Chapter 9
Root Oxygen Deprivation and Leaf Biochemistry in Trees

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Abstract Plants are aerobic organisms, that is, they depend on oxygen for their life. Therefore, oxygen deficiency impacts on the biochemical and molecular processes of the plant cell. However, plant cells have evolved inducible strategies to cope with low oxygen stress conditions. When O_2 is reduced, energy production in the form of ATP is reduced too. Cells respond to this energy crisis by switching to fermentative metabolism, producing ATP and regenerating NAD^+ through the glycolytic and fermentative pathways.

Roots are the organs most easily subject to low O_2 stress, but changes in fermentative enzymatic activities are also seen in leaves. Nevertheless, leaves already possess a constitutive expression of these enzymes. Since leaves are the plant organs less likely exposed to low O_2 conditions, they should have evolved in addition an alternative role for the enzymes usually related to fermentative metabolism. Leaves seem to have the ability to take advantage of the enzymes of a metabolic pathway commonly useful in parts of the plant which can undergo anoxia or hypoxia stress: they make use of fermentative metabolism in a different way, to limit the damage that stress condition imposes to the whole plant.

Abbreviations

ABA	Abscisic acid
ADH	Alcohol dehydrogenase
ALDH	Aldehyde dehydrogenase
ANPs	Anaerobiosis related proteins

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GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
LDH	Lactate dehydrogenase
PDC	Pyruvate decarboxylase
PPi	Inorganic pyrophosphate
Suc	Sucrose
SuSy	Sucrose synthase

9.1 Introduction

There were a few reasons in the past that drove the curiosity to investigate what happens in the leaf of a tree when the plant undergoes an oxygen deficiency stress. Trees are a difficult subject to investigate: they differ from herbaceous plants not only for size or longevity, but they also possess single features that make them unique. However, waterlogging soils can determine a heavy stress also for trees, in a longer temporal scale with respect to a herbaceous plant, and survival in prolonged hypoxic or anoxic conditions can be a severe challenge also for them. What happens in a tree leaf, when the plant experiences root oxygen deficiency?

When roots are in hypoxic or anoxic conditions, the leaf physiology may be affected by significant changes, the severity of which depends on different factors such as the duration of the stress, the plant species, or the growth stage. At the macroscopic level, reduced leaf area (Else et al. 1996) and transpiration rate (Armstrong et al. 1994), reduced rate of photosynthesis and stomatal conductance (Terazawa et al. 1992; Atkinson et al. 2008), chlorosis (Drew and Sisworo 1977), reduced photosynthetic assimilation of CO_2 (Kalashnikov et al. 1994; Yordanova and Popova 2007*),* reduced cell-wall extensibility (Smit et al. 1989), changes in leaf water content (Jackson et al. 1996), are all symptoms shown in response to flooding.

At biochemical/molecular level, since one of the damages imposed by waterlogging is the onset of an oxidative stress with generation of reactive oxygen species (ROS) (Yan et al. 1996; Biemelt et al. 2000; Blokhina et al. 2002; Garnczarska and Bednarski 2004; Wang and Jiang 2007; Balakhnina et al 2009), an early rise in antioxidant enzyme activities can generally be measured. For example, in short-term flooding, superoxide dismutase (SOD), ascorbate peroxidase (AP), and glutathione reductase (GR) activities are enhanced in maize (*Zea mays* L.) (Yan et al. 1996), and mungbean (*Vigna radiata* L.) (Ahmed et al. 2002) leaves, but with the persisting of waterlogging conditions, these enzyme activities are shown to decrease. These results, in which two different stages of behaviour can be distinguished, are generally diffused, but they are not absolute. In the flooding-sensitive pea leaves, SOD activity decreases (Zakrzhevsky et al. 1995), suggesting a possible correlation between early activation of SOD and plant adaptation to flooding stress, at least in herbaceous plants (Balakhnina et al. 2009). Otherwise, in faba bean (*Vicia faba*) leaves, even if SOD activity is enhanced at the onset of hypoxic root stress, the activity of the important antioxidant enzyme, GR, shows an equally early decrease (Balakhnina et al. 2009).

