limited capacity (Igamberdiev and Hill, 2008). According to this definition, hypoxia is, therefore, independent of the actual concentration of oxygen, but rather depends on wide variety of factors such as the rate of respiration, porosity, and thickness of the tissue.

The most studied environmental condition associated with hypoxia is flooding, since gas diffusion in water is 10,000 times slower than in air (Armstrong, 1979). However submergence does not exactly coincide with low-oxygen stress, since the first condition imply a plethora of other factors, such as ethylene entrapment, CO$_2$ accumulation, reduction in light intensity and increased amounts of reduced compounds to toxic levels (Bailey-Serres and Voeselek, 2008). Underwater, photosynthetic tissues can still provide oxygen if light conditions permit, but competition from microorganisms is usually so strong in water saturated (waterlogged) soils that, often, a hypoxic or even anoxic condition is established. In crop plants especially, this can lead to a huge reduction in yield (Setter and Waters, 2003). However, flooding is not the only determinant for hypoxia in plant tissues: densely packed cell structures or tissues with high metabolic rates can suffer from hypoxia even when external oxygen concentrations are close to that of the atmosphere (21%). Both conditions occur to developing seeds, for which, an oxygen concentration between 2 and 10 $\mu$M has been reported (Borisjuk et al., 2007; Van Dongen et al., 2004; Vigeolas et al., 2003).

Interestingly, plants growing under microgravity conditions also experience hypoxia. This is demonstrated by the increased expression of a reporter gene under the control of a promoter of a well-known anaerobiosis-induced gene, alcohol dehydrogenase (ADH), in transgenic Arabidopsis during spaceflights.

The ability to manipulate whole plants or plant organs under hypoxic or anoxic conditions can also be useful in industrial processes. Usually fruit, cut flowers, and vegetables are maintained in a controlled, low-oxygen atmosphere, to delay the metabolic processes, primarily respiration, and to prolong their shelf life. Physiological and molecular responses in fruit (oranges, avocados) have therefore been investigated (Kanelis et al., 1991; Pasentsis et al., 2007). One particular case of industrial interest in plant cell anaerobiosis is Chlamydomonas reinhardtii. This green alga in anoxia uses protons as final electron acceptors, leading to hydrogen production (Melis and Happe, 2004). Unfortunately anoxic conditions are, as expected, extremely challenging for the cell. Therefore the study of this alga and its adaptive mechanisms to low oxygen is acquiring more and more interest (Mus et al., 2007).
II. OXYGEN SENSING IN EUKARYOTS

To achieve rapid and highly tuneable responses, oxygen signaling should rely on one or more sensors capable of immediately perceiving when oxygen concentrations fall below a critical level and should be able to trigger different responses depending on the organ, tissue or cell type. A number of direct and indirect sensors probably represent the best solution to cope with a dynamic stress situation, such as a slow shift from hypoxia to complete anoxia through increasingly harsh hypoxic conditions, followed by a rapid re-oxygenation.

In mammals and yeast, where events involved in low-oxygen sensing have been extensively studied, the general sensing machinery seems to be conservatively split into different mechanisms that either directly measure the oxygen level within the cell or perceive variations in oxygen-dependent metabolites. In mammals, the hypoxia inducible factor (HIF) transcriptional complex plays a major role, since it is regulated at both a transcriptional and posttranscriptional level. Briefly, HIF is a heterodimer composed of HIF-α (hypoxia inducible), and HIF-β (constitutively expressed) subunits, all members of the bHLH-PAS (PER-ARNT-SIM) family of transcription factors (Wenger, 2002). The HIFα subunits can be hydroxylated in an oxygen dependent manner on two prolyl residues by prolyl-4-hydroxylases (PHDs) that requires molecular oxygen as co-substrate. Hydroxylated HIFα is directed to the 26S proteasome by the von Hippel–Lindau tumor suppressor protein (pVHL) that represents the recognition subunit of an E3 ubiquitin-protein ligase (Acker et al., 2006). Moreover, prolyl hydroxylation of the alpha subunits inhibits binding of the co-activator protein p300 and the cAMP response element binding CREB binding protein, necessary for assembling the RNA polymerase II complex. Reduced availability of oxygen is immediately reflected in lower rates of HIF1-α hydroxylation and degradation, giving way to a transcriptional activator complex that is translocated to the nucleus where it can regulate downstream genes (Semenza, 2007).

In fission yeast *Saccharomyces cerevisiae*, SRN1, a basic helix–loop–helix leucine zipper, is responsible for the induction of those genes required for hypoxic growth (Todd et al., 2006). In normoxic conditions SRN1 is bound to the membrane but, when the oxygen level decreases, this transcription factor is activated by proteolysis, releasing an N-terminal fragment, SRN1N, which is translocated to the nucleus. SRN1 activation by low oxygen is partially explainable by an inhibition of sterol synthesis (Goldstein et al., 2006), but it is also mediated by hypoxic stabilization. In fact, in a similar way to mammals, SRN1 is rapidly degraded, under normoxic conditions, by
OFD1, a 2-oxoglutarate dioxygenase (PHD) which hydroxylates SRN1 at a specific prolyl residue (D’Angelo et al., 2003; Hughes and Espenshade, 2008).

It is interesting to note that, apparently counter intuitively, hydroxylating enzymes acting as sensors are upregulated under low-oxygen conditions (Hughes and Espenshade, 2008). A possible explanation for this feedback mechanism is that the cell needs to rapidly adapt to normoxic conditions after re-oxygenation.

Hydroxylation and subsequent degradation of the transcriptional activator are not the only oxygen-sensing mechanisms that have been found in eukaryots. It has been suggested that reactive oxygen species (ROS) production via NADPH oxidase or mitochondrial electron transport chain (ETC) and reactive nitrogen species (RNS), via NO-synthase isoforms, act on oxygen signaling stabilizing HIF1-α (Appelhoff et al., 2004). In fact ROS and RNS contribute to changing the cellular redox state and to imbalancing the iron population from Fe^{2+} to Fe^{3+}, which is no longer a co-factor for PHDs (Semenza, 2007).

Energy charge, AMP levels, calcium (Ca^{2+}) concentrations and ion channels have also been suggested as inter-dependent modulators of hypoxic signaling in different mammal cell types (Fähl ing, 2008).

### III. OXYGEN SENSORS IN PLANTS

It is still unknown how plants do sense oxygen. Different hypotheses have been provided, involving direct oxygen binding or metabolism. However, while some of them have been excluded from the plethora of candidates for the oxygen sensing, others still need to be characterized. Indirect sensing, via signal compound(s) accumulated during oxygen shortage, is also likely to contribute to trigger or modulate the hypoxic response in the plant cell.

Gibbs and Greenway (2003) raised the question whether oxygen sensing occurs ubiquitously in all plant cells, or it is rather triggered by “anoxic cores,” that is, portions of tissue which suffer of limitations in oxygen diffusion and therefore become anoxic while surrounding tissues are still provided with oxygen for respiration (Berry and Norris, 1949). An alternative hypothesis would state that all plant cells can perceive different degrees of oxygen deficiency. Evidences for this second hypothesis come from the recent literature, since the hypoxic response in Arabidopsis protoplasts (Baena-Gonzalez et al., 2007) is largely overlapping with that of whole plants (Branco-Price et al., 2005), and the extent of induction of anaerobic genes is inversely proportional to the environmental O_{2} concentration in Arabidopsis roots (Van Dongen et al., 2008). However both hypotheses
imply the presence of a molecular sensor, that directly or indirectly, perceive the actual oxygen level in the cell.

A. DIRECT SENSING BY OXYGEN BINDING

Non-symbiotic hemoglobins were the first proteins to be put forward as oxygen sensors in plants (Appleby et al., 1988). Early objections to this hypothesis were based on the high affinity of non-symbiotic hemoglobins for oxygen (Garrocho-Villegas and Arredondo-Peter, 2008). In fact, a low-oxygen response is taking place at oxygen concentrations that does not affect the binding of oxygen to hemoglobins. Nevertheless, non-symbiotic hemoglobins are induced under low-oxygen levels and have also been characterized as playing a major role in nitric oxide detoxification and hydrogen peroxide scavenging under hypoxia (Perazzolli et al., 2004). In plants, no orthologs of the animal HIF1-α or the fungal SRN1 have been found so far. This is not surprising, since there is virtually no similarity between HIF1-α and SRN1. Also fungal and animal PHDs appear to be divergent, despite the conservativeness of the catalytic domain. Nevertheless, in plant genomes several members of the PHD family have been predicted. However so far, few have been characterized, and none have been investigated in terms of low-oxygen sensing.

Plant PHDs resemble HIF1-α PHD because they act as monomers instead of tetramers composed of two subunit types (α2β2) such as collagen PHDs. In Arabidopsis, two of the six PHDs have been characterized so far. AtPHD-1 can hydroxylate collagen and HIF1-α-type peptides, together with plant prolyl-rich peptides (Hieta and Myllyharju, 2002). Prolyl-rich sequences in plants are characteristic of cell wall proteins that include proline-rich glycoproteins, extensins, and arabinogalactanproteins. These proteins all require hydroxylation at the prolyl rich moiety to be glycosylated with arabinogalactan oligosaccharides or larger arabinogalactan polysaccharides (Lamport, 1965; Pope, 1977). Interestingly, several of these proteins are upregulated by hypoxia and anoxia in Arabidopsis and rice (Lasanthi-Kudahettige et al., 2007; Loreti et al., 2005). At-PHD-2, the second Arabidopsis PHD to be characterized, is unable to hydroxylate HIF1-α-like substrates (Tiainen et al., 2005). Several prolyl-4-hydroxylase are induced by hypoxia and anoxia under low-oxygen conditions, in rice and Arabidopsis (Branco-Price et al., 2005; Lasanthi-Kudahettige et al., 2007; Loreti et al., 2005).

Even though oxygen sensing through O2 dependent enzyme activities is conserved among animals and fungi, it may take place in a different way in
plant cells. Microarray analyses of Arabidopsis roots exposed to different hypoxic conditions, from 8% to 1% O₂ (Van Dongen et al., 2008), and of rice coleoptiles under anoxia (Lasanthi-Kudahettige et al., 2007) to low oxygen revealed that several genes encoding for enzymes, whose activities rely on oxygen availability, are upregulated. For instance, genes encoding for proteins of yet unknown function but with a high degree of similarity to the animal dioxygenase Cysteamil-dioxygenase (At5g39890 and At5g15120 in Arabidopsis, Os01g09030 and Os07g01090 in rice) are induced to a very large extent in both plant species. The highly conserved aminoacidic structure and anaerobic induction among plant species deserves investigation in terms of the role of these proteins in anaerobic signaling or tolerance.

B. INDIRECT OXYGEN SENSING

Hypoxia does not necessarily have to be sensed by the plant cell by directly measuring the oxygen concentration. As in other eukaryotes, other signal molecules could act as indirect oxygen signals.

1. Energy shortage

Good candidate molecules as signals of low oxygen are those whose concentration changes depending on a reduction in oxygen availability are ATP and ADP or the whole pool of adenosine nucleotide phosphates, which together make up the energy charge. Efforts to distinguish direct low-oxygen responses and adaptations due to changes in the energy charge have been performed using Arabidopsis, pea roots and potato tubers as models (Riewe et al., 2008; Zabalza et al., 2009).

