Adaptations of Nitrogen Metabolism to Oxygen Deprivation in Plants

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Abstract Acclimation of plants to O_2 deprivation depends on their ability to mitigate detrimental effects related to energy crisis and cytosolic acidosis. Accordingly, lactic and ethanol fermentative pathways are activated under low oxygen stress in order to regenerate NAD^+ to maintain a high glycolysis rate that becomes the major route for ATP production. Paradoxically lactic acid worsens cytosolic acidosis and ethanol fermentation drains carbon for the production of a metabolically useless dead-end product. Nitrogen metabolism is profoundly affected by O_2 deprivation. Interestingly hypoxic N metabolism not only contributes to tolerate O_2 deprivation but also mitigates negative effects of lactic and ethanol fermentation. The most salient event is the concerted modulation of alanine and glutamate pathways that allow for the substitution of ATP-dependent enzymes glutamine synthetase (GS) and asparagine synthetase (AS) by alanine aminotransferase (AlaAT) and glutamate oxoglutarate aminotransferase (NADH-GOGAT) as essential enzymes of N assimilation. This adaptation saves ATP, regenerates NAD^+, and saves carbon in the form of alanine, a C/N storage form readily remobilized upon recovery. As for acidosis amelioration, nitrogen metabolism participates in the cellular pH-stat through GABA and putrescine pathways. Alanine accumulation contributes indirectly to pH homeostasis by using pyruvate competitively with lactate dehydrogenase.

1 Introduction

Flooding of the root system is known as waterlogging and is a major cause of O_2 deprivation to plants (Bailey-Serres et al. 2012; Bailey-Serres and Voesenek 2008). When soils are saturated with water, the root environment becomes hypoxic or

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anoxic due to the insufficient diffusion of O_2 through water and the competition for O_2 with respiring microorganisms. Gases diffuse approximately 10,000 times slower in water than in air (Drew 1997; Jackson 1985) and as a consequence the flux of oxygen into plants becomes too slow to support respiration, resulting in energy deficits and, eventually, death of cells and tissues in non-adapted plants (Gout et al. 2001; Jackson and Armstrong 1999). Major disorders caused by hypoxia are related to (1) an energy crisis due to the inhibition of mitochondrial oxidative phosphorylation and subsequent reduction of the cellular ATP/ADP ratio and the adenylate energy charge ([ATP + 0.5ADP]/[ATP + ADP + AMP]) (Greenway and Gibbs 2003) and (2) cytoplasm acidification, a major determinant in intolerance to O_2 deficiency (Roberts et al. 1989) caused by the release of H^+ accompanying hydrolysis of the pools of Mg-nucleoside triphosphate (NTP) and sugar phosphates, impaired functioning of the plasma membrane H^+-pumping ATPase (Gout et al. 2001), accumulation of non-processed acidic intermediates like glycolytic compounds (Felle 1996), and a poor CO_2 removal (Saglio et al. 1999). The initial cellular reaction to cope with this energy crisis in both tolerant and intolerant species relies on the acceleration of glycolysis and lactate and ethanol fermentation to generate ATP and regenerate NAD^+. Paradoxically the onset of fermentation worsens cytoplasm acidification due to lactate synthesis by lactate dehydrogenase (LDH) (Davies 1987; Davies and Patil 1974) and the production of acetaldehyde by pyruvate decarboxylase (PDC) a highly reactive chemical affecting cellular damage by forming acetaldehyde–protein adducts (Braun et al. 1995; Jackson 1985; Jackson and Armstrong 1999). Furthermore higher rates of glycolysis and ethanol fermentation induce a faster depletion of sugar stores and thus carbon-starvation stress. Ethanol produced by alcohol dehydrogenase (ADH) is a dead-end product that either accumulates or leaks out of the tissue representing in both cases a net loss of carbon skeletons. Nevertheless it seems that the regeneration of NAD^+ by fermentative enzymes ADH and LDH is vital for hypoxia/anoxia tolerance because in the absence of NAD^+ glycolysis ceases (Albrecht et al. 2004; Ismond et al. 2003; Kursteiner et al. 2003).

The difference between tolerant and intolerant plants to O_2 deprivation is dependent on their ability to mitigate damaging effects of energy crisis and acidosis by reducing energy requirement for tissue maintenance and setting effective control of cytoplasmic pH (Greenway and Gibbs 2003). The reduction of energy consumption includes the reduction of storage compound (proteins, lipids, and starch) synthesis, preferential use of PPi-dependent enzymes like pyruvate orthophosphate dikinase (PPDK) instead of pyruvate kinase (PK) for pyruvate synthesis, and sucrose synthase pathway instead of invertase pathway for sucrose catabolism (Bailey-Serres and Voesenek 2008). Major functions involved in the control of cytoplasmic pH involve the extrusion of protons through H^+-ATPases located at the plasma membrane and through H^+-ATPases and H^+-PPiases located at the tonoplast allowing for the generation of free energy gradient for uptake of the strong cation K^+ (Greenway and Gibbs 2003): decarboxylation of organic acids contributes also to protons removal (Gout et al. 2001; Greenway and Gibbs 2003; Roberts et al. 1989). In *Oryza sativa* var arborio, a species highly resistant to O_2 deprivation,

acidification in shoots (pH 7.4–7.0) stabilized after 10 min of anoxia and alkalinization of both cytoplasm and vacuole followed thereafter. In contrast in O_2-deprivation-intolerant wheat (*Triticum aestivum* var MEK shoots), the same treatment caused a sharper and progressive cytoplasmic acidification (pH 7.4–6.6) during the anoxia period and there was no vacuolar alkalinization comparable to the one observed in the rice species.