Leaves are the plant organs that should be less likely to be exposed to anoxia or hypoxia, because they live in an atmosphere rich in oxygen. Virtually they should not ever suffer O_2 deficiency. Nonetheless, it has been found that leaves themselves are rich in the enzymes necessary for fermentation, even if there should not be any apparent need for the presence of enzymes for anaerobic metabolism. On the contrary, not only alcohol dehydrogenase (ADH) and pyruvate decarboxylase (PDC) have been found in plant leaves, but leaves of many trees possess a basal constitutive expression of enzymes usually related to fermentative metabolism (Kimmerer and MacDonald 1987).

In effect, tree leaves have been shown to produce aerobic ethanol, the product of ADH enzyme, upon exposure to atmospheric pollutants such as sulphur dioxide, or nitrogen oxides (Kimmerer and Kozlowski 1982; MacDonald et al. 1989). But, since air pollution is one of the last damaging insults caused by human beings to the Earth, this should not be the reason why trees have evolved the ability to maintain constitutive activities of ADH in their leaves.

Hence, there must be a reason why the leaf is able to modulate the activity of an enzyme already constitutively expressed, the presence of which apparently seems to be unnecessary in that organ. This phenomenon that occurs in the leaf however could be related to oxygen deficiency stress that occurs in other organs of the plant. The stress can be caused by external conditions at root level – such as an excess rainfall –, or by anatomical ones – such as limited permeability of oxygen into some internal tissues –, or by metabolic factors – such as high oxygen request by actively dividing cells.

9.2 Root O_2 Deprivation

In roots, under sub-optimal oxygen concentration, genes involved in lactic and alcoholic fermentation pathways are activated, and so are the genes implicated in ROS detoxification that could also have a role later, when the normal oxygen conditions are restored (Klok et al. 2002). Only after long periods of anaerobiosis, do morphological and anatomical changes also take part (Perata and Voesenek 2007). The first genes to be up-regulated encode for the so-called ANPs (Anaerobiosis Related Proteins), enzymes involved in fermentative pathways such as PDC, ADH, and lactate dehydrogenase (LDH) (Huang et al. 2005).

The oxygen is the terminal electron acceptor in the oxidative phosphorylation pathway. This pathway not only provides the vast majority of ATP in a plant cell but also regenerates NAD^+ from NADH. NAD^+ is of fundamental importance for glycolysis to continue: it is necessary for the enzyme GAPDH (glyceraldehyde-3-phosphate dehydrogenase) in the first step of the energy-conserving phase of glycolysis. Under oxygen deprivation, oxidative phosphorylation pathway is inhibited, all steps of the oxidative chain are electrons saturated and there is NADH

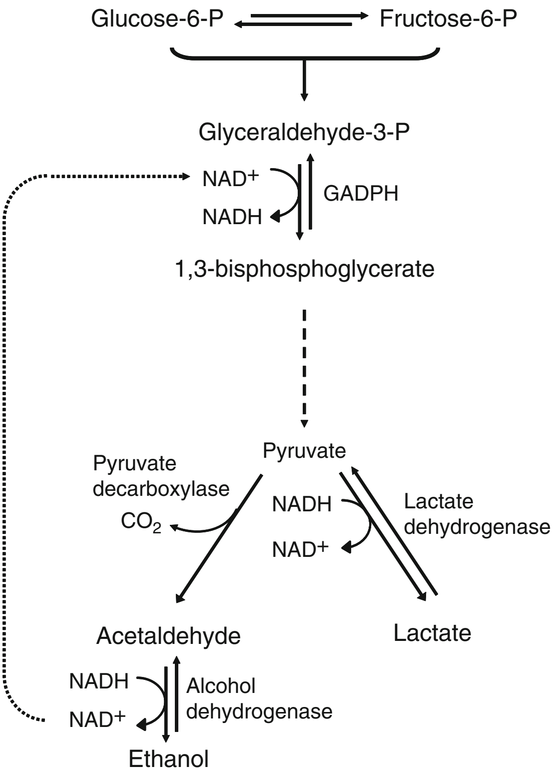

Fig. 9.1 *Schematic view of plant glycolysis and fermentation*. In the glycolysis, carbohydrates from different sources (starch, sucrose, glucose, fructose etc.) are converted to triose phosphates, and then to pyruvate. In the first reaction of the so called "energy-conserving phase of glycolysis", NAD^+ is reduced to NADH by glyceraldehyde-3-phosphate dehydrogenase, but it can be regenerated during fermentation by alcohol dehydrogenase, or lactate dehydrogenase