Although a general energy deficit is usually associated with stress since efficient energy production, structural maintenance, and development are impaired, oxygen shortage is probably the stress condition most directly linked to a reduction in energy availability. Recently, two Arabidopsis protein kinases belonging to the SnRK superfamily KIN10 and KIN11 were reported to regulate the common transcriptional response to darkness and multiple stresses that bias energy availability (Baena-Gonzalez et al., 2007). Both proteins are orthologs of yeast SNF1 and mammal AMPK, which are activated under hypoxia and ischemia and, once activated by increasing the AMP to ATP ratio, switch off energy consuming processes and activate catabolism. However, the transcriptome changes mediated by KIN10 only partially overlap with the anaerobic changes, suggesting that the SnRKs only partially contribute to the low-oxygen response.
2. Reactive oxygen and nitrogen species

ROS and, more recently RNS, have been proposed as also playing a role in oxygen sensing in plants. Two antithetical hypotheses deal with ROS production and low-oxygen signaling. According to the oldest and more intuitive hypothesis, a drop in oxygen levels is associated with a reduction in ROS synthesis. The second hypothesis, first formulated by Blokhina et al. (2001), holds that ROS is produced in the apoplast of both tolerant (*Iris pseudoacorus* and *Oryza sativa*) and intolerant (*Triticum aestivum* and *Iris germanica*) plant species under anoxic conditions. Further evidence for ROS production under hypoxic conditions has been provided by Santosa et al. (2007). They reported the emission of ethane, a volatile product deriving from membrane peroxidation, in rice seedlings subjected to flooding or a low-oxygen atmosphere. Subsequent re-oxygenation further increased the ethane emission but only for a short time (less than 2 h). A further link between ROS and low-oxygen signaling is strongly supported by H$_2$O$_2$ production by a NADPH oxidase as requirement for a controlled induction of *ADH* in Arabidopsis (Baxter-Burrell et al., 2002). Treatment using diphenyleneiodonium (DPI), an inhibitor of H$_2$O$_2$ production via NADPH oxidase, was in fact able to inhibit the hypoxic increases in ADH activity in Arabidopsis seedlings. H$_2$O$_2$ accumulation under low oxygen was demonstrated to depend on a ROP (Rh of Plants) rheostat. ROPs are monomeric proteins capable of binding GTP. When bound to GTP, ROPs are active and can be inactivated by dephosphorylation of the guanosin nucleotide. Inactivation of RopGAP4 in *Arabidopsis thaliana* causes an increase in *ADH* induction under anoxia. A link between ROS and hypoxic signaling has also been shown by the fact that ZAT12 and ZAT10, two transcription factors involved in oxidative stress responses, are also induced by low-oxygen treatment (Davletova et al., 2005; Rossel et al., 2007). Moreover, overexpression of ZAT12 leads to the repression of some anaerobic genes during treatment at 4 °C. (Vogel et al., 2005), such as *ADH*, *Sucrose synthase 4 (SUS4)* and *Non-symbiotic Hemoglobin 1 (HBI)*.

Nitric oxide (NO) has been recently shown to play a role in both signaling and tolerance to low oxygen. NO production from nitrite (NO$_2^-$), which probably occurs through the combined activities of rotenone-insensitive NAD(P)H dehydrogenases, mitochondrial complex III (ubiquinone: Cytochrome c reductase) and IV (COX), increases as a consequence of the transition from normoxia to hypoxia (Igamberdiev and Hill, 2008). This could be explained by an increased synthesis rate or by an increase in NO stability directly linked to a lack of oxygen. Molecular O$_2$ reacts quickly with NO to generate NO$_2$. Nitric oxide is then able to inhibit oxygen consumption by COX, therefore preventing the tissue from becoming anoxic (Geigenberger, 2003). Moreover NO has been shown to also inhibit oxygen
LOW OXYGEN SIGNALING AND TOLERANCE IN PLANTS

consuming reactions involved in storage metabolism and to promote mitochondrial ROS production (Amirsadeghi et al., 2006; Borisjuk et al., 2007). NO can also exert its biological role through the nitrosylation of tyrosine and cysteine and transition metals. The activities of several plant proteins involved in transcription, the central metabolism, stress responses and innate immunity have so far been shown to be regulated by S-nitrosylation (Abat et al., 2008; Grennan, 2007; Huber and Hardin, 2004; Lindemayr et al., 2006; Serpa et al., 2007).

3. pH as a low-oxygen signal

Another candidate signal for the plant cell to sense the shift from aerobic to hypoxic or anoxic condition may be represented by the cytoplasmic acidification that rapidly takes place under hypoxia concomitantly with a slower increase in external (or apoplastic) pH. Indeed, under low-oxygen conditions, production of lactate and protons leaking from the vacuole can significantly lower the cytoplasmic pH (reviewed by Magneschi and Perata (2009) and Perata and Alpi (1993)). pH changes have been suggested to act as signal from the root to the shoot during drought stress (Davies and Zhang, 1991), while cytosolic acidification may represent a precondition for gene activation in response to attacks by pathogens (He et al., 1998). The evidence that pH decrease under oxygen deprivation with a rate that is not constant, but reaches a new set point after a few hours, would support its role in signaling the oxygen (or energy) shortage. Felle (2001), using microprobes, non-invasively inserted in the sub-stomatal cavity of leaves, showed that pH changes cannot be transferred on long distance. In fact, imposing acidification to the root system or to the cut petiole was not sufficient to cause rapid and significant pH changes in the leaves. However, it cannot be ruled out that the hypoxic acidification occurring in the roots triggers a secondary signal, able to be transported to the roots. However, as proposed by Felle (2001) changes in extracellular and intracellular pH are very common responses to a wide number of stress and stimuli, and pH alone can not be sufficient to signal a specific stimulus. pH should rather be characterized as having an additional signaling effect, synergistic with a specific low-oxygen signal.

IV. LOW-OXYGEN SIGNAL TRANSDUCTION IN PLANTS

The search for signaling components in low-oxygen plant responses has been carried out mostly using genetic approaches; although some reverse genetic approaches have provided encouraging results. Provided the relatively vast
amount of data on the hypoxic response at the transcriptional level, the easiest starting points with respect to the search for signal transducer have been the functional analysis of induced proteins known to be involved in signaling in other conditions, or sharing homology with these. At the same time, analyses of the promoter sequences of well studied and uncharacterized genes was aimed at the identification of DNA elements required for their induction. These represent the ideal targets for transcription factors that play a major role in the transcriptional response to anaerobiosis in plants. Subsequent analyses on these transcription factors by means of transgenic approaches, involving either overexpression or silencing provided, in some cases, a clear demonstration of the relevance of specific transcription factors in species or variety specific manner. Analyses on miRNAs, whose expression changes during submergence stress, started to shed light at a further step in the regulation of gene expression under oxygen deprivation (Zhang et al., 2008). A different, still fruitful, approach in the identification of the hypoxic signal transduction has been based on the analyses of molecules already proved to have a similar role in other stress conditions, as it happened for calcium and phosphorylation cascades.

Several whole transcriptome analyses on different plant species and different tissues under hypoxia and anoxia have led to the identification of a number of genes that make up primary anaerobic responses (Branco-Price et al., 2005; Gonzali et al., 2005; Klok et al., 2002; Lasanthi-Kudahettige et al., 2007; Mus et al., 2007; Pasentsis et al., 2007; Van Dongen et al., 2008). Besides several genes involved in maintaining metabolisms and structures, a number of genes involved in signal transduction and gene transcription has also been identified.

A. TRANSCRIPTIONAL REGULATION OF HYPOXIC SIGNAL

1. Cis-acting elements
An extensive search for DNA elements responsible for gene induction has been carried out over the last 20 years. A first anaerobic response element (ARE) with a GGTTC core was subsequently rejected as a general element for hypoxic induction since it was also present in genes whose expression does not change under anaerobiosis (Russell and Sachs, 1989; Walker et al., 1987). The Arabidopsis ADH promoter probably represents one of the most studied DNA sequences among the anaerobic genes. It has been shown to contain a general repressor element in its 5' second half, while hypoxic regulation depends on the first 172 nucleotides that are upstream of the transcription-starting site, encompassing GT and GC boxes. The G box, which is usually associated with light-regulated genes, does not influence gene
expression under hypoxia. It has also been reported that cold and ABA regulation occur via elements that are not required for hypoxic induction (Dolferus et al., 1994).

A comparison of glycolytic enzyme promoters that are upregulated under low oxygen and flooding in different plant species has confirmed these elements, but has also identified five new ones (5′-AAACAAA-3′, 5′-AGCAGC-3′, 5′-TCATCAC-3′, 5′-GTTT(A/C/T)GCAA-3′, and 5′-TTCCCTGTT-3′), for which no binding protein has previously been reported (Mohanty et al., 2005). In rice, ADH and PDC expression has also been shown to increase biphasically: an initial expression takes place after 2 h and a second one after 12 h of flooding stress. Both have been studied with regard to the posttranslational modifications occurring at the histone octamer associated with the 5′ sequence upstream the ADH gene. The first event was shown to be associated with a change of histone 3 (H3) from di- to tri-methylation while acetylation of H3 and H4 occurred at the time of the second inductive step. Both modifications were shown to increase the binding of RNA polymerase II in the genomic region, suggesting that ADH upregulation is mediated, at least partially, at a transcriptional level (Tsuji et al., 2006).

2. Trans-acting elements
A number of transcription factors belonging to different protein families (AP2-ERF, LOB, WRKY, MYB) are coherently upregulated in rice and Arabidopsis under anoxia. So far only a few have been characterized with respect to hypoxia signaling and tolerance. AtMYB2, a member of the MyB family of transcription factors, has been shown to be able to bind the GC motif in the ADH promoter in vitro and to upregulate the GUS reporter gene expression under the control of Arabidopsis ADH promoter in protoplasts and leaves (Hoeren et al., 1998). Interestingly, MYB2 mRNA levels were shown to increase after the addition of cycloheximide, a protein synthesis inhibitor (Hoeren et al., 1998). This observation led to the hypothesis that MYB2 represents a primary actor in anaerobic gene induction, since it is independent of transcription. Further analyses at the whole transcriptome level did not identify significant changes in mRNA levels for MYB2, under hypoxic conditions (Branco-Price et al., 2005, 2008; Van Dongen et al., 2008). Unfortunately, so far no MYB2 knockout or silenced line has been characterized in terms of the ability to induce ADH and other anaerobic genes under low oxygen. Overexpression of MYB2 in Arabidopsis plants, on the other hand, showed an increase in ADH induction, but only when exogenous ABA was applied. The same was shown in plants overexpressing another transcription factor, MYC2, whose mRNA level does not change
under low oxygen and is involved in jasmonate signaling. Interestingly, plants overexpressing both transcription factors showed a constitutively enhanced expression of \textit{ADH} and other anaerobic genes irrespectively of ABA supplementation (Abe et al., 2003). This suggests that at least one member of either the Myb or the Myc family is required for hypoxic gene regulation. Other members of the Myb family have been reported to be induced under low oxygen in Arabidopsis, rice and wheat. Interestingly, \textit{TaMYB1} expression, a MYB transcription factor from \textit{T. aestivum}, was shown to be induced by low-oxygen treatment and further enhanced by light (Lee et al., 2007). The activation of these transcription factors, however, may take place via posttranslational modification or interaction with other transcription factors. DNA binding of \textit{MYB2} has in fact been demonstrated to be inhibited by \textit{S}-nitrosylation of a conserved cystein residue (Serpa et al., 2007). \textit{MYBLEU}, another member of the Myb rice family, has been proposed to play a role in gene activation under low oxygen (Locatelli et al., 2000). Heterologous expression of this transcription factor in Arabidopsis has been reported to lead to elongation in the primary roots and in the internodal region of the floral stem, together with an enhancement of tolerance under oxygen deficiency (Mattana et al., 2007).

Among the transcription factors that are upregulated under low-oxygen conditions, the AP2-ethylene responsive factor (ERF) family seems to be the most conserved, with at least one member reported to be induced in nearly all the plant species considered (Lasantha-Kudahettige et al., 2007). All the low-oxygen ERF members belong to the same group, named either VII or B2. Interestingly, rice, which is one of the few crops able to germinate in the absence of oxygen and which often experiences flooding, is the plant with the biggest VII group. Moreover \textit{Sub1a}, a variety specific ERF transcription factor, can confer tolerance to short-term flooding to intolerant varieties (Xu et al., 2006). \textit{Sub1a} overexpression in intolerant varieties was reported to induce a faster \textit{ADH} gene induction and at the same time repress the induction of genes involved in energy-expensive processes such as cell expansion (Fukao et al., 2006). The absence of \textit{Sub1a} homologues in other plant species suggests that it represents a variety specific allele that acts on a more general regulation pathway. Other transcription factors belonging to the ERFVII group have been shown to play a role in ethylene signaling as part of the response to biotic and abiotic stresses (Jung et al., 2007; Xu et al., 2007; Youm et al., 2008). Increased transcription of a gene does not necessarily result in accumulation of the encoded protein. Posttranscriptional mechanisms may also regulate gene expression by regulating the levels of a specific mRNA or its translational rate through the activity of microRNAs (miRNAs). They are approximately 21-nt-long, noncoding RNA produced
by excision from a stem-loop precursor. Despite their critical role in mediating development and response to stresses (Jones-Rhoades and Bartel, 2004), only few analyses of miRNAs associated with low oxygen or flooding conditions have been published. Zhang et al. (2008) recently reported the change in expression of more than 100 miRNAs maize roots after 12, 24, and 36 h flooding. miRNAs which target sucrose degradation and carbohydrate breakdown were repressed after 24 and 36 h flooding. Surprisingly, one miRNA (osa-miR528-like) which regulates genes involved in ROS and acet-aldehyde detoxification was upregulated. Among the upregulated miRNAs, zma-miR166 may regulate the root meristem cell differentiation under submergence stress in maize, since this miRNA can target transcription factors such as Rolled leaf 1 (Rdl1), a homologue of the Arabidopsis HD-ZIP. Moreover repression of zma-miR167 and zma-miR168, which target auxin responsive factors (ARFs) at the early submergence stage, was suggested to mediate the formation of adventitious rooting in the hypocotyl of submerged maize seedlings.