Cellular acclimation to O_2 deprivation is an important issue, to which nitrogen metabolism may contribute. In the present chapter; the contribution of several aspects of nitrogen metabolism, including nitrate reduction and amino acids as well as polyamine metabolism, to the cellular response to hypoxia in plants is presented and discussed.

2 Modulation of Nitrogen Metabolism Upon O_2 Deprivation

2.1 Nitrate, Nitrite, and Nitric Oxide

Nitrate reductase (NR) is a cytosolic enzyme that catalyzes NADH-dependent nitrate reduction into nitrite. NR gene expression and enzyme activity were shown to increase significantly in response to O_2 deprivation in several plant species, e.g., *Arabidopsis thaliana*, *Medicago truncatula*, and *Oryza sativa* (Allègre et al. 2004; Botrel and Kaiser 1997; Lasanthi-Kudahettige et al. 2007; Loreti et al. 2005; Stoimenova et al. 2007). In *Cucumis sativus* increased NR activity correlated with an increase in transcript levels of NR and its cofactor-binding domain genes *FAD* (*FAD binding*) and *CYP51G1* (*Heme binding*). Furthermore, it was proposed that the activation of NR activity was probably induced by both new enzyme synthesis and dephosphorylation of the phosphor-NR protein because the expression of the *PP2A* gene that encodes the phosphor-NR phosphatase increased several-fold under hypoxic stress (Shi et al. 2008). It has also been proposed that cytosolic pH acidification may increase NR activity because of the low pH optimum of this enzyme (Botrel and Kaiser 1997; Stoimenova et al. 2007).

Altogether these findings point out to an important role played by NR under hypoxic conditions with species exhibiting greater ability to tolerate O_2 deprivation showing higher nitrate reductase activity. Indeed, tobacco plants with low NR activity displayed several metabolic disorders linked to hypoxia stress with enhanced ethanol and lactate production and increased acidification of the cytosol. Conversely, supplying maize seedlings with nitrate during anoxia maintains a slightly higher cytosolic pH than that in the control seedlings (Libourel et al. 2006). In *Cucumis sativus*, increased NR activity upon hypoxia was accompanied by decrease in NO_3^- and increase in NO_2^- contents indicating that nitrite reduction under hypoxic conditions does not match the increased nitrate reduction process and that the benefit of nitrate supply was not due to a metabolic effect (Shi

et al. 2008). Nitrate reduction may rather contribute to cellular acclimation to low O_2 deprivation by regenerating NAD^+ from NADH.

The effect of nitrite was also investigated. For this aim maize seedling roots were fed nitrite under hypoxic condition. It appeared that the provision of micromolar levels of nitrite was effective on the cytosolic pH adjustment indicating that nitrite is also implicated in cellular acclimation to O_2 deprivation. Since very low levels of nitrite effected cellular response to hypoxia its role is unlikely to be limited to the regeneration of $NAD(P)^+$ and might rather be linked to a regulatory mechanism (Libourel et al. 2006) such as NO emission that may activate mitochondrial ROS production and Ca^{2+} release (Ma et al. 2012; Talwar et al. 2012; Zhang et al. 2007). During O_2 deficit electrons generated by the oxidation of NAD(P)H by the Ca^{2+}-sensitive NAD(P)H dehydrogenases on the inner mitochondrial membrane surface are proposed to be accepted by nitrite at complex III (ubiquinone:cytochrome *c* reductase) or IV (COX) of the mitochondrial electron transport chain, producing the signal molecule NO and contributing to ATP synthesis due to proton pumping at the sites of complex III or COX (Planchet et al. 2005; Stoimenova et al. 2007). Alternatively nitrite may participate to NO emission by being reduced in the cytosol by nitrate reductase (Kaiser et al. 2002; Sakihama et al. 2002; Yamasaki et al. 1999). Due to the high K_m (100 mM) of nitrate reductase for nitrite, emission of NO is competitively inhibited by nitrate (Rockel et al. 2002). However, the rate of NO production by nitrate reductase increases each time nitrate reduction exceeded nitrite reduction such that nitrite accumulated; typically this is the case under low oxygen condition, e.g., NO emission could be established in leaves exposed to hypoxia/anoxia in the dark and in detached leaves fed nitrite through the petiole and maintained in the dark (Kaiser et al. 2002). Accordingly the inactivation of nitrate reductase by maintaining the enzyme in its phosphorylated state by the inhibitors of PP2A decreased the rate of NO emission (Kaiser et al. 2002). Nonsymbiotic hemoglobin, which genes expression was found to be greatly induced by low oxygen in flooded roots, was proposed to scavenge NO in hypoxic tissues by catalyzing its turnover to nitrate (Dordas et al. 2003a, b; Igamberdiev and Hill 2004; Igamberdiev et al. 2004). When hemoglobin (Hb) is coupled with nitrite reductase activity in hypoxic cells, this forms the Hb/NO cycle, in which excess NAD(P)H is oxidized (Hebelstrup et al. 2012). Additional positive effect of this reaction is the limitation of nitrogen loss under NO emission (Hebelstrup et al. 2012). Over-expression of Hb in alfalfa maintained ATP levels, ATP/ADP ratio, and increased survival during hypoxia compared to wild type and Hb-silenced plants in which ATP and ATP/ADP ratio declined under low oxygen condition (Dordas et al. 2003a). NO emission was 2.5-fold higher in Hb-silenced plants compared to Hb-over-expressers suggesting the involvement of Hb in plant response to hypoxia through the modulation of NO emission and the loss of nitrogen (Dordas et al. 2003a).