accumulation. The GAPDH, lacking its cofactor, comes to a halt: glycolysis thus cannot continue (Fig. 9.1). To overcome these problems, the cell metabolism then switches to one or more forms of fermentative metabolism (Davies et al. 1974; Albert et al. 1983; Ricard et al. 1994). This impairs cellular metabolism: the efficiency of energy production in the form of ATP is strongly reduced, intracellular pH is lowered, and there is an accumulation of lactate and ethanol (Drew 1997). The plant cell starts to ferment pyruvate (formed from glycolysis) to lactate, through LDH. This fermentation is often transient, since it might results in a dangerous acidification of intracellular pH by lactic acid. This lowering in pH quickly leads to a switch from lactic to ethanolic fermentation, helped by different pH optima of the involved enzymes. Indeed, at acidic pH LDH is inhibited while PDC is activated, leading first to the production of acetaldehyde, and then to ethanol through the subsequent action of ADH.

9.2.1 Root O_2 Deprivation: Effects on Leaves

In roots, *Adh* gene expression increases under oxygen deprivation stress. In leaves, *Adh* gene seems to be constitutively expressed, at least in poplars (Kreuzwieser et al. 1999) and in some other tree species (Harry and Kimmerer 1991). It's also known that in the leaf there's a response to root flooding which includes enzymatic activities, and ethanol and possibly acetaldehyde leak. But under oxygen deprivation *Quercus palustris*, a flood-tolerant tree, and *Quercus rubra*, flood-intolerant species, produce about the same amount of ethanol; also, the flood-tolerant *Betula nigra* produces less ethanol than the flood-intolerant *Betula lenta* (Kimmerer and MacDonald 1987). Little or no ethanol can be measured from the leaves of herbaceous plants upon exposure to root hypoxia (Freeling and Bennett 1985; Kimmerer 1987; Kimmerer and MacDonald 1987).

So, which kind of relation exists between ADH and ethanol, and between them and root flooding stress? The thing becomes clearer if we consider the hypothesis that there must be also an alternative physiological role of ADH in the leaves of plants, maybe related to aerobic conditions. Whereas in hypoxic tissues such as roots, germinating seeds, or even stems, ADH (gene and enzyme) is needed in order to guarantee the continuity of glycolysis producing ethanol and oxidizing NADH in the process (Tadege et al. 1999), in the aerobic leaves its expression is constitutive and independent of the condition of the roots (Kreuzwieser et al. 2001).

Moreover, focusing on *Adh* gene, if foliar *Adh* plays an exclusive role in flooding stress, its expression should be modulated. However, in the leaves of many of herbaceous species, such as soybean or maize seedlings, *Adh* gene seems to be not even inducible at all.

9.3 The Role of ADH

It is also true that ethanol produced in the roots when soil is depleted of molecular O_2 can exit from the roots and enter the xylem transpiration stream. In this way, ethanol can reach the foliar mesophyll. However, if the ADH role is to metabolize this xylem-translocated fermentation product, a higher activity in leaves from flooded plants or from flood-tolerant plants would be expected (Kimmerer 1987).

On the other hand, ADH not only mediates the acetaldheyde reduction in a two-step pathway in cooperation with PDC but can also operate in the reverse direction, promoting the ethanol oxidation and thereby producing acetaldehyde. In effect, both acetaldehyde and ethanol have been found in the transpiration stream of trees (Crawford and Finegan 1989; MacDonald and Kimmerer 1991). ADH might thus operate in a sequential action with aldehyde dehydrogenase (ALDH) that converts acetaldehyde into acetate (Fig. 9.2).

The mechanism is similar in animals where ethanol is converted to acetaldehyde through ADH, and then to acetate through ALDH (Williamson and Tischler 1979).