3. Transcriptional regulation of the anaerobic response in Chlamydomonas

Low-oxygen signaling in Chlamydomonas cells requires a specific discussion. Among genes induced by anoxic treatment in Chlamydomonas, only those belonging to the general energy starvation response were upregulated. However no anoxia-regulated transcription factors in Chlamydomonas shared homology with those induced in multicellular plants. On the other hand, many of the enzymes involved in glycolysis, fermentation and stress responses were the same, indicating a conserved response from unicellular algae to higher plants (Mus et al., 2007). Hypoxic responses in Chlamydomonas have been reported to mirror that of copper deficiency in the induction of Cytochrome Cpx1 (encoding coprogen oxidase, a tetrapyrrole biosynthesis enzyme) and Crd1 (encoding a putative di-iron enzyme) (Eriksson et al., 2004). Moreover treatments with HgCl2, an inhibitor of the copper deficiency response, also blocked the hypoxic response. A physiological connection between copper- and oxygen-deficiency-induced gene expressions was therefore proposed (Moseley et al., 2000; Quinn and Merchant, 1995). A common copper response element, called CuRe, whose core sequence is GTAC and is present in the promoter, has been shown to be necessary and sufficient to stimulate gene expression under copper deficiency. This element was shown to be necessary but not sufficient for low-oxygen induction, which also required the presence of a hypoxic response element (HyRE), which shares the same core sequence as the CuRe.

A trans-acting element capable of regulating copper deficiency responses has been associated with the copper response regulator locus (CRR1).
Mutation in the *err1* locus leads to inhibition of low copper responses but only part of the low-oxygen response, since the hydrogenases gene was still induced under low oxygen. Cloning of the *err1* locus led to the identification of a putative transcription factor with a plant-specific DNA-binding domain called SBP, ankyrin repeats, and a C-terminal Cys-rich region, which is similar to a Drosophila metallothionein. Since *err1* only slightly increases with copper deficiency, it has been proposed that it acts as a positive regulator after being activated at the protein level (Kropat *et al.*, 2005).

**B. OTHER ELEMENTS INVOLVED IN HYPOXIC SIGNALING**

Signal cascades in stress physiology usually end up with the activation of transcription factors which, in turn, activate genes involved in adaptation and promote tolerance to the biological system. However, the path from the signal to the activation of transcription factors is mediated by several effectors that need to interact with each other and with components of the developmental program. A great number of proteins and small molecules that play this role have been extensively studied in relation to abiotic stresses different from anaerobiosis. The major components in abiotic stress signaling have been hypothesized as being phosphorylation/dephosphorylation events, variations in Ca^{2+} concentrations and ROS.

In mammalian cells, a decrease in oxygen levels activates voltage-gated plasma membrane channels triggering an influx of Ca^{2+}, while stimulating an efflux from the mitochondria (Fählmg, 2008). It has been shown that ruthenium red (RR), an inhibitor of calcium flux from organelles, is sufficient to block the induction of *ADH1* and *SH1* (encoding a sucrose synthase isoform) in maize seedlings and reduced their ability to survive short time flooding. On the other hand, the supplementation of calcium prevented RR effects (Subbaiah *et al.*, 1994b). Other authors then found that Ca^{2+} is necessary for anaerobic induction of *ADH* in Arabidopsis and rice (Chung and Ferl, 1999; Tsuji *et al.*, 2000).

Taking advantage of fluorescence imaging and photometry of Ca^{2+} in maize suspension-cultured cells, Subbaiah *et al.* (1994a) demonstrated an immediate increase in calcium levels in cytosol, which was fully reversible a few seconds after re-oxygenation. This first increase in calcium level was followed by a second, more persisting one. The existence of a double, temporally distinct, Ca^{2+} increase in Arabidopsis seedlings was also reported by Sedbrook *et al.* (1996) who used a calcium sensitive luminescent protein Aequorin. Repeated anoxic treatment, with the addition of a calcium chelator EGTA and PM calcium channel blockers hampered the first spike in Ca^{2+} concentration.
The hypothesis that a calcium increase in the cytosol comes from mitochondria stemmed from the observation that these organelles are probably the first to be influenced when oxygen levels drop. Observations that Ca$^{2+}$ accumulates in the cytoplasmic periphery of mitochondria using confocal fluorescence microscopy strongly enforced this hypothesis (Subbaiah et al., 1998). The mechanism through which Ca$^{2+}$ is released from mitochondria still needs elucidation. However, treatment with caffeine, which promotes calcium releases without membrane depolarization, induced ADH in normoxic conditions. Exposing caffeine treated cells to anoxia, leads to a further increase in calcium, suggesting that Ca$^{2+}$ is released through an initial, caffeine sensitive and Na$^+$ or H$^+$-Ca$^{2+}$ antiport and, later, via mitochondrial membrane depolarization (Subbaiah et al., 1998). Greenway and Gibbs (2003) proposed that a variation in pH of 0.5, which occurs a few minutes after the onset of anoxia, is sufficient for calcium to move against its electrochemical gradient using energy provided by an influx of protons in the mitochondria (Greenway and Gibbs, 2003; Gunter et al., 1994).

Calcium was also shown to be involved in the induction of a cystein protease (calpaine), starting from the root apex and then spreading to the root axis in maize roots (Subbaiah et al., 2000). Calcium is not considered to play a role on proteolytic activity in plant calpaines, since these proteins lack the calcium-binding domain IV (Margis and Margis-Pinheiro, 2003; Wang et al., 2003). However, the induction of this protease has been proposed as being responsible for cell death occurring at the root tip and, in fact, de-tipping maize roots improved root tolerance to anoxic stress (Subbaiah et al., 2000).

Oxygen consumption at the ETC is also probably Ca$^{2+}$-mediated. In potato tubers, calcium was shown to inhibit the AOX pathway mitochondria energized by NADH or succinate, but only when the cytochrome pathway was inhibited by cyanide (Mariano et al., 2005). Moreover, in the absence of cyanide, calcium stimulates NO degradation via the activation of NADPH dehydrogenase, therefore preventing the inhibition of COX (complex IV) (De Oliveira et al., 2008).

Despite the fact that mitogen-activated kinases (MAPKs) play a role in almost all signaling and developmental programs in plants (Colcombet and Hirt, 2008). Indeed, although these kinases have been shown to modulate hypoxic signaling in mammals (Fähling, 2008), little is known about their role with respect to oxygen deficit in plants. A minimal MAP kinase cascade consists of three levels: MAP3Ks, which phosphorylate MAP2Ks which, in turn, phosphorylates MAPKs which activate different signaling components (Colcombet and Hirt, 2008). Not many genes encoding for MAP kinases of phosphatases are upregulated under low-oxygen conditions in Arabidopsis
Fig. 1. Integrative view of the different pathways proposed to be involved in hypoxic signaling.

(Branco-Price et al., 2008; Loreti et al., 2005). This is however not surprising, since a rapid signal transduction should rely more on existing peptides rather than waiting for new components to be synthesized, especially when translation is impaired because of energy deficit. Two MAPKs are already upregulated after 2 h hypoxia in Arabidopsis: M KK9 and M KK11. M KK9 was reported to stimulate ethylene synthesis and abiotic stress responses through the activation of MPK3 and MPK6 (Xu et al., 2008). M KK11, together with M YB2, have been reported to be regulated by ABA in a ROP10 dependent manner (Xin et al., 2005) (Fig. 1).

V. LOW-OXYGEN RELATED STRESSES: ENERGY DEFICITS AND CONSEQUENCES

The main cellular stress caused by low oxygen availability consists in a reduced respiration and, therefore, lower energy production, resulting in slower metabolic processes. Recently, studies in pea and Arabidopsis roots suggested that plant cell adjust respiration to oxygen availability to prevent, or at least postpone, anoxia (Zabalza et al., 2009). When respiratory activity is reduced or blocked, energy production is limited to the glycolytic process. To avoid slowing in the glycolytic flux, plant cells activate fermentative pathways whose end products, if accumulated, could be detrimental to the
plant survival. Especially, cytosolic acidification has been observed as a consequence of the energy deficit, although it is unclear whether this is a cause or a consequence of cell death under low-oxygen conditions. Moreover, return to normoxia following a hypoxic or anoxic condition also implies a new stress condition, because of the production or reactive oxygen and nitrogen species.

A. COX, AOX, AND IMPAIRED ENERGY PRODUCTION

In all aerobic organisms, a decrease in oxygen concentrations inside the cell results in reduced energy production via oxidative phosphorylation. This is because $O_2$ represents the ideal last electron acceptor in the mitochondrial electron transport chain (mETC) (Geigenberger, 2003).

Two types of mitochondrial terminal-oxidases play a role in the oxidative phosphorylation pathway: the cyanide-sensitive COX and cyanide-insensitive alternative oxidase (AOX). The COX complex (IV) consists of at least nine peptides, of which the three largest are mitochondrial encoded, and whose activity is linked to active ATP synthesis (Millar et al., 2004). The AOX complex on the other hand, is a simple homodimer encoded by genomic genes and surprisingly its activity is not coupled to ATP production. The expression of $AOX1a$ is instead increased in conditions that impair mitochondrial activity, such as abiotic stresses (Clifton et al., 2005). AOX may play a role in avoiding oxidative stress and modulating metabolic flexibility in plants (Plaxton and Podestá, 2006; Watanabe et al., 2008).

When oxygen levels drop, the COX function is reduced since its $K_m$ for oxygen is in the order of 10 $\mu$M (Affourtit et al., 2001; Millar et al., 2004) and it is inhibited by NO (Dordas et al., 2003). Zabalza et al. (2009) suggested that adaptation of respiration under hypoxia takes place at the COX level, since its activity in pea roots follows a biphasic trend, as the respiratory activity does, while AOX activity decreases just linearly. Under hypoxia, energy production within the cell is provided mainly by glycolysis, which requires constant NAD$^+$ regeneration to work efficiently (Perata and Alpi, 1993). The energy status of the cell can be measured by two parameters: adenylate energy charge (AEC), which indicate the proportion of high energy phosphate bonds in the nucleotide reserve pool ($AEC = ATP + 0$, $5ADP$ AT $P + ADP + AMP$), and ATP production, expressed as a percentage of sugar catabolism in the air. These two parameters are both necessary since AEC alone represents the energy status in the plant only until there is no further decrease in the nucleotide pool (Greenway and Gibbs, 2003). In complete anoxic conditions, energy production, based on rates of ethanol formation, can vary between 3 and 37.5% when compared to production in
normoxic conditions. These variations depend on the glycolytic flux and the amount of polysaccharides (starch or sucrose) used as a starting source (Greenway and Gibbs, 2003). Several authors have reported an increased glycolytic ATP production, when conditions become completely anoxic, defined as a “Pasteur effect,” but this is not enough to prevent the energy deficit (Gibbs et al., 2000; Neal and Girton, 1955; Vartapetian, 1982). Moreover, slowing of glycolysis during prolonged (24–64 h) anoxic treatment has been proposed to occur in rice coleoptiles (Colmer et al., 2001) and aged storage tissue of red beet (Zhang and Greenway, 1994), estimated by observing reduced ethanol accumulation.

B. DRAWBACKS OF METABOLIC ADAPTATIONS TO HYPOXIA

Continuous substrate availability and regeneration of oxidized NAD are required for an efficient glycolysis. This is achieved mainly through fermentative pathways: a short initial lactic and longer lasting ethanolic fermentation (Perata and Alpi, 1993). Lactic acid accumulation can be toxic to the plant tissues since its dissociation would contribute in decreasing cytosolic pH, while ethanol barely reaches dangerous concentrations in the cells since the membranes are permeable, provided a gradient in maintained (Davies, 1980). However, ethanol can probably reach high values in bulky tissues such as tubers, rhizomes or seeds sealed by the testa. Concentrations of around 70 umol g$^{-1}$ fresh weight have been measured for *I. germanica* rhizomes treated for 16 days in anoxic conditions in a humid atmosphere (Monk et al., 1984). Evidence from carrot protoplasts cultures, showed that ethanol toxicity is directly linked to acetateode production. The addition of 4.15–5.35 mM ethanol to aerobic cultures delayed cell growth, however treatment with 4-methylpyrazole, an inhibitor of ADH, prevented the toxic effects of exogenous ethanol up to 40–80 mM, while toxic effects were obtained by directly adding acetateode (Perata and Alpi, 1991; Perata et al., 1984). Acetateode, produced by ethanol oxidation, can also be a problem for the plant when aerobic conditions are restored since it may react with proteins and DNA (Perata et al., 1992) or act as an electron donor for ROS production via xantine oxidase (Mustroph et al., 2006). Acetateode production upon re-oxygenation may represent a pathway for consuming H$_2$O$_2$ via catalase mediated ethanol peroxidation (Zuckermann et al., 1997). Oxygen shortage also lead to a drop in cytoplasmic pH in almost all the plant systems studied so far, though this can vary among species, varieties, organs, and tissues (Felle, 2005, 2006). Initial cytoplasmic acidification has been proposed to be caused by the accumulation of organic acids, such as lactic acid (Vartapetian and Jackson, 1997), which dissociates rapidly. However,
Saint-Ges et al. (1991) showed that, in anoxically shocked maize root tips, a decrease in pH preceded a peak in lactate concentration. Gout et al. (2001) linked the initial drop in cytoplasmic pH to the hydrolysis of NTP to NMP, while Greenway and Gibbs (2003) suggested the strong inhibition of vacuolar H\(^+\) ATPase as the cause of this phenomenon. H\(^+\) influx together with K\(^+\) efflux across the plasma membrane and tonoplast, and organic acids accumulation in the cytoplasm are the most likely causes of acidification when anaerobic conditions last more than few hours.