2.2 Alanine and GABA

Nitrogen assimilation and amino acids metabolism are profoundly affected by O_2 deprivation and energy shortage (Fig. 1). One of the most salient effects of hypoxia is the accumulation of alanine. In *Medicago truncatula* seedlings it has been shown that these changes were related to the stimulation of the expression of the mitochondrial alanine aminotransferase (*mAlaAT*) isogene and the accumulation of the encoded protein mAlaAT (Ricoult et al. 2005, 2006). Expression of the gene encoding the mitochondrial alanine glyoxylate aminotransferase (*AGT*) was inhibited by hypoxia while the gene encoding the cytosolic *AlaAT* was not expressed in young seedlings. AlaAT activity, as determined in vivo by using $^{15}NH_4$ labeling, increased in hypoxic seedlings (Limami et al. 2008; Ricoult et al. 2006). Alanine metabolism was investigated more thoroughly by feeding seedlings with either ^{15}N-glutamate or ^{15}N-alanine upon normoxic and hypoxic conditions (Ricoult et al. 2006). Feeding embryo axis with ^{15}N-glutamate or ^{15}N-alanine under normoxic conditions showed that mAlaAT catalyzed a reversible reaction allowing for synthesis of alanine with glutamate as amino donor and synthesis of glutamate with alanine as amino donor.

The same experiment showed that glycine synthesis occurred at the expense of either glutamate or alanine indicating that besides alanine glyoxylate aminotranferase (AGT) a glutamate glyoxylate aminotranferase was also operating. Feeding seedlings either ^{15}N-glutamate or ^{15}N-alanine under hypoxia showed that mAlaAT activity was directed towards alanine synthesis using glutamate as amino donor while the reaction of glutamate synthesis using alanine as amino donor was inhibited (Ricoult et al. 2006). The results indicate that mAlaAT isoform is regulated at both transcriptional and posttranslational levels. This dual mode of regulation by hypoxia allowed for an increase in the enzyme content through an increase in the expression of the coding gene and at the posttranslational level for the orientation of the equilibrium of the catalyzed reaction towards alanine synthesis. As a result ^{15}N-alanine was 4 times higher in hypoxic seedlings than in the control and alanine accumulated as the major amino acid instead of asparagine (Limami et al. 2008).

In vivo AGT activity in the direction of glycine synthesis was inhibited by hypoxia as shown by the fact that almost no labeled glycine was detected in seedlings when they were fed ^{15}N-alanine (Ricoult et al. 2006). Total absence of glycine (both labeled and unlabeled) in hypoxic seedlings fed ^{15}N-alanine means that glutamate glyoxylate transaminase did not compensate for the lack of AGT activity probably because glutamate was competitively recruited for alanine and GABA synthesis by mAlaAT and GDC (glutamate decarboxylase). Labeling experiment showed also that there was a dramatic decrease in de novo synthesis of glutamine and particularly asparagine which is the most abundant amino acid in *Medicago truncatula* (Glevarec et al. 2004). Both the enzymes glutamine synthetase (GS) and asparagine synthetase (AS) are ATP-dependent; inhibition of their activities is probably related to the energy crisis. Consistently, more than just a