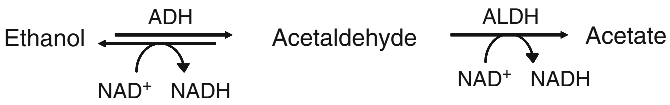

Fig. 9.2 *Oxidative pathway of ethanol metabolism.* In leaves, ethanol can be converted back to acetaldehyde by alcohol dehydrogenase, and further oxidized to acetate by aldehyde dehydrogenase

But the real analogy is between ethanol/acetate production in plants, and the lactate metabolism in animals. When muscles are under stress, lactate is produced, and enters the bloodstream. After the physical activity, in the recovery period, with O_2 disponibility, lactate is converted back to glucose by gluconeogenesis, and then respired aerobically (Lehninger 1982). In other words, similar mechanisms belong to organisms of both Plant and Animal Kingdoms. Animals possess the ability to recover the anaerobic metabolism product (lactate) and convert it into a molecule involved in the aerobic metabolism (glucose) when the stress is over. Plants, similarly, possess the ability to recover the anaerobic metabolism product (ethanol) and convert it into a molecule involved in the aerobic metabolism (acetate). Moreover, differently from animals, plants operate when the stress is not still over, and in organs not directly affected (the leaves).

The physiological significance of these reactions may be a recycling of the root-derived ethanol produced under conditions of oxygen deficiency (Kreuzwieser et al. 2001). Acetate, activated as acetyl-CoA, can enter then into many pathways of the general aerobic metabolism (MacDonald and Kimmerer 1993; Bode et al. 1997; Kesselmeier and Staudt 1999).

Not only the leaves, but also young stems possess the ability to metabolize ethanol produced in hypoxic tissues, preventing loss of carbon and helping to maintain an efficient respiration process during the stress. In effect, very high ADH activity was also measured in the vascular cambium of trees, and tree stems (Kimmerer and Stringer 1988; MacDonald and Kimmerer 1991). So, in flooded trees fermentation occurs in the roots, and possibly it may occur also along the stems (Eklund 1990; Harry and Kimmerer 1991). Ethanol that reaches the leaves carried by the transpiration stream can be metabolized by ADH before it can escape from the plant (MacDonald et al. 1989; MacDonald and Kimmerer 1990, 1991). In other words, constitutive expression of *Adh* gene in leaves of trees may be a strategy to avoid ethanol escape, and safeguard the carbon and energy invested in ethanol.

9.4 Carbon Recovery

Many studies have demonstrated that acetaldheyde is effectively emitted by leaves of trees such as spruce (Hahn et al. 1991), oak, pine (Kesselmeier et al. 1997) and poplar (Kreuzwieser et al. 1999). In general, there are significant variations in acetaldehyde concentrations in forests during the day (Enders et al. 1992; Steinbrecher et al. 1997), and flooded plants show higher acetaldheyde emission rates during the day and lower at night (Kreuzwieser et al. 2001). It has been proposed that this acetaldehyde is the result of the oxidation in leaves of the ethanol produced in flooded roots, and then transported to the leaves via the transpiration stream.

In order to confirm this hypothesis, excised poplar leaves were incubated in a buffer plus ethanol, with the addition of inhibitors of the enzymes responsible of acetaldehyde metabolism: ADH and ALDH (Kreuzwieser et al. 2001). The inhibition of ADH led to a collapse of acetaldehyde emission from the leaves. This means that ADH also generates the conversion of ethanol into acetaldehyde in the leaves, originating acetaldehyde emissions. A little enhanced ethanol emission is also noted, probably due to an ethanol accumulation as a consequence of inhibited ethanol oxidation. By contrast, treatment of excised poplar leaves with ethanol and the ALDH inhibitor caused significantly increased emission both of ethanol and acetaldehyde. Acetaldehyde oxidation was in fact inhibited, and this led to acetaldehyde and ethanol accumulation in the leaf tissue (Kreuzwieser et al. 2001). The loss of carbon in the form of ethanol or acetaldehyde therefore, should be considered as a gap in the metabolization of fermentation products coming from the roots.

Even if ADH is responsible for acetaldehyde production from ethanol, other factors seem to be related to a higher diurnal acetaldehyde emission from the leaves of flooded trees. There might be a further link between oxygen deficiency and ADH and acetaldehyde emission. It could be supposed that during the day, the higher acetaldehyde emission measured may be promoted by the opening of stomata. In fact, acetaldehyde is not an easily volatilizable molecule because of its polar nature; it may only be emitted from leaves via the stomata and not via the cuticle.