It is still not clear whether acidosis, in anoxic cells, is a cause or a consequence of cell death (Greenway and Gibbs, 2003; Roberts et al., 1984; Tadege et al., 1998; Xia and Roberts, 1994); however, tolerance is strongly associated with a reduction in cytosolic acidification: in both excised shoots of anoxia tolerant rice (O. sativa cv. Arborio) and wheat (T. aestivum cv. MEK) a quick drop in cytosolic pH was observed within the first 30 min of anoxic treatment. However, while in rice this did not drop below pH 7.1, in wheat it reached values a pH value of 6.6 (Menegus et al., 1991).

C. THE RE-OXYGENATION STRESS

A return to the aerobic state from low-oxygen conditions leads to further, mainly oxidative, stress possibly due to ROS and NOS production caused by mitochondria malfunctioning (Smirnoff, 1995). ROS and NOS can react with proteins, inhibiting their functions with nucleic acids, and with polyunsaturated lipids generating a peroxidation chain reaction, which leads to general membrane damage. In rice, ROS detoxifying enzymes show a reduced expression when coleoptile grows under anoxic conditions rather than in air (Lasanthi-Kudahettige et al., 2007). However, it has also been reported that flooded seedlings, when exposed to normoxia, restored catalase activity to a greater extent than aerobic controls, suggesting ROS production upon re-oxygenation (Biemelt et al., 1998). The synthesis of catalase may represent a waste of energy under anoxia (Fukao and Bailey-Serres, 2004; Magneschi and Perata, 2009); however, it is also possible that ROS scavenging is even detrimental in low oxygen, since it hampers a possible signal for regulation and adaptation.

VI. METABOLIC ADAPTATION TO ENERGY CRISIS

When plants, or some of their organs, cannot avoid hypoxic conditions, they try to metabolically adapt to cope with the stress.
As mentioned previously, energy production under anaerobic conditions mainly depends on the glycolytic pathway, which, in turn, requires the regeneration of NAD\(^+\). As respiration is reduced, pyruvate is rapidly accumulated in concentrations similar to the \(K_m\) of pyruvate decarboxylase (PDC), whose affinity for the substrate is usually lower than that of pyruvate dehydrogenase (PDH) (Pronk et al., 1996).

Therefore, accumulated pyruvate can be used as a substrate in fermentative pathways that regenerate NAD\(^+\). Pyruvate has also been shown to be strongly linked to the ability of plants to adjust oxygen consumption by respiration (Zabalza et al., 2009). A shift from pyruvate utilization by the TCA cycle to the fermentative pathways is associated with a decrease in PDH mRNA translation (Branco-Price et al., 2005) and an upregulation of PDH kinase, which inactivates PDH (Marillia et al., 2003).

Almost all plants studied so far showed an increase in ethanolic and, at lower levels, lactic fermentations under hypoxia and anoxia, although to different extents and with different effects on tolerance. Therefore, despite its conservativeness in nearly all the eukaryotes, fermentation alone is not necessarily able to confer resistance. A crucial point may rather be the efficiency in mobilizing the carbohydrate reserves during hypoxia. Other reactions, producing mainly alanine and \(\gamma\)-aminobutyric acid (GABA), may also contribute to NAD\(^+\) regeneration (Bailey-Serres and Voesenek, 2008). A further, recently suggested, role for the fermentative pathways is to regulate the pyruvate level within the cell, therefore modulating the respiration rate under hypoxia (Zabalza et al., 2009). As for many other stress conditions, efficient metabolic adaptation to low oxygen requires time. Therefore a gradual acclimation from mild hypoxia such as \(O_2\) levels at which respiration is reduced to half of the maximum rate to more extreme conditions, often has a positive effect on tolerance (Saglio et al., 1988). The environmental relevance of this phenomenon with respect to flooding is questionable since it depends on the type of the soil and the temperature (Gibbs and Greenway, 2003). However it may be of great importance in case of slow hypoxia onset, such as the one caused by growth of bulky tissues or envelopes (Borijusk et al., 2007).

A. LACTATE SYNTHESIS AND ACCUMULATION

Lactic fermentation occurs through the reduction of pyruvate by lactate dehydrogenase (LDH) with the concomitant oxidation of NADH to NAD\(^+\). A rapid activation of LDH has been observed in almost all plant species (e.g., *O. sativa*, *Zea mays*, *Solanum lycopersicum*, *Solanum tuberosum*) (Christopher and Good, 1996; Rivoal and Hanson, 1994; Rivoal et al., 1991;
Sweetlove et al., 2000). Glycolytic flux to lactate is quite low compared with that to ethanol in several plant species, with rates usually not higher than 1 μmol g^-1 fresh weight h^-1. Lactic acid accumulation can be harmful for the cell, since it quickly dissociates lowering cytosolic pH. However, lactate production may contribute to a posttranslational regulation of the fermentative pathways. According to this hypothesis, lactate-induced acidification would adjust pH to an optimal value for PDC activity, therefore channeling NAD regeneration toward ethanol synthesis via ADH (Davies, 1980). However, in maize roots, increased ethanolic fermentation is observed prior to cytosolic acidification (Saint-Ges et al., 1991). However, in some species, such as in Solanaceae, anaerobic LDH isoforms may play a role in pyruvate regeneration during long-lasting hypoxic conditions. In fact, in Solanum lycopersicum, hypoxia inducible LDH1 has been shown to possess greater activity toward pyruvate synthesis, whereas, constitutive LDH2 catalyzes the reaction in direction of lactate production (Germain et al., 1997a). The observation of LDH-silenced potato tubers is in agreement with this hypothesis, since transgenic tubers contain twofold more lactate than wild types (Sweetlove et al., 2000). Lactate is continuously produced in Arabidopsis tissues 2 h after the onset of hypoxia and its level rises further after 9 h. However, a high cytosolic accumulation of this metabolite is probably prevented by lactate extrusion via a hypoxia-inducible nodulin intrinsic protein NIP2;1 (Choi and Roberts, 2007). A similar function can be assumed for some pleiotropic drug resistance (PDR) type ATP-binding cassette (ABC) transporters, whose expression in rice is indeed regulated by lactate and other weak acids (Moons, 2008). Considering these premises, lactate production does not likely to play a major role in low-oxygen intolerance, since its production is restricted in time and the small amount of lactate produced during the first hours of hypoxia is easily expelled from the cell. Moreover, overexpression of LDH in Arabidopsis significantly increases root tip survival and root tip growth compared with the wild-type plants in non-hypoxically pre-treated tissues submitted to anoxic stress (Dolferus et al., 2008). The observation that increased LDH activity also stimulates ethanolic fermentation led Dolferus et al. (2008) to hypothesize that lactic fermentation in Arabidopsis is either required to initiate or to favor the ethanolic fermentation.

B. ETHANOL PRODUCTION

Unlike lactic fermentation, ethanolic fermentation consists of two reactions: the conversion of pyruvate to ethanol proceeds through the coupled reactions of PDC and ADH. Of the two enzyme activities, PDC controls
anaerobic sugar catabolism in several plant species, as shown with different transgenic approaches. Tobacco leaves overexpressing PDC produced 10–20-fold more ethanol than wild types under anoxia (Bucher et al., 1994). Transgenic Arabidopsis overexpressing either PDC1 or PDC2 showed about 50–150% ethanol concentrations compared to wild types after 24 h in a 5% O₂ atmosphere, whereas ADH overexpression led to very small increases (10–20%) (Ismond et al., 2003). In addition, overexpression of both PDC isoforms, but not ADH, enhanced survival rates under low-oxygen conditions (Ismond et al., 2003). In general, except for ADH knockout mutants, where ADH activity is completely abolished, no correlation has been observed between ADH activity (measured in vitro) and ethanol production (Roberts et al., 1989). This suggests that ADH activity is not crucial to anoxia tolerance in ideal situations such as those in laboratories. It can be hypothesized that maximum activity, on the other hand, is crucial in environmental conditions where plants experience different stress conditions at the same time, or when ADH is required after re-oxygenation, to utilize ethanol as a carbon source (Gibbs and Greenway, 2003).

C. OTHER PRODUCTS OF THE ANAEROBIC METABOLISM

Alanine and GABA are the other two products of the anaerobic metabolism (Bailey-Serres and Voesenek, 2008; Magneschi and Perata, 2009). Alanine is produced by the transfer of an amino group from glutamate to pyruvate, with the generation of α-ketoglutarate. The continuous removal of α-ketoglutarate contributes to NAD(P)⁺ regeneration and hinders the reverse reaction toward alanine degradation. This can be achieved by the glutamine synthetase/glutamate synthase cycle GS-GOGAT (Reggiani et al., 1988) or the glutamate dehydrogenase pathway (Fan et al., 1997). The observation that NH₄⁺ incorporation in the GS-GOGAT pathway would consume 1 ATP molecule per pyruvate molecule converted to alanine, whereas GDH does not consume ATP and regenerates 1 NADP⁺ molecule seems to favor the glutamate dehydrogenase pathway (Gibbs and Greenway, 2003).

In fact, a modest increase in GDH2 mRNA translation has been observed in Arabidopsis (Branco-Price et al., 2008).

Glutamate is involved in several anaerobic metabolic reactions, as demonstrated by its decreased content after 2 h hypoxia in Arabidopsis (Branco-Price et al., 2008). It can act as an amino group donor in aspartate transamination, with the production of the TCA intermediate oxalacetate, which is then converted to malate thus generating NAD⁺. Aspartic-transaminase mRNA has been reported to be both induced and actively translated under hypoxia (Branco-Price et al., 2008). Alternatively, glutamate can be
decarboxylated by glutamate decarboxylase to generate γ-Aminobutyric acid (GABA), with a concomitant H⁺ consumption and therefore counteracting the cytosolic acidification caused by lactic fermentation (Aurisano et al., 1995). GABA transaminase (GABA-T) metabolizes GABA to succinic semi-aldehyde (SSA) coupling consumption of α-ketoglutarate and additional conversion of pyruvate to alanine (Breitkreuz et al., 2003). SSA is possibly reduced to γ-hydroxybutyrate by a NADPH consuming reaction catalyzed by GHB dehydrogenase. None of these enzymes seem to be regulated at the transcriptional or translational level (Branco-Price et al., 2008); therefore GABA accumulation ought to be explained by posttranslational activation of GDH or inactivation of GABA-T. Upon re-oxygenation SSA is probably converted to succinate, and then channeled to the TCA cycle by succinic semialdehyde dehydrogenase (SSADH) in a NAD⁺-consuming reaction. SSADH activity has been reported to inhibit ROS formation (Bouché et al., 2003). In agreement with this, while SSADH is induced under low oxygen, it is highly translated only upon re-oxygenation, when ROS are likely to be formed. A link between hypoxic accumulated GABA and secondary metabolism has been proposed by Liu and Castelfranco (1970), who hypothesized reactions in pea seedlings involving ethanol incorporation with the formation of ethyl-β-glucoside, which are part the cell wall.