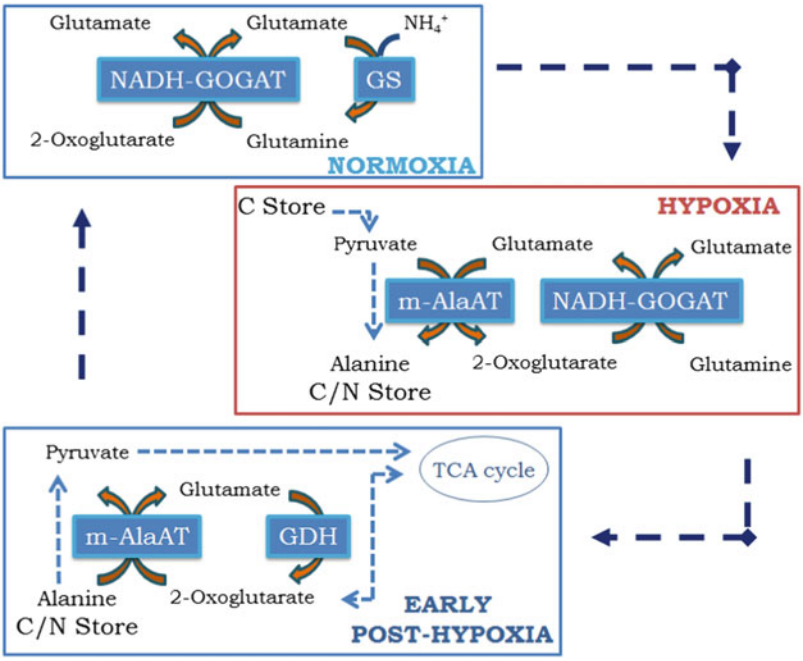

Fig. 1 Schematic representation of the central role of alanine metabolism during hypoxia and early post-hypoxia periods as revealed by ^{15}N labeling experiments in young *Medicago truncatula* seedlings. *Dashed lines* in Hypoxia and Early Post-hypoxia boxes represent fluxes of carbon from carbon storage compounds to alanine and further mobilization into Krebs cycle during post-hypoxia recovery period

change in *AlaAT* expression and activity significant changes were induced by hypoxia in amino acids metabolism that resulted in the accumulation of alanine as the major amino acid instead of asparagine in *Medicago truncatula*.

Changes in amino acids metabolism with alanine accumulation as the culminant event may contribute to mitigate the most damaging consequences of O_2 deprivation; i.e., an energy crisis and acidosis. Synthesis of alanine is accompanied by the generation of 2-oxoglutarate which can be further metabolized to succinate, via the TCA cycle enzyme succinate CoA ligase, thereby providing additional ATP per molecule of sucrose metabolized (Rocha et al. 2010). Accumulation of alanine upon hypoxia as a C/N storage compound saves ATP that otherwise is needed for the synthesis of asparagine or glutamine and saves C3 skeletons avoiding a shortage in carbon availability. Ethanol is a dead-end product that either accumulates or leaks out of the tissue representing in both cases a net loss of carbon. As a matter of fact the increase in the flow of carbon to ethanol by overexpressing *PDC* in *Arabidopsis thaliana* was effective in improving anoxia tolerance only in plants growing on a full nutrient Murashige and Skoog medium containing 3 % (w/v) of sugar (Ismond et al., 2003; Albrecht et al., 2004). Under sugar-limiting conditions as in the field, due to higher rates of glycolysis and ethanol fermentation, a faster

depletion of sugar stores leads to decreased survival upon O_2 deprivation (Ismond et al. 2003). Finally, although cytoplasmic pH homeostasis relies on the cellular pH-stat, synthesis of alanine contributes to a limitation of cytoplasmic acidification by lactate because of the competition for pyruvate by both lactate fermentation and alanine synthesis pathways (Menegus et al. 1991; Reggiani et al. 2000; Ricoult et al. 2005, 2006).

Alanine synthesis and accumulation is thought to occur upon O_2 deprivation through another metabolic pathway. Alanine may accumulate as a byproduct of the GABA shunt that involves three reactions catalyzed by glutamate decarboxylase (GDC), GABA transaminase (GABA-T), and succinic semialdehyde dehydrogenase (SSADH). GABA is derived from the decarboxylation of glutamate by GDC thereby contributing to cellular pH-stat under hypoxia stress as glutamate decarboxylation is a proton consuming reaction that reduces the weak acid content and increases pH (Drew 1997; Greenway and Gibbs 2003). GDC is regulated by H^+ and Ca^{2+} which levels increases under hypoxia. Consistently GDC activity increases as the cytoplasmic pH declines and decreases as the pH recovers (Carroll et al. 1994). Ca^{2+} with calmodulin as Ca/CAM complex binds to the enzyme, thereby relieving it from autoinhibition (Bouché et al. 2004, 2005). Succinic semialdehyde (SSA) is produced from GABA via GABA-T that can use either pyruvate or 2-oxoglutarate as amino acceptor. It seems however that under hypoxic conditions the enzyme uses preferentially pyruvate thus leading to alanine synthesis. In favor of this hypothesis alanine accumulation was observed under hypoxic conditions in Arabidopsis T-DNA knockout mutants affected in *AlaAT* (Miyashita et al. 2007; Miyashita and Good 2008) indicating that the production of alanine might depend on another metabolic pathway. However GABA-T null mutants accumulated only slightly less alanine upon hypoxia compared with wild-type plants (Miyashita et al. 2007; Miyashita and Good 2008) suggesting that AlaAT and GABA-T pathways might be redundant at least under hypoxic conditions.