However, it has also been demonstrated that stomatal conductance does not directly influence acetaldehyde emission. Manipulating stomatal conductance by light intensity or ABA (two known factors inducing stomatal closure) did not result in altered acetaldehyde emission. Therefore, only acetaldehyde concentration can directly influence acetaldehyde emission. When is acetaldehyde concentration higher? In hypoxic/anoxic soil condition? During flooding stress, more ethanol than in aerobic condition reaches the leaves, where it is oxidized generating acetaldehyde as an intermediate in the pathway to acetate conversion. It is also true that leaf ethanol concentration increases depending on the transpiration rate. Since the transpiration is controlled by stomata, stomatal conductance may indirectly influence acetaldehyde emission from leaves by controlling ethanol transport (Kreuzwieser et al. 2001).

Moreover, acetaldehyde is a high phytotoxic compound. At the beginning it was also thought that ethanol was the toxic compound, however, later, studies have not proven its toxicity (Jackson et al. 1982; Atkinson et al. 2008). Recent works now highlight how acetaldehyde rather than ethanol possibly causes the observed anoxia-related injuries on growth and development of flooded plants (Ap Rees 1980; Perata and Alpi 1991). Therefore acetaldehyde accumulation must be avoided, or it should be further metabolized, to prevent self-poisoning. Acetaldehyde loss from the leaves has thus to be considered a problem of efficiency between production and metabolization of this molecule (Kreuzwieser et al. 1999).

9.5 Differential mRNA Translation

There is another point that deserves a little more attention. Why is ADH activity enhanced in leaves of flooded trees, while the *Adh* gene expression seems to be not altered?

As discussed above, an enhanced ADH activity may be required in order to manage all the ethanol transported into the xylem flux. This role is confirmed by the greater concentration of the ethanol-derived oxidation product, acetaldehyde, and by the increased ethanol concentrations in the xylem sap (up to 200 fold after 24 h of flooding, Kreuzwieser et al. 2009). However, microarray experiments on poplar do not report any other isoform of *Adh* that could be induced in leaves in response to anoxic/hypoxic root conditions (Kreuzwieser et al. 2009). Moreover, it should be kept in mind that a lack in the gene induction does not necessarily imply a lack of activity in the corresponding enzyme. In fact, there is more than one level in the regulation of gene expression. Besides the differential transcriptional induction of genes in response to a stress, the translation can also be selectively regulated (Branco-Price et al. 2008; Kreuzwieser et al. 2009).

Transcript and metabolite profiling was performed to characterize the different molecular and physiological responses under conditions of oxygen deprivation in flooding tolerant and non tolerant plants (Branco-Price et al. 2005, 2008; Loreti et al. 2005; Lasanthi-Kudahettige et al. 2007; Van Dongen et al. 2008; Kreuzwieser et al. 2009). The first result that appears confirms that the genes involved in changes in transcript abundance are different between roots and leaves. That means that the most significant variation in gene expression belongs to the roots, whereas changes in the metabolite levels are found in both organs. Regarding root behaviour, as soon as a root senses the oxygen deficiency, it begins to modify its internal metabolism in order to maintain energy availability, and to activate the pathways that permit an adequate supply of ATP. In Arabidopsis roots for example, the peak in *Adh* transcript abundance was observed 2–4 h after hypoxia onset (Liu et al. 2005). In roots of poplar trees, the consistent induction of *Adh* is maintained for the whole flooding period. Once again, even if *Pdc* transcript level in poplar roots is 15 times higher after 5 h of hypoxia, PDC activity has only a threefold increase (Kreuzwieser et al. 2009). This lack of proportional correlation between gene expression and enzyme activity may suggest a reduced translation efficiency (Branco-Price et al. 2008), or some post-transcriptional or post-translational regulation, as suggested by Kreuzwieser et al. 2009.