D. RESERVES MOBILIZATION TO FUEL THE GLYCOLYTIC FLUX

Provided that there is a group of efficient reactions that regenerates NAD⁺, glycolysis coupled to the fermentative metabolism requires constant carbohydrate supplementation. In storage organs such as cereal grains, potato tubers, and Acorus calamus rhizomes, hexose provision requires efficient starch mobilization via starch degrading enzymes, such as endo-amylases, exo-amylases, debranching enzymes, and starch phosphorylase (Magneschi and Perata, 2009; Perata and Alpi, 1993; Smith et al., 2005; Stitt, 1990). Starch phosphorylase may act on starch degradation in the late phases of low-oxygen stress, as demonstrated by its three- to sixfold increase in activation after 2 days of flooding treatments in rice. This enzyme could play a role in stress tolerance, since rice intolerant varieties did not show any increase in Starch phosphorylase (Das et al., 2000). Rice seeds, but not wheat, barley, oat, and rye which are unable to germinate under anoxia, induce α-amylase genes in low-oxygen conditions (Guglielminetti et al., 1995). α-Amylases in rice have been grouped into three subfamilies: AMY1 (A–B–C), AMY2 (A) and AMY3 (A–B–C–D–E–F) (Magneschi and Perata, 2009). Isoforms encoded by AMY1A are present both in aerobic and anaerobic seedlings, while AMY3D mRNA is anoxia-specific (Loreti et al., 2003). This has been
explained by a differential regulation at a transcriptional level: *AMY1A* is induced by gibberellins (GA) in air but not in anoxia, whereas *AMY3D* does not depend on GA since its promoter lacks the distinctive cis-acting element that confers GA-responsiveness (Morita et al., 1998; Loreti et al., 2003; Perata et al., 1997). On the other hand, anaerobic induction of *AMY3D* is repressed by high sugar levels, suggesting that under aerobic conditions, GA-dependent activation of *AMY1A* maintains high sugar levels in the aleurone layer, thus preventing *AMY3D* induction. Sugar dependent regulation of *AMY3D* depends on cis-acting DNA elements contained in its promoter, named SRC (sugar repression core) (Chen et al., 2006) and on transacting transcription factor *MYBS1* (Lu et al., 2002). *MYBS1* is more expressed in anoxic rice coleoptiles compared to aerobic controls (Lasanthi-Kudahettige et al., 2007). It has also been shown that both *AMY3D* and *MYBS1* are activated by the general regulator OsSnRK1 (Ismond et al., 2003; Lu et al., 2007), belonging to the same superfamily of the Arabidopsis KIN10 and KIN11, and already reported to play a role in sugar and energy depletion signaling (Baena-Gonzalez et al., 2007).

During germination in rice seeds, starch-derived glucose is transferred to the scutellum, where sucrose synthesis takes place (Nomura et al., 1969). Although this anabolic step is energy expensive, it may be required to transport carbon units to the growing tissues of the developing seedlings, thus providing substrate for glycolysis (Magneschi and Perata, 2009). Anoxic sucrose synthesis in rice seeds and Arabidopsis seedlings depends on the expression of sucrose-phosphate synthase and glucose-6-phosphate isomerase, but not on sucrose synthase (Bertani et al., 1981; Guglielminetti et al., 1995, 1999; Ricard et al., 1991). In cereals unable to germinate under anoxia, no sucrose synthesis has been observed under anoxia (Guglielminetti et al., 1999).

The supplementation of exogenous sucrose but not glucose improves survival under anoxia (Germain et al., 1997a; Loreti et al., 2005). Sucrose degradation, to provide hexose-6-phosphates for glycolysis, is achieved through two distinct pathways in plant cells: the sucrose synthase (Susy) pathway, which is bi-directional but favored in the catabolic direction, and the unidirectional invertase pathway. Under low-oxygen conditions, sucrose synthase isoforms are induced in almost all the plants examined, while invertase is repressed (Branco-Price et al., 2008; Lasanthi-Kudahettige et al., 2007). Degradation of sucrose to fructose 6-P and UDP-glucose by Susy only requires one molecule of pyrophosphate if the UTP produced is directly used by fructokinase to phosphorylate fructose or to regenerate ATP from ADP via NDP-kinase (Guglielminetti et al., 1995). In contrast, the invertase pathway requires two ATP molecules per sucrose molecule.
degraded (Mustroph et al., 2005). Therefore sucrose synthase isoforms are probably induced and Susy activity is stimulated while genes encoding for invertases are repressed. The advantage conferred by sucrose synthase under hypoxia was demonstrated by the reduced ATP levels observed in transgenic potato tubers overexpressing bacterial invertase, compared to wild types under 8% O₂ (Bologa et al., 2003). The existence of more than a single hypoxia-inducible Susy isoform in several species suggests that functional redundancy is required to ensure low-oxygen tolerance in plant tissues. In fact, Arabidopsis mutants, whose hypoxia-inducible Susy genes (SUS1 and SUS4) have been knocked-down, showed an increased susceptibility to root flooding (Bieniawska et al., 2007). A sugar or energy starvation signal may mediate anaerobic upregulation of SUS1 and SUS4, since sucrose supplementation was shown to reduce their induction (Loreti et al., 2005). However, previous microarray analyses in sucrose starved Arabidopsis cells did not identify significant changes in SUS1 mRNA transcription and translation, whereas SUS4 transcription was strongly repressed (Contento et al., 2004; Nicolai et al., 2006). A non-sucrolytic role for anaerobic isoforms of the Susy family has also been proposed (Subbaiah et al., 2006) following the observation of intra-mitochondrial localization for two Susy isoforms in maize root tips together with their interaction with voltage-dependent anion channel. Subbaiah et al. (2006) hypothesized that these proteins could modulate fluxes in and out of mitochondria in an anoxia- and tissue-specific manner (Subbaiah et al., 2006).

Glucose and fructose need to be phosphorylated by hexokinases to be channeled to the glycolytic pathway. The anoxic induction of a fructokinase (OsFK2) in rice seedlings has been reported (Guglielminetti et al., 2006), together with a hexokinase OsHXK7 whose induction is probably mediated by a sucrose starvation signaling pathway (Cho et al., 2006; Lasanthi-Kudahettige et al., 2007). Hexokinase activity exerts great control over the glycolytic pathway under anoxia in excised maize root tips (Bouny and Saglio, 1996) and tomato roots (Germain et al., 1997b). Since sucrose degradation is presumed to mainly occur via a Susy pathway, glucose kinase(s) may also play a role in utilizing glucose produced either by invertase or derived from amylases activities. The generation of fructose-1,6bisP from fructose-6P can be catalyzed by the unidirectional phosphofructokinase PFK which uses ATP as a phosphate group donor, or via the bidirectional phosphofructokinase PFP which uses pyrophosphate (PP). Together with PDC, PFK may regulate carbon flux during anaerobiosis in several plant species (Faiz-ur-Rahman et al., 1974; Gibbs et al., 2000; Mohanty et al., 1993). PFP is activated under anoxia in rice (Mertens et al., 1990; Mohanty et al., 1993) but the direction of the reaction it catalyzes is uncertain. The role of these
substrate cycles between PFK and PFP is related to that of another cycle, mediated by pyruvate kinase (PK) and pyruvate orthophosphate dikinase (PPDK). Depending on PPi consumption/production rates in the cell, these two cycles perhaps work in opposite directions. Interestingly, PPDK is also upregulated by low oxygen in rice at the mRNA and protein level (Lasanthi-Kudahettige et al., 2007; Moons et al., 1998).

E. MITOCHONDRIAL FUNCTION UNDER LOW-OXYGEN CONDITIONS

Under oxygen deprivation mitochondria do not necessarily stop functioning and, though with some changes in the enzyme composition, they maintain their ultrastructure even in anoxic conditions, as showed in rice and *Echino- cloa* seedlings (Couee et al., 1992; Fox and Kennedy, 1991). Nitrate has been shown to have a positive effect on mitochondria, and it has been proposed, but not proved, to take the place of oxygen as an alternative electron acceptor in the mtETC (Vartapetian et al., 2003) or playing a role in NAD (P)H oxidation (Igamberdiev et al., 2004). Further NAD(P)H regeneration is ensured by the reduction of nitrate to nitrite, catalyzed by nitrate reductase (Igamberdiev et al., 2004), competing with production of ethanol. Interestingly, a reduction in anoxic cytoplasmic acidification has been obtained by supplementing maize root segments with nitrate (Libourel et al., 2006). Supplementing nitrate to transgenic tobacco roots with reduced nitrate reductase (NR) levels, on the other hand, showed an increased lactate and ethanol production under anoxia, suggesting competition with the fermentative pathways (Stoimenova et al., 2003). Nitrate reductase (NR) is significantly induced in Arabidopsis and rice under anoxia (Lasanthi-Kudahettige et al., 2007; Loreti et al., 2005) but not hypoxia (Branco-Price et al., 2008). Further NR activation, at the posttranslational level, is mediated by pH acidification (Kaiser and Brendle-Behnisch, 1995). Nitrite dependent NAD (P)H oxidation is catalyzed via two Ca^{2+} dependent, rotenone-insensitive NAD(P)H dehydrogenases, which transfer electrons to the ubiquinone pool at the inner membrane. As mentioned previously Ca^{2+} releases from mitochondria are triggered within few minutes of anoxic imposition. Possible explanations for Ca^{2+} involve either the H^{+}–Ca^{2+} antiport, enhanced by pH acidification, or an adenilate kinase-dependent change in Mg^{2+} concentration which, in turn, modulates calmodulin and opens Ca^{2+} pores (Igamberdiev and Kleczkowski, 2003). Electron transfer from the ubiquinone pool to nitrate, associated with NO generation, has been proposed to be mediated by complex III (Cytochrome b reductase) and IV (COX) (Igamberdiev and Hill, 2008). AOX has also been proposed to mediate NO production from nitrite (Gupta et al., 2005; Tischner et al., 2004), however there
is no strong evidence supporting this hypothesis (Igamberdiev and Hill, 2008). Nitrite to NO reduction in mitochondria from algae and higher plants has been observed under strict anaerobic conditions (Gupta et al., 2005; Planchet and Kaiser, 2006), and may generate the proton motor force required for ATP synthesis (Stolmenova et al., 2007). To prevent the inhibition of mtETC by NO accumulation, plants under hypoxic conditions have the advantage of non-symbiotic hemoglobins. The induction of members of this subfamily under low-oxygen conditions has been reported for a number of plant species (Lasanthi-Kudahettige et al., 2007; Loreti et al., 2005; Taylor et al., 1994). Non-symbiotic hemoglobins have been proposed to react with NO and oxygenate it to regenerate nitrate, thus becoming an oxidized form of ferric Hb–Fe$^{3+}$. Ferric Hb–Fe$^{3+}$ is reduced to ferrous Hb–Fe$^{2+}$ by free ascorbate with the production of the strong oxidant monodehydroascorbate (MDHA). Moreover, ascorbate and MDHA can scavenge peroxynitrite, which is formed by the reactions of NO and superoxide and can modulate protein activity via nitrosylation (Barone et al., 2003). Cytosolic MDHA reductase has been demonstrated (Igamberdiev et al., 2006) to rapidly enhance ferric Hb$^{2+}$ regeneration which then binds oxygen and starts the cycle again. Interestingly, a gene encoding MDHAR has been shown to be induced under low-oxygen conditions in Arabidopsis (Loreti et al., 2005). MDHA can also be reconverted to ascorbate by the mitochondrial transport chain (Li et al., 2002), as with succinate at the level of complex II (Szarka, 2007).

VII. DEALING WITH OXYGEN SHORTAGES: AVOIDANCE STRATEGIES

To avoid the energy crisis caused by oxygen deprivation, plants developed a number of constitutive or inducible tolerance strategies depending on their growing environment. Plant species that experience periodic and long-lasting flooding (e.g., Rumex palustris and Ranunculus sceleratus) and species able to germinate in flooded soils (e.g., O. sativa and Potagemoton pectinaus) have indeed evolved a number of strategies that enable photosynthetic tissues to reach the surface of the water and therefore provide oxygen to the organs remaining under water (Bailey-Serres and Voesenek, 2008; Voesenek et al., 2006). The group of traits underlying these strategies, elongation, adventitious rooting, and aerenchyma formation, has been named low-oxygen escape syndrome (LOES; Pierik et al., 2008). Among the three main strategies aimed to avoid low oxygen discussed in this section, fast elongation is quite controversial since it requires a considerable energy expense. Therefore, stimulation or inhibition of underwater elongation can both have adaptive
value depending on the condition where the hypoxic environment is established. Interestingly, there is a consistent overlap in the signal molecules, mainly phytohormones, which regulate these processes.

A. LEAF GAS FILMS

In terrestrial plants, gas exchanges can also take place across the cuticola that, however, can represent an impediment to O₂ uptake and CO₂ release in the leaf during the night, while in the day the opposite (O₂ release and CO₂ uptake) is required to prevent restriction of photosynthesis (Colmer and Pedersen, 2008). Some wetland species developed hydrophobic cuticles able to retain a layer of air when submerged. These gas films are also defined as plant plastrons for their similarity with gas layers of aquatic insects that enlarge gas–water interface between the tracheary elements and the water (Vogel, 2006). The leaf gas layers may act in a similar way facilitating CO₂ collection during the day and O₂ collection during the night. Moreover, Colmer and Peders (2008) speculated that the presence of gas layers could help leaves to keep the stomata open (Mommer et al., 2005). So far, no evidence for this hypothesis has been provided. However, several reports described the significant contribution provided by leaf gas films to internal aeration in partially flooded deep water rice (Raskin and Kende, 1983), a tolerant rice type under complete submergence (Pederse et al., 2009).