2.3 Glutamate

Enzymes of primary nitrogen assimilation involved in glutamate synthesis or using glutamate as amino donor were shown to be differently affected by O_2 deprivation in *Medicago truncatula* seedlings (Limami et al. 2008). Expression of genes encoding the ATP-consuming enzymes GS and AS and activities of these enzymes were inhibited by hypoxia stress. NADH-dependent glutamate synthase (*NADH-GOGAT*) expression was inhibited by hypoxia stress, while NADH-GOGAT activity increased. Conversely glutamate dehydrogenase (*GDH*) expression was up-regulated by hypoxic stress, while GDH activity, determined either in vitro or by native PAGE staining, was down-regulated. In vivo $^{15}NH_4^+$ labeling in the presence and absence of the GS-inhibitor methionine sulfoximine (MSX), used in combination with GC–MS amino acids analyses, indicated that the residual GDH activity was not contributing to glutamate synthesis upon hypoxic conditions.

In parallel to these investigations a blend of metabolic experiments using the incorporation of $^{15}NH_4^+$ and ^{15}N-amino acids during hypoxic stress showed that the pools of newly synthesized glutamate (^{15}N-glutamate) in normoxic and hypoxic *Medicago truncatula* seedlings were very similar indicating that the glutamate content was subjected to a very tight control. Consistently it is suggested that rather than just an activation of alanine synthesis, the adaptive reaction of the plant to hypoxic stress consists of a concerted modulation of nitrogen flux through both glutamate and alanine synthesis pathways.

It appears as if the decrease in glutamate utilization by GS and AS—probably due to the lack of ATP—was compensated for by increased NADH-GOGAT and AlaAT activities, as revealed by increased amounts of newly synthesized alanine (^{15}N-alanine) during hypoxic stress. Therefore, it is likely that the reductive amination of 2-oxoglutarate by NADH-GOGAT during hypoxic stress fulfills two major roles. The first is the synthesis of glutamate, the substrate of AlaAT, and the second is the oxidation of NADH when oxidative phosphorylation is totally or partially inhibited by the lack of oxygen, thus making NAD^+ available to enable glycolysis to proceed (Limami et al. 2008).

Finally the discrepancy between the levels of *GDH* gene expression and enzyme activity questions the significance of the up-regulation of the expression of *GDH* in *Medicago truncatula* immediately following hypoxic stress? It has been suggested that the return to aerobic conditions is anticipated in plants subjected to hypoxic stress by expressing genes whose products have functions during the subsequent recovery period (Drew 1997). Consistently, it is proposed that *GDH1* was up-regulated by hypoxia-induced carbon stress, in anticipation that the product of its transcription would regenerate 2-oxoglutarate by deaminating glutamate during the subsequent post-hypoxic recovery period (Fig. 1) (Limami et al. 2008). Higher rates of glycolysis and ethanolic fermentation are known to lead to carbon stress due to the faster depletion of sugar stores in hypoxic tissues (Ismond et al. 2003). Up-regulation of the expression of *GDH* genes has been observed under various conditions associated with carbon stress, i.e., senescing leaves, low light and dark, and C/N imbalance due to excess ammonium nutrition (Masclaux-Daubresse et al. 2005; Melo-Oliveira et al. 1996; Skopelitis et al. 2006). The function of GDH in these conditions is assumed to be the oxidative deamination of glutamate that provides C skeletons (2-oxoglutarate) and reducing power (for review, see Forde and Lea 2007). The same pattern of regulation was observed for the hypoxia-inducible *AlaAT1* in *Arabidopsis thaliana* (Miyashita et al. 2007). *AlaAT1* was up-regulated at the transcriptional level during hypoxic stress while the major role of the encoded enzyme was shown to be the conversion of alanine into glutamate during the post-hypoxic period.

2.4 Polyamines

The first step in polyamines biosynthesis in higher plants is the decarboxylation of either arginine by arginine decarboxylase (ADC) or ornithine by ornithine decarboxylase (ODC). Ultimately, both reactions lead to putrescine, the diamine precursor of spermidine and spermine. The latter are formed by sequential addition of an aminopropyl moiety onto putrescine and spermidine in reactions catalyzed by spermidine synthase and spermine synthase (Shelp et al. 2012).

Accumulation of putrescine, rather than spermidine or spermine, was associated in several plant species to hypoxia tolerance. Greater capacity of putrescine accumulation was observed in species like rice and barnyard grass which are well adapted to hypoxic environment than that in anoxia-intolerant species (Reggiani et al. 1989). In several graminae species acclimation to O_2 deprivation was associated to putrescine accumulation as a result of the induction of ADC and ODC and concomitant inhibition of degradation of putrescine by diamine oxidase (DAO). Furthermore, ethylene—which synthesis is boosted by O_2 deprivation (Bailey-Serres and Voesenek 2008)—is known to inhibit spermidine and spermine synthesis by competition for a common precursor, the aminopropyl moiety donor *S*-adenosylmethionine (SAM) (Amir 2010). Hypoxia-induced shoot elongation in the flood-tolerant grassweed *Scirpus mucronatus* coincided with increased putrescine content. Alternatively the inhibition of putrescine synthesis through the inhibition of ADC and ODC resulted in an inhibition of shoot elongation upon O_2 deprivation. This inhibitory effect was reversed by exogenous putrescine treatment pointing out to the important role this diamine may play in acclimation to O_2 deprivation (Lee and Kende 2001, 2002). Similarly rice coleoptiles elongation under anoxia was also associated with putrescine accumulation, inhibited by an ADC inhibitor and reestablished by exogenous putrescine treatment (Reggiani et al. 1989, 2000). Root hypoxia tolerance was increased and injury linked to O_2 deprivation was alleviated by putrescine application to tomato plants (Nada et al. 2004). Altogether these findings strongly suggest that putrescine may play a protective role in acclimation to O_2 in plants (Lee and Kende 2001, 2002). Putrescine and to a very lesser extent spermidine but not spermine accumulated in waterlogged *Medicago truncatula* roots as well as the precursors ornithine and arginine (Fig. 2, Diab and Limami, unpublished data).

