

Review

Molecular Mechanisms in Understanding Anoxia Tolerance in Rice Seeds under Submergence and Their Implication in Rice Biotechnology

Malay Kumar Adak ^{1,*}, Abir Das ¹ , Ankita Kundu ¹, Mitali Chatterjee ² and Mirza Hasanuzzaman ^{3,*} 

¹ Plant Physiology and Molecular Biology Research Unit, Department of Botany, University of Kalyani, Kalyani 741235, West Bengal, India

² Rice Research Station, Directorate of Agriculture, Government of West Bengal, Chinsurah 712102, West Bengal, India

³ Department of Agronomy, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka 1207, Bangladesh

* Correspondence: mkadak09@gmail.com (M.K.A.); mhzsauag@yahoo.com (M.H.)

Abstract: Submergence in rice fields creating inundation stress and realizing anoxia or hypoxia is a problem in agriculture. Seeds under this oxygen deficit are faced with fermentative respiration, where the end product would be poisoning the tissue viability. This is more aggravated in direct seeded rice cultivation with the accumulation of lactate as a poison. This review is concerned with the basic insights into anoxia tolerance in seeds and possible strategies to reduce anoxic shock through the modification of metabolism preceded by gene expression. The major concern of anoxic germination is starch metabolism and downstream physiological realization to facilitate escape or quiescence strategy, overcoming submergence stress. The coleoptiles facing hypoxic stress mated with transcripts for oxidative traits, energy metabolism, and proteins for membrane peroxidation in support of energy metabolism are the most important. Hypoxic genes are recovered from traditional *indica* and *japonica* land races of rice, and show changes in glycolytic flux and sugar sensing. Anoxic germination and seedling vigor are based on a combinational regulation of oxidative stress and fermentative catabolism. De novo antioxidant and antioxidative enzyme production can support improved seed germination in this condition. Pre-harvest spouting with seed-coat-induced dormancy, hormonal ratios, and hydrolyses would be of concern. Therefore, comprehensive analysis aimed to understand rice seed priming for better gas exchange, diffusion, temperature sensitivity, ion uptake, redox balance, and others. Still, in-depth insights are being awaited for better understanding the physiological and molecular basis using a multi-omics approach for better seed priming to overcome the anoxic/hypoxic revelation mostly acquainted with submergence stress.

Keywords: α -amylase; QTLs; snorkel; CIPK15; GAs; ADH; PDC; bZIPs; redox; multi-omics



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1. Introduction

Expansion in the human population has been a serious problem for food security worldwide. The climate changes resulting in unseasonal precipitation could increase the frequency of flash or continued floods [1]. The consequences include water logging in agricultural lands, therefore resulting in crop failure and leading to economic loss. Water logging in various depths causes complete or partial submergence, which causes the vulnerability of crops regarding their survival and of seed germination regarding productivity. Rice (*Oryza sativa* L.) happens to be the most staple multi-seasonal, multifold cereal crop throughout the world that supports world hunger [2]. Rice, a semi-aquatic plant, requires a significant amount of water and tissue hydration throughout its developmental stages. The germination of rice seeds requires more soil moisture than other crops, up to the level of full submergence. Seeds of rice require hypoxia or anoxia under inundation

conditions for germination [3]. The germination of rice seeds under hypoxic conditions induces one type of carbohydrate metabolism for starch and mobilization via enzymatic induction. The ability of rice seeds to tolerate hypoxia or anoxia also offers direct seeding in agronomic practices [4]. Direct seeding is more economic than seedling transplantation, where the availability of complete submergence is frequent. Herein, the chances of weed intolerance from floods are highly possible and obstructed by infestation from pests [5]. No matter the species, rice seeds are sensitive to submergence for germination and few cultivars have a better germination rate or seedling tolerance to hypoxia. Tolerant rice seeds exhibit better coleoptile elongation, the induction of carbohydrates, fermentative pathways, and reduced oxidative damages indicating stress tolerance [6]. There are specific quantitative trait loci (QTL) obtained from segregating the populations of rice for better hypoxic germination. Likewise, the QTL qAG9-2 from the cv. *Japonica* sub-species is important [7]. Mapping other locus populations has meant that *OsTPP7* in qAG9-2 has been cloned and validated with transcript expression for hypoxic tolerance [8]. *OsTPP7* expresses its activity for trehalose 6-phosphate phosphatase used for sucrose synthesis, as well as carbohydrate translocation to coleoptile under anoxia [9]. Regarding upland and low land rice, traits are differentially shown via anoxia and seeds are also demarcated via hypoxic germination. For low land rice cultivars, typical gene expression is based on hypoxic germination with the promoter activity of *OsCBL10*. Under this promoter, a number of genes are exhibited with up- or downregulation for some protein kinases for phosphorylation [10]. In transgenic rice, natural variations are found from tolerant rice genotypes, where a low calcium flow in root membranes promotes α -amylase activity more than non-tolerant lines.