The origin of water repellency has been intensively studied since it is relevant to agricultural spray-application processes (Wagner et al., 2003). An extensive study on over 200 plant species revealed that a wide variety of morphologies serves as a basis for surface roughness that causes water repellency (Neinhuis and Barthlott, 1997). Trichomes, papillae-structured epidermal cells and the biochemical composition of waxes and wax crystals are supposed to be the major determinants of this property (Wagner et al., 2003). Cuticular waxes are composed by a mixture of very-long chain fatty acids (VLCFA), embedded in cutin polymers (Kunst and Samuels, 2003). Understanding the genetic control of these properties would probably provide a useful tool toward the improvement of plant resistance to flooding (Pederse et al., 2009), in a way similar to that provided by the discovery of the Sub1a gene (Xu et al., 2006). Studies on cuticular wax biosynthesis in rice and Arabidopsis identified some genes encoding enzymes directly involved in VLCFA synthesis (Chen et al., 2003; Greer et al., 2007; Rowland et al., 2006; Yu et al., 2008) and a clade of AP2/EREBP transcription factors involved in the regulation this process (Aharoni et al., 2004).
B. FAST ELONGATION

The fast growth of shoots under flooding conditions resembles in many aspects the shade avoidance syndrome (SAS), since (petiol and leaf) elongation is preceded by the imposition of a hyponastic habitus that enables the leaf blade to reach the water’s surface in the shortest time possible (Cox et al., 2003; Mommer et al., 2006). The enhanced shoot elongation is known to be mediated by ethylene (Jackson, 2008; Vreeburg et al., 2005). Ethylene accumulation within tissues under submergence has been reported in fast elongating species such as *Ranunculus sceleratus* (Samarakoon and Horton, 1984) and *R. palustris* (Voesenek et al., 1993) as well as in non-elongating species (Banga et al., 1997). So far no clear demonstration has been provided as to whether submergence induced ethylene accumulation is just a consequence of gas entrapment or enhanced synthesis. In fact, most reports on anaerobic ethylene biosynthesis involve measurements after a hypoxic or anoxic treatment (Khan et al., 1987; Peng et al., 2001). Indeed, since ethylene synthesis is oxygen dependent, it would be reasonable to expect its inhibition under anoxia, although under hypoxia synthesis of this hormone could be possible or even enhanced. Indeed low-oxygen conditions strongly induce ACC synthase (ACS) and ACC oxidase (ACO) genes in several species (Peng et al., 2005; Vrijezen et al., 1999; Zhou et al., 2001) and O$_2$ for ethylene synthesis can be provided following transport from above-water tissues. Hypoxia also induces the ethylene ETR2 receptor in Arabidopsis (Loreti et al., 2005) and OsERL1 in rice (Lasanthi-Kudahettige et al., 2007). Since ethylene signaling acts through a negative regulation by the receptors, an increase in the levels of these proteins would hamper ethylene signal during hypoxia. Instead, the induction of ethylene sensors may be explained as a block for ethylene sensitivity when tissues return to normoxic conditions (Bailey-Serres and Voesenek, 2008). In partial agreement with this, a comparison of total and polysome-associated mRNA in *Arabidopsis thaliana* seedlings treated with 2 and 9 h hypoxia showed that an increase in the mRNA level for ETR2 was not associated with the same increase in translation. However, after 1 h of re-oxygenation, the association of this mRNA with polysomes further decreased instead of increasing as expected (Branco-Price et al., 2008).

Ethylene promotes elongation also by enhancing ABA metabolism to phaseic acid while, at the same time, genes encoding ABA biosynthetic enzymes are repressed (Bailey-Serres and Voesenek, 2008; Benschop et al., 2005). In rice, ABA breakdown is mediated by an ABA 8'-hydroxylase, whose expression depends on ethylene, while inhibition of ABA biosynthetic enzymes is not ethylene dependent (Saika et al., 2007). A decrease in ABA
levels results in an increase in gibberellins (GA) activity, either following conversion of inactive GA forms to active, as in *Rumex*, or resulting from increased cell sensitivity to GA, as in rice internodes (Bailey-Serres and Voesenek, 2008). Petiole and leaf elongation rely on cell expansion and division and both mechanisms are positively regulated by ethylene and GA (Gray, 2004). Cell wall-modifying enzymes, such as expansins and xyloglucan endotransglycosidases have been reported to be regulated at the transcriptional and posttranslational level by flooding and, in maize and rice, directly by ethylene or low-oxygen treatment (Lasanthi-Kudahettige et al., 2007; Lee and Kende, 2002; Peschke and Sachs, 1994). In *R. palustris*, ethylene also triggers proton efflux into the apoplast, facilitating the actions of extensins (Vreeburg et al., 2005). In rice, GA seems to regulate cell division though the upregulation of cyclin (*CyeOs1, CycOs2*), cyclin dependent kinase and replication protein A1 (Lorbiecke and Sauter, 1998). Since all these processes require a lot of energy, a supply of carbohydrates deriving from starch or sucrose degradation and the efficient translocation of photosynthates is required. Therefore, shoot elongation provides only an ecological advantage when flooding lasts few days and the water level can be reached by at least the leaf tips plant, whereas it can be detrimental to plant survival in case of short period of flooding followed by re-aeration. In this perspective, the prevention of elongation triggered by submersion may provide a consistent acclimation value in several plant species living in environments characterized by frequent, though short-lasting, flooding events. For instance, some rice varieties belonging to the *indica* group, which can endure short-lasting submergence, have been shown to possess a specific allele, associated with the locus named SUB1, which is responsible for almost 70% of their flooding tolerance (Fukao et al., 2006). Introgression of this locus via marked assisted selection in an intolerant indica variety turned it into flooding tolerant (Xu et al., 2006). The SUB1 tolerance alleles contain three genes encoding for ERF transcription factors, called *SUB1A, SUB1B*, and *SUB1C. SUB1C*, which is present in all varieties, is GA responsive and positively regulates the expression of several expansins. *SUB1A-1*, the allele present only in tolerant varieties, is sufficient alone to provide flooding tolerance. In fact, overexpression of this gene in a submergence-intolerant *japonica* variety conferred enhanced flooding tolerance (Fukao et al., 2006). SUB1A-1, directly or indirectly, enhances the expression of the negative regulators of GA signaling SLR1 and SLRL1 (Fukao and Bailey-Serres, 2008). SLR1 may hinder the activity of the transcriptional activators, similarly to one of its Arabidopsis orthologs does with phytochrome interacting factor 3 and 4 (PIF3 and PIF4) (De Lucas et al., 2008) thus preventing induction of a subset of GA responsive genes, such as SUB1C.
Furthermore SLR1 also contains an N-terminal DELLA domain that mediates its GA-dependent degradation (Ueguchi-Tanaka et al., 2007). On the contrary, SLRL1 does not possess any DELLA domain and therefore it is not degraded in response to GA. SUB1A is also able to restrict ethylene production under submergence, limiting the ethylene-mediated enhancement of GA-responsiveness (Fukao and Bailey-Serres, 2008) therefore enabling the plant to save energy by inducing a quiescent state until the water level decreases and normoxic conditions are restored (Xu et al., 2006). Moreover, in some species such as R. palustris, the morphology of newly, underwater developed leaves changes quite dramatically, with a lower starch content, thinner cuticle and chloroplasts oriented toward the epidermis. These changes allow higher rates of CO₂ assimilation, lower CO₂ compensation points and facilitate O₂ transfers into the shoots, as shown in acclimated plants compared with non-acclimated ones (Mommer et al., 2006).

C. LOW OXYGEN-INDUCED ADVENTITIOUS ROOTING

Flooding events often result in the partial submergence of the plant and some species have evolved the ability to produce new roots closer to the surface of the water. As adventitious rooting is a process associated with several productive applications, such as rootstock propagation or in vitro plant regeneration, this process has been extensively studied. However, there have been no conclusive studies explaining this phenomenon under hypoxic conditions. For instance, several studies have reported the involvement of almost all known plant hormones in this process, but it is still not clear whether hypoxia alone can induce adventitious roots production, or whether ethylene accumulation, high humidity or high CO₂ level is also required.

Adventitious root formation begins with an increase in cell proliferation from quiescent cells modulated by a number of stimuli, including auxin, peroxidase activity, and ABA (De Klerk et al., 1999; Moncousin and Gaspar, 1983; Tari and Nagy, 1996). Rapid cell duplication generates a mass of metabolically active cells, called root primordium, whose structure resembles that of the root apical meristem (Malamy and Benfey, 1997). It is not known whether endogenous auxins are required for adventitious rooting under flooding conditions. However ABA levels have been reported to decrease in root tissues in hypoxic conditions (Smit et al., 1990). After this phase, cell replication decelerates (Friedberg and Davidson, 1971; MacLeod and McLachlan, 1975) and the primordium elongates mainly by expansion, passing through the parental tissue and penetrating the epidermis (Malamy and Benfey, 1997). This requires the degradation of pericycle and epidermis cell walls to enable the adventitious root to emerge unwounded. Cell wall-modifying
proteins such as expansins (Cho and Kende, 1997), subtilisin-like proteases (Neuteboom et al., 1999), pectate lyases (Laskowski et al., 2006), and endo-β-1,4-glycanases (Kimpara et al., 2008) may play a role in this process. It is hard to observe the induction of genes encoding these enzymes using the methods currently available since adventitious rooting processes require strictly tissue-specific gene regulations. In addition, a modulation of their activity is also likely to occur at a posttranslational level (Yoshida and Komae, 2006). However, the induction of expansins in hypoxic and anoxic conditions has been reported both in rice and Arabidopsis (Branco-Price et al., 2008; Lasanthi-Kudahettige et al., 2007). It has also been demonstrated that underwater ethylene accumulation together with a reduction in ABA levels and a concomitant increases in active GA level shows a synergistic effect on epidermal cell death further facilitating the emergence of adventitious roots (Steffens et al., 2006).

D. AERENCHYMA FORMATION

Adventitious rooting and leaf morphology modifications could not provide any real advantage to the flooded plant if oxygen, either produced by photosynthesis or taken up by the aerobic tissues, is not transported to the under water organs.

Gas flow through the intracellular spaces is probably able to provide enough oxygen to sustain root respiration and elongation in short roots (shorter than 3 cm) (Greenwood, 1967). Cell porosity is contributing considerably, as demonstrated by the correlation between increased fractional root porosity (FRP) and the ability to elongate (Justin and Armstrong, 1987). FRP depends on a number of parameters including cell packing, cortical cell configuration, ratio between porous and non-porous tissue and proportion of different configuration types. Cubic cell arrays were shown to have higher FRP when compared to hexagonal cell packing (Justin and Armstrong, 1987). As expected, the longer the root grows, the less oxygen is transported to the root tip, as demonstrated by Armstrong et al. (1983) on pea plants whose roots were growing on O₂ free medium. Oxygen levels reached values around 2.5% at the tip of pea roots 9–11 cm long. Moreover, lateral roots considerably reduced oxygen levels at the tip of the main root, where it reached values close to anoxia. Gas transport through submerged tissues is enhanced by the formation, in roots, hypocotyls and petioles, of the so called aerenchyma, a modification of parenchyma cells that generates enlarged air chambers, cavities or enlarged air spaces, which work as gas exchange channels (Chaffey, 2007; Evans, 2004). Aerenchyma can be formed through two different processes, defined as schizogeny, lysigeny, or schizo-lysigeny
and, lately also, expansigeny (Drew et al., 2000). The generation of schizo-
genous aerenchyma involves cell separation via cell wall reorganization, whereas lysigenous aerenchyma is produced as a consequence of pro-
grammed cell death (Campbell and Drew, 1983; Evans, 2004;
Gunawardena et al., 2001). Expansigeny, on the other hand, occurs via cell
division and cell enlargement and is not followed by cell separations and cell
collapse or death (Seago et al., 2005). Aerenchyma formation as Arabidopsis
was ignored until recently, when reports by Muhlenbock et al. (2007)
revealed for the first time that 12-week-old plants showed hypoxia-induced
lysigeny, although only in tissues where secondary growth was occurring.
It had been previously proposed that failure in aerenchyma formation in
young plants depended on the impossibility to entrap ethylene in lignified
xylematic structures (Evans, 2004). Therefore, the developmental stage of the
plants appears to play a role in their ability to form aerenchyma. Lysigenous
aerenchyma formation in Arabidopsis seems to require a combination of
light, H$_2$O$_2$ and ethylene signals, which affect stomatal conductance in leaves
(Mateo et al., 2004).