The beneficial role of putrescine is still poorly understood. As a cation and one of the compounds thought to produce basic equivalents it was suggested that the diamine can contribute to balancing the anoxic production of organic acids and to the homeostatic buffering mechanism for stabilizing intracellular pH. Putrescine accumulated in anoxic rice coleoptiles to concentrations comparable in magnitude to the sum of concentrations of lactic acid and succinic acid (Reggiani et al. 1989).

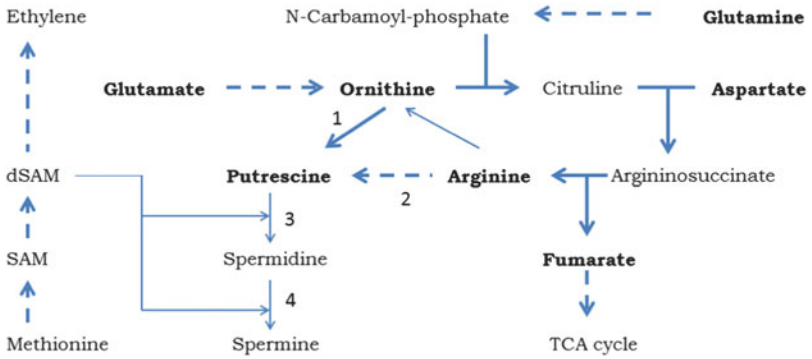

Fig. 2 Schematic representation of putrescine, spermidine, and spermine biosynthesis pathway and its relation with ethylene biosynthesis through the common precursor, decarboxylated *S*-adenosylmethionine (dSAM). In *bold characters* are shown the compounds that accumulated in waterlogged roots of *Medicago truncatula*. Putrescine is synthesized directly by ornithine decarboxylase (1) or through agmatine and *N*-carbamoylputrescine by arginine decarboxylase (2). Reactions 3 and 4 are catalyzed, respectively, by spermidine synthase and spermine synthase

3 Conclusion

Modulation of nitrogen metabolism is an important component of the acclimation of plants to waterlogging-induced O_2 deprivation. Hypoxic N metabolism not only contributes to energy crisis and acidosis amelioration but also counteracts detrimental effects of lactic and ethanol fermentation. A large bulk of information was gathered during the last decades on the adaptive response of each of N and C primary metabolisms to O_2 deprivation. However, a broad picture of the interaction between both metabolisms under hypoxic conditions including secondary metabolism is still needed for a full understanding of the metabolic adaptation of plants to low O_2 stress. For this aim an integrative approach including metabolomic and transcriptomic analysis would be suitable.

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References

Albrecht G, Mustroph A, Theodore C (2004) Sugar and fructan accumulation during metabolic adjustment between respiration and fermentation under low oxygen conditions in wheat roots. Physiol Plant 120:93–104

Allègre A, Silvestre J, Morard P, Kallerhoff J, Pinelli E (2004) Nitrate reductase regulation in tomato roots by exogenous nitrate: a possible role in tolerance to long-term root anoxia. J Exp Bot 55:2625–2634

Amir R (2010) Current understanding of the factors regulating methionine content in vegetative tissues of higher plants. Amino Acids 39:917–931