Substantial development has been made in the perception and understanding of the basis of tolerance in the early vegetative stage that initiates germination in rice, unlike other crops [11]. Significant genetic variabilities are observed for specific land races on germination ability under flooded soil. Herein, we make an effort to review the physiological and molecular insights, and offer a revision to the explanation of genetic consistency supporting tolerance to flooding for germination. We place emphasis on substitute paths and related gene expression over energy crises under anaerobic respiration. With suitable evidence from experiments conducted on rice and related aquatic species, these experiments emphasize hormonal signaling in seed germination under flooding conditions. Thus, an attempt to identify knowledge gaps in future studies with whole metabolomes on germination under submergence has been elucidated. Knowledge on adaptive traits and pathways is expected to support breeding strategies for direct seeding systems in *kharif* seasons. This in downstream situations could be an alternative method for waterlogging tolerance in cereals like rice. Conclusively, we establish a comprehensive insight in this review related exclusively to rice for anoxic seed germination and growth. This is also elucidated via multi-omics approaches which could be exercised in a breeding program.

2. Physiological Relevance of Seeds' Response to Anoxia

The germination of seeds, establishment of seedlings, and development into plants are subjected to flooding stress. Therefore, seed development under anoxic conditions would be sensitive to anoxia or hypoxia [12]. When flooding occurs during seed germination (under complete submergence), a distinct shift into anaerobic metabolism is observed. Rice plants that are semi-aquatic in nature can experience withstanding anaerobic respiration mostly for maintaining respiration, meaning seed tissues are viable under the water. Coleoptiles of sprouting seeds are exposed to anoxia, and total carbohydrate metabolism is shifted toward anaerobic respiration, mostly via alcoholic fermentation. A number of genes like alcohol dehydrogenase (*ADH*) and pyruvate decarboxylase (*PDC*) are the most important in inducing fermentation metabolism [13]. Even rice seeds, which are dormant in nature, also remain non-viable under water. The dormancy of rice seeds and their further progress under water are regulated by an endogenous ratio of abscisic acid (ABA) to gibberellic acid (GA) [14]. The major signaling gene(s) for ABA metabolism in

rice show their expression with varied modalities through the anaerobic phases. The genes that are predominantly involved include *OsABI5*, *OsDSG1*, *OsNCED3*, *OsVP1*, *OsPDS*, and *OsZDS* for ABA metabolism [15]. These are actual key genes governing seed dormancy and germination, and are mostly required for the signaling and metabolism of ABA [16]. In germinating seeds, GA is regulated in an energy dependent flux in terms of its signaling and subsequent energy-yielding process. These are facilitated by GA₃ oxidase and GA₂₀ oxidase gene(s) for catabolic oxidation with a number of homologs [17]. Though the precise regulatory mechanism of genes remains inconclusive, environmental factors like oxygen and carbon dioxide tension, light intensity, relative humidity, the turbidity of water, and diurnal changes in temperature are other regulatory factors [18]. As for examples, a prolonged submergence with high relative humidity causes more dormancy, with the expression of ABA metabolic genes being suppressed by GAs. This is the reason behind submergence-prone areas in South East Asia during early summer, where pre-harvested sprouting under the submergence condition of rice cultivation has been recorded. A significant amount of work, through next generation sequencing (NGS), has been a breakthrough regarding whole genomes contributing to germinating seeds under anaerobic conditions [19]. Transcription and its consequent metabolomics enable specific sequences in a more precise manner in the regulation of seed dormancy under oxygen depletion. In most cases, specific QTLs are deciphered with more of a contribution of ABA and GA signaling in seeds and these develop molecular markers resistant to anoxic germination.