The induction of enzymatic activities involved in cell wall degradation
(cellulases) and cell death in maize adventitious roots depends on ethylene
production under hypoxia but not anoxia (He et al., 1996). Moreover, these
processes are inhibited in hypoxic roots by antagonists of inositol phospho-
lipids, Ca$^{2+}$-calmodulin and protein kinases. On the other hand they are
stimulated by the chemical activation of G-proteins, an increase in cytosolic
Ca$^{2+}$, or an inhibition of protein phosphatases (He et al., 1996).

From a genetic point of view, the induction of aerenchyma is subject to a
tissue-specific program involving lesion stimulating disease 1 (LSD1), a zinc
finger transcription regulator together with enhanced disease susceptibility 1
(EDS1) and phytoalexin deficient 4 (PAD4), two lipase-like proteins. These
three proteins can interact with each other and are all involved in responses
to pathogen infection, photo-oxidation and leaf senescence (Muhlenbock
et al., 2007; Ochsenbein et al., 2006; Parker et al., 1996).

The expression of LSD1, EDS1, and PAD4 is induced by an increase in
CO$_2$ concentration, together with phosphorus depletion, which is also asso-
ciated with flooded and hypoxic environments (Drew et al., 1979; Topa and
Cheeseman, 1994). A search on microarray databases showed that LSD1 is
also moderately upregulated by low-oxygen treatments in Arabidopsis young
plants (4 and 7 days old) in low-oxygen conditions. The longitudinal O$_2$
transfer from shoots to the root apex can be enhanced by the formation, in
adventitious roots, of an external cell layer characterized by low radial
permeability to O$_2$, a barrier to radial O$_2$ loss (ROL) (Armstrong and
Beckett, 1987; Colmer, 2003). While in some wetland plants this barrier to
ROL is constitutively present in adventitious roots (e.g., Juncus effusus, Echinochloa crus-galli, Schoenoplectus validus) (McDonald et al., 2001, 2002; Visser et al., 2000) in others, for example, in O. sativa and some wild Hordeum species, the ROL barrier is induced by hypoxia (Colmer et al., 1998; Garthwaite et al., 2003; Jackson and Colmer, 2005) (Fig. 2).

VIII. FUNCTIONAL MAINTENANCE OF THE CELL AND ENERGY SAVING

Cell survival under oxygen deprivation relies on the ability to maintain a minimal functionality that minimizes energy costs and sustains membrane integrity and cellular compartmentation. To cope with the energy crisis, ATP-expensive processes such as protein synthesis are strongly reduced, while the limited amount of ATP produced is used to counteract the detrimental cytosolic acidification by fuelling the $\text{H}^+$ transport to the vacuole. Presence of anaerobic proteins preceding an anoxic stress improves the tolerance of the tissues, as shown by experiments on hypoxic acclimation. Surprisingly, also heat acclimation can improve anoxia tolerance, probably because of the induction of proteins, such as the heat shock proteins (HSP) that respond to both stimuli.
A. ADAPTATION OF THE TRANSLATIONAL MACHINERY TO THE ENERGY SHORTAGE

Despite the possibility of producing energy under low-oxygen conditions, plant tolerance also relies on the ability to reduce energy requirements for the maintenance of minimal cell functions and prevention of cell death. A decrease in transcription of most of the aerobic proteins associated with different aerobic cell functions has in fact been reported in rice and Arabidopsis (Branco-Price et al., 2008; Lasaniti-Kudahettige et al., 2007). In Arabidopsis, a reduction in the association of mRNA encoding for aerobic proteins with polysomes has already been reported after 2 h of hypoxic stress, which reverses within 1 h of re-oxygenation. A general analysis of genes known to be involved in cytosolic or organelar translation revealed an average threefold repression of these proteins under hypoxia, suggesting an energy saving mechanism similar to that observed for mild dehydration stress (Kawaguchi and Bailey-Serres, 2005; Kawaguchi et al., 2004) and sucrose starvation (Nicolai et al., 2006). A bioinformatic analysis was conducted to find out whether differential ribosome loading under hypoxia could be caused by specific RNA motifs in the 5’ or 3’ untranslated regions (TRL). General features such as short UTR sequences (75–250 nt) and low GC content positively correlate with polysome association during hypoxia (Branco-Price et al., 2005). The UTR sequences spanning the ADH coding sequences have been extensively studied with respect to their ability to regulate mRNA translation. It was reported that a 17 nt element in the 5’UTR of ADH mRNA was able to increase its translation rate without affecting its stability under hypoxia and heat stress (Bailey-Serres and Dawe, 1996; Mardanova et al., 2007). mRNA translation in eukaryotes involves the assembly of the translation initiation complex on the mRNA through an interaction of the eIF4E subunit with the 5’ m7GpppN cap. The translation initiation complex then recruits the small ribosomal subunit to scan the mRNA for a favorable AUG start codon. The amount of eIF4E can be limiting for translation and hypoxia has been shown to reduce transcription and translation of the eIF4E isoforms. When cap-dependent protein synthesis is impaired, translation has been proposed to be achieved by a recruitment of the initiator complex at an internal ribosome entry site (IRES) (Baird et al., 2006; Komar and Hatzoglou, 2005; Macejak and Sarnow, 1991). ADH mRNA contains IRESs but they have been proved to contribute only marginally to overall translation in tobacco cells (Mardanova et al., 2008). However these experiments were done in aerobic cells, when there was no decrease in eIF4E levels. Further investigation, under anaerobic conditions would probably help toward an understanding of the importance of IRES in ADH translation during hypoxia.
B. CONTROL OF PH ACIDIFICATION DURING OXYGEN DEPRIVATION

Despite its possible role as a signal in hypoxic cells, cytoplasm acidosis could be extremely threatening for cell viability if not efficiently controlled. Greenway and Gibbs (2003) described the possible involvement of a biochemical pH stat to mitigate acidification. In other words, a set of biochemical reactions that compensate for the synthesis of weak organic acids. A mechanism that has been suggested to play this role includes the decarboxylation of acids, supported by the formation of γ-aminobutyric acid via glutamate decarboxylation (Drew, 1997). This relates to the consumption of nitrate and the accumulation of cations, whose paradigm is putrescine biosynthesis from arginine in rice (Reggiani et al., 1989). The importance of putrescine in rice coleoptile has been shown by the use of [α-difluoromethylarginine (DFMA) an inhibitor of putrescine synthesis, which was able to prevent anoxic coleoptiles elongation (Reggiani et al., 1989) while putrescine supplementation increased survival of wheat roots under anoxia (Reggiani et al., 1990). The formation of NH₄⁺ and its retention within the cell may also play a role in pH maintenance: high levels of NH₄⁺ have been reported in hypoxic tomatoes (Horchani et al., 2008) and NH₄⁺ efflux from rice seedlings was also observed (Menegus et al., 1993). A general strategy of anoxia tolerant plants to reduce energy costs to compensate for the proton leakage into the cytoplasm is to increase vacuolar pH (Greenway and Gibbs, 2003), by decreasing the loading of undissociated acids in the tonoplast. A further reduction of proton leakage from vacuoles into the cytosol in tolerant plants is probably due to a switch from vacuolar ATPase to P₅₃ase to pump H⁺ back into the vacuole (Brauer et al., 1992). The inhibition of H⁺–ATPase possibly depends on low ATP availability (Saint-Ges et al., 1991) but this is still under discussion (Greenway and Gibbs, 2003), while the induction of H⁺–PP₅₃ase has been reported at the protein and mRNA level in rice seedlings (Carystinos et al., 1995). A drop in cytoplasmic pH may also play a role in low-oxygen adaptation: Webster et al. (1991) related the inhibition of translation under anoxia to cytoplasmic acidification, demonstrating in vitro that translation is blocked by low pH values. Greenway and Gibbs (2003) suggested that pH is a fine tuner of ethanolic fermentation, and Tournaire-Roux et al. (2003) demonstrated that anoxic aquaporin closure depends on decreasing pH.

C. HYPOXIC AND HEAT TREATMENTS LEAD TO ACCLIMATION TO ANOXIA

Mild hypoxic pre-treatments appear to improve tolerance to subsequent anoxic treatment in Arabidopsis, rice, wheat, and tomatoes (Ellis and Setter, 1999; Ellis et al., 1999; Saglio et al., 1988; Waters et al., 1991). This acclimation seems to require protein synthesis since the addition of
the translation inhibitor cycloheximide prevented the hypoxic acclimation. Possible explanations for the acquired low-oxygen tolerance include the efficient synthesis of proteins involved in stress tolerance before the drastic energy crisis begins, and protein synthesis is impaired. Protein turnover has also been observed to be reduced in germinating lettuce seeds under anoxia (Pradet and Raymond, 1983).

Similar to the increased anoxia tolerance induced by hypoxic acclimation, mild heat treatments (38 °C, 1.5 h) have also been shown to induce anoxia tolerance in Arabidopsis seedlings. An anoxic pre-treatment on the other hand, followed by heat stress did not result in any improved tolerance to heat stress (Banti et al., 2008). Analyses of global transcriptional and translational changes under hypoxia and anoxia in rice and Arabidopsis clearly demonstrated the induction of a large number of HSP and other heat-related genes, which are also often induced under conditions that involve oxidative stress (Loreti et al., 2005; Swindell, 2006). Moreover, Loreti et al. (2005) showed that sucrose supplementation to Arabidopsis seedlings enhances their tolerance to anoxia. This ameliorative effect does not account for the higher substrate availability for glycolysis, since it also occurred in ADH knockout mutants (Banti et al., 2008). Sucrose-related increased tolerance, on the other hand, may be associated with increased HSP induction under anoxia, since sucrose enhances the anoxia-inducibility of HSP-encoding genes (Banti et al., 2008). In higher plants five major families of HSPs/chaperones have been described (Nover and Scharf, 1997): the HSP70 family, the chaperonins (HSP60), the HSP90 family, the HSP100 family, and the low molecular mass (12–40 kDa) small HSP (sHSP) family. HSPs act as molecular chaperones (Lindquist, 1986), assisting protein folding, assembly, translocation and degradation. A comparison of transcriptome profiling under different low-oxygen conditions showed that after a few hours of anoxia, a number of HSPs belonging to different families were induced. Two hours of hypoxia, on the other hand, were only able to upregulate small HSPs, and only later (9 h) were members of the HSP90 and 100 induced. sHSPs are believed to prevent, in an ATP independent manner, protein aggregation (Lee et al., 1997; Löw et al., 2000; Smýkal et al., 2000) caused by heat stress but also during developmental transitions such as during chromoplast biogenesis (Lawrence et al., 1997), flower development, (Dafny-Yelin et al., 2008), embryogenesis (Barcala et al., 2008), and germination (Banti et al., 2008). The highly conserved HSP100/ClpB chaperone system, on the other hand, is involved in reverting protein aggregation. However this mechanism depends on ATP availability, required for substrate binding, together with its hydrolysis to ADP, to release the disaggregated polypeptides (Balogi et al., 2008; Bosl et al., 2005). Based on distinct functions and different expression
patterns under low-oxygen conditions, it is tempting to speculate that the early induction of small HSP is aimed at preventing protein aggregation. Subsequent induction of HSP10/90 may be required to solubilize protein aggregates when the sHSP preventive activity is not sufficient. In animal systems, protein aggregation occurs under anoxic conditions (Clegg, 2007) and can be caused by oxidative stress, by a direct reaction with ROS or indirectly by lipid peroxidation that further affects proteins. Amino acid oxidation results in the addition of carbonyl groups that alter protein conformation, increasing hydrophobicity and enhancing non-specific protein–protein interactions (Davies, 1995). Besides acting as molecular chaperone, sHSPs also exhibit interactions with lipids in prokaryotes, plants, and mammalian systems that modulate membrane properties (Nakamoto and Vigh, 2007; Tsvetkova et al., 2002). This could suggest a positive effect of sHSP on membrane integrity maintenance during an energy crisis, together with a further protection of lipid peroxidation from re-oxygenation. Interestingly, sHSP translation rates remain either unchanged or even increased upon re-oxygenation with respect to hypoxia, while most of the protein involved in anaerobic metabolism is rapidly dissociated from polysomes (Branco-Price et al., 2008).