This alteration of gene expression in response to the stress is a way to adapt internal cell metabolism to the new temporary condition of low energy disponibility. Naturally, the first way to preserve energy dissipation is to reduce the transcription of those mRNAs that are not strictly necessary to the survival of the cell, such as those needed in DNA synthesis or cell division (Gibbs and Greenway 2003). But recently it has been demonstrated that this is not the only way (Branco-Price et al. 2005, 2008; Kreuzwieser et al. 2009). Changes have been observed in the association between mRNA and polysomes. Polysomes (polyribosomes) are large cytoplasmic assemblies made up of several ribosomes. During low oxygen stress, it has demonstrated a decrease in polysome complexes inside the cells, which reflect a decrease in initiation of protein synthesis (Branco-Price et al. 2008). In addition, not all the mRNAs have the same affinity to associate with polysomes: this capability depends on numerous factors such as the length of the 5′-UTR, the ability to form secondary structures, etc. (Kawaguchi and Bailey-Serres 2005). So, a little sub-set of mRNAs still maintains association with polysomes under stress conditions. This led to the conclusion that the regulation in protein synthesis observed can also occur at translational level, even in absence of variation in mRNA transcription. mRNA translation is in fact an extremely energy requiring step, since it needs multiple molecules of ATP and one of GTP in removing secondary mRNA structure and in the recognition of the initiation codon. It seems advantageous a regulation of protein synthesis at level of the initiation phase. Moreover, the fact that protein synthesis can be regulated without the request for a decrease in transcript abundance permits the quick re-recruitment of these not down-regulated mRNA to polysomes, as soon as oxygen comes back (Branco-Price et al. 2008).

9.6 Effects on Cell Metabolism

A comparison of the response of a flooding-sensitive plant with that of a flooding-tolerant one reveals important differences in the number of genes selectively expressed during oxygen deprivation stress. Over 5,000 genes show significantly altered expression in the hypoxic stressed poplar tissue, the flooding-tolerant, whereas similar conditions alter much less the transcript abundance of Arabidopsis, the flooding-sensitive, where only approximately 150 genes change (Klok et al. 2002; Liu et al. 2005). In spite of this difference, transcriptional factors represent a high percentage of altered genes in both species. This differential expression of transcriptional factors suggests a regulation of the transcriptional network, aimed at a stress adaptation in response to hypoxia (Kreuzwieser et al. 2009).

It seems that the stimulation of glycolysis and the supply of sugars can be important for tolerating anoxia, as suggested by Drew in 1997. On the other hand, also the inhibition of energy consuming processes such as biosynthesis of cellulose, hemicellulose, and cell wall proteins may be of some help. The synthesis of secondary cell wall seems to be not a priority in hypoxic or anoxic conditions, when the life of the plant is at risk, and can be postponed if the plant has survived, at the end of the stress.

But as the situation of oxygen deprivation continues, in contrast to the early activation response, genes in roots start to be down-regulated. This behaviour is a common response to survival, found both in herbaceous flooding-tolerant species such as rice (*Oryza sativa*) (Lasanthi-Kudahettige et al. 2007), and in flooding-tolerant trees such as grey poplar (Kreuzwieser et al. 2009).

When poorly drained soil becomes waterlogged, the roots of plants experience a deficiency in O_2 disponibility, because of slow diffusion of O_2 in the water-filled pore space of the soil. When oxygen becomes unavailable, glycolysis remains the primary source of energy for the cell, while fermentation recycles the NADH produced by glycolysis. The efficiency of fermentation, measured as net synthesis of ATP, is very low with respect to the full respiration of carbohydrates in the mitochondrion, since most of the energy available in the sugars remains inside the not fully oxidized products ethanol and/or lactate. In fact, fermentation yields only 2–3 mol ATP mol^{-1} glucose (Mancuso and Marras 2006), compared with 24–36 mol ATP mol^{-1} glucose in O_2 presence (Gibbs and Greenway 2003). Because of this, it may happen to verify an increased rate of glycolysis, in order to balance the lower energy yield of alcoholic fermentation. This is known as the Pasteur effect (Gibbs et al. 2000). As a result, under prolonged hypoxic conditions the activation of glycolytic enzymes can be observed (Setter et al. 1997; Liu et al. 2005; Loreti et al. 2005), and the consequent high rate of fermentation increases the demand for carbohydrates.

The hypothesis that carbohydrate supplies for an enhanced glycolysis rate becomes critical for survival in oxygen deficiency stress is supported by some studies that show an improved sugar supply to flooded roots, precisely to compensate the higher request for oxidable substrate under these conditions. This behaviour might make one think that an optimal carbohydrate supply could be important for survival under prolonged hypoxic conditions. Indeed, many flood-sensitive species show a reduced carbohydrate concentration in their roots (Vu and Yelenosky 1991), but their survival can be improved by providing them with exogenous sugars (Waters et al. 1991; Loreti et al. 2005).