3. Hormonal Interplay between Seed Dormancy and Germination in Rice under Water

It is evident that seed dormancy and germination play a key role in plant initial survival against submergence. Growth regulators like ABA and GA and their ratio compete with each other, favoring either dormancy or germination depending on the physiological needs [20]. ABA suppresses the physiologically assisted genes for germination whereas GAs can erase the effects. Embryo activity at the cellular level overcoming mechanical distances via tests is important. All of the activity of developing embryos is regulated by GA-assisted genes. The latter, with their increasing rates of transcription and translation, may support ABA-suppressed activities of quiescence strategies [21]. In seeds, a precise concentration of GA is maintained by a balance between biosynthesis and its turnover via oxidative degradation. Regulation for de novo biosynthesis is also important in the context of GA activity on seedling development. This becomes more complicated given that under water a significant level of reactive oxygen species (ROS) and Ca²⁺-dependent signaling are prerequisites. This may integrate downstream pathways for anoxia tolerance and the germination of seeds [22]. This is explained more by the fact that ROS can upregulate a number of ABA-metabolizing gene (particularly *ABA13* and *AP2*) transcription factors (TFs). Auxins (Au) are also involved to allow the binding of a few TFs, and thereby they induce downstream genes related to ABA metabolism. Au, like GA involvement, in this aspect may concern other TFs like *AP2* and *DELTA* and other proteins. These often cause GA oxidation pathways involving genes like *GA20ox*, *GA3ox*, and *PIL5* TF [23]. After the ripening of embryos in seeds, a number of genes are involved, like *RGA*, *RGL2*, and *GAI*, from different insensitive mutants to GA. For the up- or downregulation of ABA-inducing genes, growth substances are involved including jasmonic acid (JA), brassinosteroid (BR), ethylene (ET), salicylic acid (SA), etc. [24]. ABA, through its auxin inductive cascade, can induce a number of genes via some auxin response factors (ARF10/16). These factors are involved to allow the binding of other TFs. Likewise, ABA can induce MYB96 factor, which otherwise induces TF ABI4. The latter is responsible for the suppression of CYP707A1/2 factor activity, which is likely to bind the α -amylase promoter and promotes seed germination [25]. ABA can induce other TFs (*ABI5*, *BIN2*, *PKS5*, etc.) which also inhibit seed germination. All of these are related to the precise concentration of ROS, which collectively are key balancing factors for gene regulation. ABA, under anaerobic conditions, is more active with regard to its regulation of catabolism as well. On the seeds and vegetative parts of plants, a number of ABA receptor components are grouped and these regulate protein phosphatase 2C (PP2C)

when ABA is present. PP2C is inactive under aerobic conditions but is activated with sucrose non-fermenting 1 (SNF1)-related kinase 2. The latter allows for specific response element binding TFs [26]. Increased dormancy is also related to ABA, where a particular factor, DELAY OF GERMINATION 1 (DOG1), becomes the master regulator of the primary dormancy activator. This is in turn regulated by a specific PP2C, activated by ABA [27]. DOG1 modifies ABA signaling where PP2C acts on seeds, particularly regarding those depending on ABA hypersensitive germination. Even after grain development, *OsDOG1*-like gene expression is also involved for immature seed development. This is co-dominantly expressed with an over expression of *OsNCED2* and *OsABA8'OH3*. Seed germination under water is independent of primary dormancy, where susceptible varieties can germinate but fail through downstream development. Not only embryos under submergence stress but also heat-stressed embryos are dependent on DNA methylation. The DNA methylation of ABA-catabolizing genes and the α -amylase promoter are also subjected to anaerobic stress [28]. Another two genes, *ABA deficient 4* (*ABA4*) and *Neoxanthin deficient 1* (*NXD1*), are required for ABA biosynthesis and its regulation [29]. Therefore, more studies on ABA-GA cross talk in the germination phenomenon with molecular insights into submergence or anoxic germination are required (Figure 1).