Bailey-Serres J, Voesenek LA (2008) Flooding stress: acclimations and genetic diversity. Annu Rev Plant Biol 59:313–339
Bailey-Serres J, Fukao T, Gibbs DJ, Holdsworth MJ, Lee SC, Licausi F, Perata P, Voesenek LA, van Dongen JT (2012) Making sense of low oxygen sensing. Trends Plant Sci 17:129–138
Botrel A, Kaiser WM (1997) Nitrate reductase activation state in barley roots in relation to the energy and carbohydrate status. Planta 201:496–501
Bouché N, Fait A, Zik M, Fromm H (2004) The root-specific glutamate decarboxylase (GAD1) is essential for sustaining GABA levels in Arabidopsis. Plant Mol Biol 55:315–325
Bouché N, Yellin A, Snedden WA, Fromm H (2005) Plant-specific calmodulin-binding proteins. Annu Rev Plant Biol 56:435–466
Braun KP, Cody RB, Jones JDR, Peterson CM (1995) A structural assignment for a stable acetaldehyde-lysine adduct. J Biol Chem 270:11263–11266
Carroll AD, Fox GG, Laurie S, Phillips R, Ratcliffe RG, Stewart GR (1994) Ammonium assimilation and the role of [gamma]-aminobutyric acid in pH homeostasis in carrot cell suspensions. Plant Physiol 106:513–520
Davies DD (1987) The role of lactate dehydrogenase isozymes in controlling the cytosolic pH of plant cells. Isozymes Curr Top Biol Med Res 16:193–207
Davies DD, Patil KD (1974) Regulation of 'malic' enzyme of Solanum tuberosum by metabolites. Biochem J 137:45–53
Dordas C, Hasinoff BB, Igamberdiev AU, Manac'h N, Rivoal J, Hill RD (2003a) Expression of a stress-induced hemoglobin affects NO levels produced by alfalfa root cultures under hypoxic stress. Plant J 35:763–770
Dordas C, Rivoal J, Hill RD (2003b) Plant haemoglobins, nitric oxide and hypoxic stress. Ann Bot 91 Spec No: 173–178
Drew MC (1997) Oxygen deficiency and root metabolism: injury and acclimation under hypoxia and anoxia. Annu Rev Plant Physiol Plant Mol Biol 48:223–250. doi:10.1146/annurev.arplant.48.1.223
Felle HH (1996) Control of cytoplasmic pH under anoxic conditions and its implication for plasma membrane proton transport in Medicago sativa root hairs. J Exp Bot 47:967–973. doi:10.1093/jxb/47.7.967
Forde BG, Lea PJ (2007) Glutamate in plants: metabolism, regulation, and signalling. J Exp Bot 58:2339–2358
Glevarec G, Bouton S, Jaspard E, Riou MT, Cliquet JB, Suzuki A, Limami AM (2004) Respective roles of the glutamine synthetase/glutamate synthase cycle and glutamate dehydrogenase in ammonium and amino acid metabolism during germination and post-germinative growth in the model legume Medicago truncatula. Planta 219:286–297
Gout E, Boisson A, Aubert S, Douce R, Bligny R (2001) Origin of the cytoplasmic pH changes during anaerobic stress in higher plant cells. Carbon-13 and phosphorous-31 nuclear magnetic resonance studies. Plant Physiol 125:912–925
Greenway H, Gibbs J (2003) Mechanisms of anoxia tolerance in plants. II. Energy requirements for maintenance and energy distribution to essential processes. Funct Plant Biol 30:37
Hebelstrup KH, van Zanten M, Mandon J, Voesenek LA, Harren FJ, Cristescu SM, Møller IM, Mur LA (2012) Haemoglobin modulates NO emission and hyponasty under hypoxia-related stress in Arabidopsis thaliana. J Exp Bot 63:5581–5591
Igamberdiev AU, Hill RD (2004) Nitrate, NO and haemoglobin in plant adaptation to hypoxia: an alternative to classic fermentation pathways. J Exp Bot 55:2473–2482
Igamberdiev AU, Seregélyes C, Manac'h N, Hill RD (2004) NADH-dependent metabolism of nitric oxide in alfalfa root cultures expressing barley hemoglobin. Planta 219:95–102
Ismond KP, Dolferus R, De Pauw M, Dennis ES, Good AG (2003) Enhanced low oxygen survival in Arabidopsis through increased metabolic flux in the fermentative pathway. Plant Physiol 132:1292–1302
Jackson M (1985) Ethylene and responses of plants to soil waterlogging and submergence. Annu Rev Plant Physiol Plant Mol Biol 36:145–174

Jackson M, Armstrong W (1999) Formation of aerenchyma and the processes of plant ventilation in relation to soil flooding and submergence. Plant Biol 1:274–287

Kaiser WM, Weiner H, Kandlbinder A, Tsai CB, Rockel P, Sonoda M, Planchet E (2002) Modulation of nitrate reductase: some new insights, an unusual case and a potentially important side reaction. J Exp Bot 53:875–882

Kursteiner O, Dupuis I, Kuhlemeier C (2003) The pyruvate decarboxylase1 gene of Arabidopsis is required during anoxia but not other environmental stresses. Plant Physiol 132:968–978

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

Lee Y, Kende H (2001) Expression of beta-expansins is correlated with internodal elongation in deepwater rice. Plant Physiol 127:645–654

Lee Y, Kende H (2002) Expression of alpha-expansin and expansin-like genes in deepwater rice. Plant Physiol 130:1396–1405

Libourel IG, van Bodegom PM, Fricker MD, Ratcliffe RG (2006) Nitrite reduces cytoplasmic acidosis under anoxia. Plant Physiol 142:1710–1717

Limami AM, Glevarec G, Ricoult C, Cliquet J-B, Planchet E (2008) Concerted modulation of alanine and glutamate metabolism in young Medicago truncatula seedlings under hypoxic stress. J Exp Bot 59:2325–2335. doi:10.1093/jxb/ern102

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

Ma F, Lu R, Liu H, Shi B, Zhang J, Tan M, Zhang A, Jiang M (2012) Nitric oxide-activated calcium/calmodulin-dependent protein kinase regulates the abscisic acid-induced antioxidant defence in maize. J Exp Bot 63:4835–4847