However, even if maintaining adequate levels of fermentable sugars can help for long term survival, there are various exceptions to this trend. Some herbaceous plants as well as trees show a build-up of sugars in roots (Albrecht et al. 1993; Castonguay et al. 1993; Huang and Johnson 1995), possibly due to a stop in root growth in order to save energy, with a consequent reduced carbon demand (Angelov et al. 1996). This is another survival strategy (metabolic depression strategy), as seen in *Vitis*, in order to slow down metabolic activity, and to depress the rate of ATP use (Mancuso and Marras 2006). Furthermore in some species, an ATP deficiency seems to delay glycolysis, and sugars again accumulate rather than decline (Fukao and Bailey-Serres 2004).

To assure cell survival, the roots of flooded plants can require a higher carbohydrate supply to sustain the increased rate of glycolysis necessary for ATP production. In this case the need for sugars can be satisfied by an increase in transport of sucrose (Suc) via phloem from leaves to roots. There is experimental evidence of an increase in Suc concentration in the phloem sap of hypoxically treated plants, accompanied by a decrease in Suc concentration in leaves (Kreuzwieser et al. 2009). Some species such as peas, pumpkin, or several herbaceous plants, have shown that they need a continuous supply of sugars from shoot to root to survive under flooding (Jackson and Drew 1984; Saglio et al. 1980; Webb and Armstrong 1983).

On the other hand, there are also cases in which flooding decreased carbohydrate translocation, as in alfalfa (*Medicago sativa* L.) and *Lotus corniculatus* L. (Barta

1987). This trend may be due to a request reduction for carbohydrate by roots (Wample and Davis 1983), but also to an impaired phloem translocation (Saglio 1985; Topa and Cheeseman 1992a). The reduction of carbohydrate request is probably caused by the slowed root metabolism under hypoxia which in turn generates a decreased demand for sugars from leaves (Hsu et al. 1999; Wample and Davis 1983) and therefore a reduced rate of carbohydrate translocation (Barta 1987). This could be one reason why under flooding conditions starch accumulates in leaves, although the photosynthetic rate declines (Vu and Yelenosky 1991; Wample and Davis 1983). In flooding treatment, carbohydrate content (including the contribution of starch) can thus increase in leaves, as for sunflower (Wample and Davis 1983), purple flower alfalfa (Barta 1988), sweet orange (Vu and Yelenosky 1991), pine (Topa and Cheeseman 1992a, b), and bitter melon (Liao and Lin 1994), *H. annuus* (Wample and Davis 1983), *M. charantia* (Liao and Lin 1994), or *S. samarangense* (Hsu et al. 1999).

9.7 Conclusions

Much evidence suggests that leaf biochemistry in trees is severely affected by soil waterlogging, causing a noticeable decline in the rate of photosynthesis and transpiration (Kreuzwieser et al. 2004). Phloem transport can also be affected, and soluble sugars accumulate in the leaves instead of providing the roots with carbohydrates. On the other hand, roots need adequate levels of fermentable sugars for long term survival. When sugars are not coming from the leaves, some roots can utilize the starch accumulated there. In some species in fact, starch can be degraded and converted into fermentable substrates for anaerobic metabolism (Perata et al. 1992; Liao and Lin 2001).

As oxygen falls under normoxic conditions, there is a widespread decrease in ATP-consuming processes, mostly in the ones involved in biosynthesis and growth. Other pathways which cannot be suppressed are by-passed by alternative ones, but less energy-consuming. This is the case of sucrose degradation. Sucrose is degraded by invertase and hexokinase to generate fructose-6-phosphate, the first intermediate of glycolysis. This reaction requires two molecules of ATP. When oxygen concentrations are low, this pathway is replaced by sucrose synthase (SuSy) and UDP-glucose pyrophosphorylase, which require only one molecule of inorganic pyrophosphate (PPi). The cost is even lower if one thinks that PPi is generated by a wide range of reactions as a waste product (Geigenberger 2003; Magneschi and Perata 2009).

There is still much to learn about the molecular and biochemical adaptations in a plant to low oxygen condition, and the way each tissue responds to it. It is evident that a coordinate and cooperative response is required for the tolerance to the stress. It involves changes in numerous pathways and metabolic processes, starting from the oxygen sensing system(s) and the consequent signal transduction pathway(s) that still remain unknown.

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