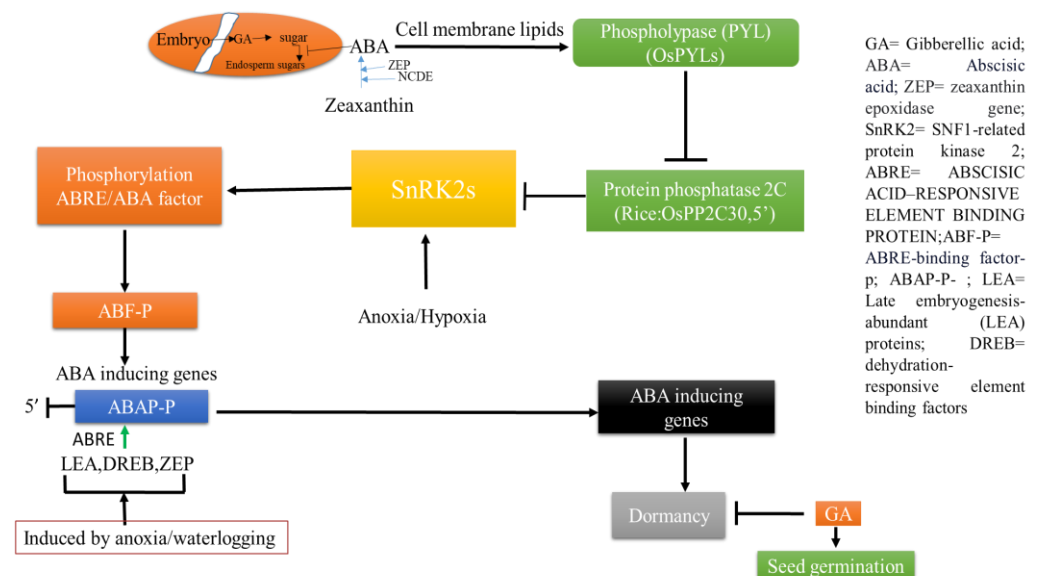


Figure 1. Hormonal interplay between seed dormancy and germination under inundation stress.

4. Development of Rice Seedlings Facing Anoxic Conditions

Soil flooding may be the most vulnerable abiotic constraint in rice cultivation after seed germination. Seedling growth becomes important with the duration and depth of water stagnation. Seeds of taller plants almost fail to germinate under anaerobic conditions whereas rice germinates in oxygen-depleted soil under partial or full submergence [30]. Rice seeds starchy in grain material could tolerate anaerobiosis, where fermentative catabolism is a prime event. Thereby, seeds can maintain a high rate of carbohydrate turnover under oxygen deficiency. A decline in oxygen partial pressure causes negative effects in other cereals like oat and barley, where root emergence is normal [31]. In contrast, even though root growth is suppressed, shoot growth increases where there is oxygen demand under submergence in the case of rice. Even depleted oxygen can increase the final length of coleoptiles that exceeds that of aerobically grown seeds. Under this condition, coleoptiles may be sustained, but the root and primarily the leaves do not grow successfully. Coleoptiles thrive under a prolonged anaerobic period via the suppression of tissue growth, which matches the mechanism of snorkel effects [32]. The diffusion of oxygen into the roots downstream supports the establishment of more seedlings under direct seeding [33]. There are many reasons that exist behind the phenomenon of the snorkel effect in support of seedlings for a better transfer of oxygen dissolved in water [34]. For germinated seedlings,

a negative correlation is still recorded between the elongation of water and survival. This is a metabolic exhaustion of reserve carbohydrates competing with maintenance respiration of tissues versus the elongation of coleoptiles [35]. However, it has also been supported that Au is rapidly over expressed under aerobic conditions, which otherwise induces ET biosynthesis. The latter induces the senescence of coleoptile tissues and thereby the shoot emerges out as a coleoptile. However, the Au and anaerobic growth of the coleoptile in connection with ET remains inconclusive in regard to a lack of consistency for the Au and anaerobic growth of coleoptiles.