IX. CONCLUSIONS

Oxygen sensing and signal transduction in plants is undoubtedly much less explored when compared to other eukaryotes. However, advances in analysis techniques together with the increasing interest in applications of low-oxygen treatments in crop science and technology have recently contributed considerably to a better understanding of the molecular mechanisms underlying plant adaptation to anaerobiosis. Exponential increase in genome sequences for crop plants is also extremely beneficial, allowing the comparison of tolerant and intolerant species. Translational biology to formulate new hypotheses seems extremely promising, as demonstrated by frequent comparisons and overlaps with other responses to different conditions, in plants as in other eukaryotes. The emerging crosstalk between reactive oxygen- and nitrogen-species and signaling pathways especially deserves further investigation. However, the more traditional signaling components such as the role of oscillations in calcium concentrations and phosphorylation cascades also require better understanding. Future research will probably focus on the cross talk mechanisms between the three transcriptionally active organelles, nucleus, chloroplast, and mitochondria, since the last two are linked with oxygen, one in terms of its production and the other its consumption.
Although much attention has been paid to the mechanisms behind gene activation under hypoxia and anoxia, there has been practically no focus on how general transcriptional repression is achieved. Despite the relatively small amount of knowledge gained, compared with other stress conditions, very encouraging results have already been provided regarding applications for the marker assisted selection of flood tolerant rice cultivars (Xu et al., 2006). Distinguishing between tissue and cell specific responses and adaptations in different species would probably help to understand what strategies really affect individual fitness and thus, which mechanism is worth transferring in to breeding programs.
### Definitions of the acronyms referring to genes or proteins cited in the text

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Full name</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACO</td>
<td>ACC oxidase</td>
<td>Catalyze ethylene synthesis</td>
</tr>
<tr>
<td>ACS</td>
<td>ACC synthase</td>
<td>Catalyze synthesis of ACC</td>
</tr>
<tr>
<td>ADH</td>
<td>Alcohol dehydrogenase</td>
<td>Catalyze the interconversion between ethanol and acetaldehyde</td>
</tr>
<tr>
<td>AMY1A</td>
<td>α-Amylase isoform 1A</td>
<td>Catalyze breakdown of starch</td>
</tr>
<tr>
<td>AMY3D</td>
<td>α-Amylase isoform 3D</td>
<td>Catalyze breakdown of starch</td>
</tr>
<tr>
<td>AOX</td>
<td>Alternative oxidase</td>
<td>Catalyze electron transfer to oxygen</td>
</tr>
<tr>
<td>AP2-ERF</td>
<td>Apetala2-ethylene responsive factor</td>
<td>Binding to DNA sequences</td>
</tr>
<tr>
<td>bHLH</td>
<td>Basic helix loop helix</td>
<td>Binding to DNA sequences</td>
</tr>
<tr>
<td>COX</td>
<td>Cytochrome c oxidase</td>
<td>Catalyze electron transfer to oxygen</td>
</tr>
<tr>
<td>CREB</td>
<td>cAMP response element binding</td>
<td>Transcriptional co-activating protein</td>
</tr>
<tr>
<td>CRR1</td>
<td>Copper response regulator 1</td>
<td>Locus containing elements that mediate copper deficiency response in <em>Chlamydomonas reinhardtii</em></td>
</tr>
<tr>
<td>CycOs1</td>
<td>Cyclin of <em>Oryza sativa</em> isoform 1</td>
<td>Regulates cell cycle progression</td>
</tr>
<tr>
<td>EDS1</td>
<td>Enhanced disease Susceptibility 1</td>
<td>Component of the R-gene mediated disease resistance</td>
</tr>
<tr>
<td>eIF4E</td>
<td>Eukaryotic translation initiation factor 4E</td>
<td>Initiates protein synthesis from a mRNA molecule in eukaryots</td>
</tr>
<tr>
<td>ETR2</td>
<td>Ethylene receptor 2</td>
<td>Involved in ethylene sensing and signal transduction</td>
</tr>
<tr>
<td>GDH</td>
<td>Glutamate dehydrogenase</td>
<td>Catalyzes the conversion of glutamate to α-ketoglutarate</td>
</tr>
<tr>
<td>GHB</td>
<td>γ-Hydroxybutyrate dehydrogenase</td>
<td>Catalyze the conversion of γ-hydroxybutyrate to succinate semialdehyde</td>
</tr>
<tr>
<td>HB1</td>
<td>Non-symbiotic heomoglobin 1</td>
<td>Catalyze scavenging of NO</td>
</tr>
<tr>
<td>HIF</td>
<td>Hypoxia inducible factor</td>
<td>Mediates the transcriptional reprogramming of the hypoxic response in animal cells</td>
</tr>
<tr>
<td>HSPs</td>
<td>Heat shock proteins</td>
<td>Prevent or dismantle protein aggregates caused by denaturation (heat or oxidation)</td>
</tr>
<tr>
<td><strong>KIN10</strong></td>
<td><strong>SNF1 kinase homolog 10</strong></td>
<td>Conserved energy sensor, controlling convergent reprogramming of transcription in response to seemingly unrelated stress conditions that deplete sugar and energy supplies</td>
</tr>
<tr>
<td>-----------</td>
<td>-----------------------------</td>
<td>---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>KIN11</strong></td>
<td><strong>SNF1 kinase homolog 11</strong></td>
<td>Conserved energy sensor, controlling convergent reprogramming of transcription in response to seemingly unrelated stress conditions that deplete sugar and energy supplies</td>
</tr>
<tr>
<td><strong>LDH</strong></td>
<td><strong>Lactate dehydrogenase</strong></td>
<td>Catalyzes interconversion of lactate to pyruvate</td>
</tr>
<tr>
<td><strong>LSD1</strong></td>
<td><strong>Lesion simulating Disease 1</strong></td>
<td>Top of form</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negatively regulates a plant cell death pathway</td>
</tr>
<tr>
<td><strong>MDHAR</strong></td>
<td><strong>Monodehydroascorbato reductase</strong></td>
<td>Catalyze the regeneration of reduced MDHA</td>
</tr>
<tr>
<td><strong>MKK11</strong></td>
<td><strong>Map kinase kinase 11</strong></td>
<td>Involved in signal transduction through phosphorylation cascades</td>
</tr>
<tr>
<td><strong>MKK9</strong></td>
<td><strong>Map Kinase Kinase 9</strong></td>
<td>Involved in signal transduction through phosphorylation cascades</td>
</tr>
<tr>
<td><strong>MPK3</strong></td>
<td><strong>MAP protein kinase 3</strong></td>
<td>Involved in signal transduction through phosphorylation cascades</td>
</tr>
<tr>
<td><strong>MPK6</strong></td>
<td><strong>MAP protein kinase 6</strong></td>
<td>Involved in signal transduction through phosphorylation cascades</td>
</tr>
<tr>
<td><strong>MYB2</strong></td>
<td><strong>Myb transcription factor 2</strong></td>
<td>Mediates the transcriptional response to abscissic acid</td>
</tr>
<tr>
<td><strong>MYB51</strong></td>
<td><strong>Myb transcription factor acting on the Sugar response complex isoform 1</strong></td>
<td>Mediates transcriptional reprogramming caused by gibberellins and sucrose starvation in rice</td>
</tr>
<tr>
<td><strong>NIP2;1</strong></td>
<td><strong>Nodulin 26 intrinsic protein</strong></td>
<td>Lactate transporter</td>
</tr>
<tr>
<td><strong>OFD1</strong></td>
<td><strong>2-OG-Fe(II) dioxygenase</strong></td>
<td>Accelerates Sre1N degradation in the presence of oxygen</td>
</tr>
<tr>
<td><strong>OsERL1</strong></td>
<td><strong>Oryza sativa ethylene receptor 2-like 1</strong></td>
<td>Involved in ethylene sensing and signal transduction</td>
</tr>
<tr>
<td><strong>p300</strong></td>
<td></td>
<td>Transcriptional co-activating protein</td>
</tr>
<tr>
<td><strong>PAD4</strong></td>
<td><strong>Phytoalexin deficient 4</strong></td>
<td>Mediates salicylic acid signaling</td>
</tr>
<tr>
<td><strong>PAS</strong></td>
<td><strong>PER-ARNT-SIM</strong></td>
<td>Protein domain that functions as sensory module for oxygen tension, redox potential or light intensities</td>
</tr>
<tr>
<td><strong>PDC</strong></td>
<td><strong>Pyruvate decarboxylase</strong></td>
<td>Catalyze conversion of pyruvate to acetaldehyde</td>
</tr>
<tr>
<td><strong>PDH</strong></td>
<td><strong>Pyruvate dehydrogenase</strong></td>
<td>Catalyzes conversion of pyruvate to acetyl-CoA</td>
</tr>
<tr>
<td><strong>PFK</strong></td>
<td><strong>Phosphofructokinase</strong></td>
<td>Phosphorylates fructose 6-P</td>
</tr>
<tr>
<td><strong>PHD</strong></td>
<td><strong>Prolyl-4-hydroxylase</strong></td>
<td>Catalyze the oxygen dependent degradation of HIFα</td>
</tr>
<tr>
<td><strong>PIF3</strong></td>
<td><strong>Phytochrome interacting factor 3</strong></td>
<td>Mediates gene transcriptional regulation by light</td>
</tr>
<tr>
<td>Acronym</td>
<td>Full name</td>
<td>Function</td>
</tr>
<tr>
<td>---------</td>
<td>-----------</td>
<td>----------</td>
</tr>
<tr>
<td>PIF4</td>
<td>Phytochrome interacting factor 4</td>
<td>Mediates gene transcriptional regulation by light</td>
</tr>
<tr>
<td>pVHL</td>
<td>von Hippel–Lindau tumor suppressor protein</td>
<td>Component of the protein complex responsible for ubiquitination and degradation of HIF</td>
</tr>
<tr>
<td>ROP10</td>
<td>Rho of Plants isoform 10</td>
<td>Involved in modulation of ADH expression in response to hypoxia in <em>Arabidopsis thaliana</em></td>
</tr>
<tr>
<td>SH1</td>
<td>Sucrose synthase 1</td>
<td>Catalyze sucrose catabolism in maize (isoform 1)</td>
</tr>
<tr>
<td>SLR1</td>
<td>Slender rice 1</td>
<td>Repress transcriptional activity of bHLH transcription factors</td>
</tr>
<tr>
<td>SLRL1</td>
<td>Slender rice 1 like isoform 1</td>
<td>Repress transcriptional activity of bHLH transcription factors</td>
</tr>
<tr>
<td>SNF1</td>
<td>Sucrose non-fermenting 1</td>
<td>Essential for release from glucose repression in eukaryotes</td>
</tr>
<tr>
<td>SnRKs</td>
<td>Snf1-related protein kinases</td>
<td>Involved in signal transduction through phosphorylation cascades</td>
</tr>
<tr>
<td>SRE1</td>
<td>Sterol regulatory element binding protein homolog 1</td>
<td>Activates large part of the transcriptional response to hypoxia in yeast</td>
</tr>
<tr>
<td>SSADH</td>
<td>Succinic semialdehyde dehydrogenase</td>
<td>Catalyzes the oxidation of succinate semialdehyde to succinate</td>
</tr>
<tr>
<td>SUB1</td>
<td>Submergence 1</td>
<td>Locus responsible for inhibition of flooding stimulated elongation and determining submergence tolerance in rice</td>
</tr>
<tr>
<td>SUS1</td>
<td>Sucrose synthase 1</td>
<td>Catalyze conversion of sucrose to fructose and UDP-glucose (isoform 4 of <em>Arabidopsis thaliana</em>)</td>
</tr>
<tr>
<td>SUS4</td>
<td>Sucrose synthase 4</td>
<td>Catalyze conversion of sucrose to fructose and UDP-glucose (isoform 4 of <em>Arabidopsis thaliana</em>)</td>
</tr>
<tr>
<td>TaMYB1</td>
<td>Myb transcription factor 1 of <em>Triticum aestivum</em></td>
<td>Mediates transcriptional reprogramming caused by drought stress</td>
</tr>
<tr>
<td>ZAT10</td>
<td>Zinc finger protein of <em>Arabidopsis thaliana</em></td>
<td>Mediates the transcriptional reprogramming caused by oxydative stress</td>
</tr>
<tr>
<td>ZAT12</td>
<td>Zinc finger protein of <em>Arabidopsis thaliana</em></td>
<td>Mediates the transcriptional reprogramming caused by oxydative stress</td>
</tr>
</tbody>
</table>
REFERENCES


shunt is required to restrict levels of reactive oxygen intermediates in plants. *Proceedings of the National Academy of Sciences of the United States of America* **100**, 6843–6848.


Menegus, F., Cattaruzza, L., Mattana, M., Beffagna, N. and Ragg, E. (1991). Response to anoxia in rice and wheat seedlings: Changes in the ph of
intracellular compartments, glucose-6-phosphate level, and metabolic rate. *Plant Physiology* 95, 760–767.


Mustroph, A., Albrecht, G., Hajirezaei, M., Grimm, B. and Biemelt, S. (2005). Low levels of pyrophosphate in transgenic potato plants expressing *E. coli*


Proceedings of the National Academy of Sciences of the United States of America 81, 6029–6033.