Masclaux-Daubresse C, Carrayol E, Valadier MH (2005) The two nitrogen mobilisation- and senescence-associated GS1 and GDH genes are controlled by C and N metabolites. Planta 221:580–588

Melo-Oliveira R, Oliveira IC, Coruzzi GM (1996) Arabidopsis mutant analysis and gene regulation define a nonredundant role for glutamate dehydrogenase in nitrogen assimilation. Proc Natl Acad Sci U S A 93:4718–4723

Menegus F, Cattaruzza L, Mattana M, Beffagna N, 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 Physiol 95:760–767

Miyashita Y, Good AG (2008) Contribution of the GABA shunt to hypoxia-induced alanine accumulation in roots of Arabidopsis thaliana. Plant Cell Physiol 49(1):92–102

Miyashita Y, Dolferus R, Ismond KP, Good AG (2007) Alanine aminotransferase catalyses the breakdown of alanine after hypoxia in Arabidopsis thaliana. Plant J 49:1108–1121

Nada K, Iwatani E, Doi T, Tachibana S (2004) Effect of putrescine pretreatment to roots on growth and lactate metabolism in the root of tomato (Lycopersicum esculentum Mill.) under root-zone hypoxia. J Jpn Soc Hortic Sci 73:3

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

Reggiani R, Hochkoeppler A, Bertani A (1989) Polyamines in rice seedlings under oxygen-deficit stress. Plant Physiol 91:1197–1201

Reggiani R, Nebuloni M, Mattana M, Brambilla I (2000) Anaerobic accumulation of amino acids in rice roots: role of the glutamine synthetase/glutamate synthase cycle. Amino Acids 18:207–217

Ricoult C, Cliquet J-B, Limami AM (2005) Stimulation of alanine amino transferase (AlaAT) gene expression and alanine accumulation in embryo axis of the model legume Medicago truncatula contribute to anoxia stress tolerance. Physiol Plant 123:30–39

Ricoult C, Echeverria LO, Cliquet JB, Limami AM (2006) Characterization of alanine aminotransferase (AlaAT) multigene family and hypoxic response in young seedlings of the model legume Medicago truncatula. J Exp Bot 57:3079–3089

Roberts JKM, Chang K, Webster C, Callis J, Walbot V (1989) Dependence of ethanolic fermentation, cytoplasmic pH regulation, and viability on the activity of alcohol dehydrogenase in hypoxic maize root tips. Plant Physiol 89:1275–1278

Rocha M, Licausi F, Araujo WL, Nunes-Nesi A, Sodek L, Fernie AR, van Dongen JT (2010) Glycolysis and the tricarboxylic acid cycle are linked by alanine aminotransferase during hypoxia induced by waterlogging of Lotus japonicus. Plant Physiol 152:1501–1513. doi:10.1104/pp. 109.150045

Rockel P, Strube F, Rockel A, Wildt J, Kaiser WM (2002) Regulation of nitric oxide (NO) production by plant nitrate reductase in vivo and in vitro. J Exp Bot 53:103–110

Saglio P, Germain V, Richard B (1999) The response of plants to oxygen deprivation : role of enzyme induction in the improvement of tolerance to anoxia. In: Lerner HR (ed) Plant responses to environmental stresses. Marcel Dekker, New York, pp 373–393

Sakihama Y, Nakamura S, Yamasaki H (2002) Nitric oxide production mediated by nitrate reductase in the green alga Chlamydomonas reinhardtii: an alternative NO production pathway in photosynthetic organisms. Plant Cell Physiol 43:290–297

Shelp BJ, Bozzo GG, Trobacher CP, Zarei A, Deyman KL, Brikis CJ (2012) Hypothesis/review: contribution of putrescine to 4-aminobutyrate (GABA) production in response to abiotic stress. Plant Sci 193–194:130–135

Shi K, Ding X-T, Don D-K, Zhou Y-H, Yu JQ (2008) Putrescine enhancement of tolerance to root-zone hypoxia in Cucumis sativus: a role in increased nitrate reductase. Funct Plant Biol 35:48

Skopelitis DS, Paranychianakis NV, Paschalidis KA, Pliakonis ED, Delis ID, Yakoumakis DI, Kouvarakis A, Papadakis AK, Stephanou EG, Roubelakis-Angelakis KA (2006) Abiotic stress generates ROS that signal expression of anionic glutamate dehydrogenases to form glutamate for proline synthesis in tobacco and grapevine. Plant Cell 18:2767–2781

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

Talwar PS, Gupta R, Maurya AK, Deswal R (2012) Brassica juncea nitric oxide synthase like activity is stimulated by PKC activators and calcium suggesting modulation by PKC-like kinase. Plant Physiol Biochem 60:157–164

Yamasaki H, Sakihama Y, Takahashi S (1999) An alternative pathway for nitric oxide production in plants: new features of an old enzyme. Trends Plant Sci 4:128–129

Zhang A, Jiang M, Zhang J, Ding H, Xu S, Hu X, Tan M (2007) Nitric oxide induced by hydrogen peroxide mediates abscisic acid-induced activation of the mitogen-activated protein kinase cascade involved in antioxidant defense in maize leaves. New Phytol 175:36–50