5. Fermentative Mechanism: Pathways for Anaerobic Seeds' Germination in Rice

Under limited oxygen concentration, mitochondrial respiration is inversely proportional to glycosylate metabolism, which produces NADH. Following entry into mitochondria, NADH undergoes re-oxidation at the start of fermentative pathways. The lactate and toxic substrate ethanol produced with a minimum gain of ATP is a bottleneck for germination under water [36]. Lactate, a substrate of lactate dehydrogenase (LDH), is typically toxic to the aleurone membrane in inducing amylase activities by GA, whereas PCD produces ethanol along with the activity of ADH. Seeds may be tolerant to anoxia by reverting the central glycolytic pathways. Ethanol may either be oxidized to acetyl dehydrogenase or diffused out of the seed coat, accumulating within spaces of grains and glumes [37]. This also induces dormancy in terms of ecological consideration for submergence tolerance. The minimum amount of ATP (2 moles) over the normal tricarboxylic acid cycle (32 moles) is a limitation for anaerobic respiration [38]. Therefore, a specific set of protein expression and their corresponding regulation are selection criteria for the better germination of seeds in the anaerobic mode. Distinct proteins like sucrose-phosphate synthase, PDC, and ADH are the most important in accessing breeding programs where submergence induces anoxia [39]. Carbohydrate metabolism in rice seeds under prolonged anoxic conditions creates the most effective screening index. There are specific modalities of the regulation of genes on the anaerobic response elements upstream of the promoters, characterizing the coding of anaerobic proteins [40]. These, in turn, become the factors to control the paths of fermentation for specific genes. From the sequence alignment, *cis*-elements also bear the homology for anoxia-inducible genes in plants, similar to bacteria. Rice plants tolerant to anoxia can accommodate ATPs; however, for a long time, this has occurred via fermentative catabolism, as long as the hypoxia is maintained without affecting the embryo tissues. This can tolerate alcohol from fermentation unless feedback inhibition on pyruvate decarboxylase is accomplished [41]. This is explained more with rice seeds, where the speedy consumption of sugars may accelerate the principal glycolytic flux and fold under anoxia. Interestingly, anoxia-tolerant coleoptiles from few rice genotypes can produce less ATP under the same conditions. Unlike other plants, deficits in energy can be recovered with a high value when the same tissue is transferred to aerobic conditions [42]. This may imply the potential of coleoptiles of rice under anoxia, which succeeds with a high value of energy charge [43]. In contrast, ethanol production is the most crucial for hypoxia tolerance, as proved by the alcohol dehydrogenase 1 (*adh1*) mutant. In other plant species, no consistent correlation for ADH activity and tolerance has been observed. It has also been observed for others that catalytic enzymes are those that might be downregulated and thereby strongly stand for biochemical markers for anoxia with polymorphic variations [44]. In anoxic seed germination, two rice genes, *ADH1* and *ADH2*, are characterized by a point mutation, where a particular amino acid becomes the factor. For reduced ADH activity, mutant rice coleoptile becomes slow in growth and elongation under water logging. This is well established in plants transformed with *ADH1*—antisense construct [45]. Therefore, *ADH1* activity becomes the most important for coleoptile elongation under submergence, which compensates for lower ATP production. Under submergence, rice roots may overcome the activity of ADH due to an oxygen deficiency. Therefore, it appears to be another marker for root survival. Not only ADH but also PDC recording several fold increases in activities may suggest that the limiting factor for ethanolic fermentation is pyruvate

decarboxylase [46]. Moreover, anoxia firstly increases amino acids via the over expression of the alanine-amino-transferase catalyzing trans-amination of pyruvate to alanine. In germinated seedlings, the maximum amount of pyruvate is converted to ethanol and lactate is converted to alanine. This means that ethanolic fermentation may demand pyruvate consumption with a reduction in NADP^+ , with its recycling through oxidation. These collectively minimize the cytoplasmic acetolysis of coleoptiles, a recurrent event under submergence vulnerability. Therefore, under submergence in real conditions is prolonged pre-adopted anoxia that can exhaust the carbohydrate pool and also negatively impact rice seedlings. Still, tolerant genotypes maintain a high fermentation rate of coleoptiles along with a constant flow of soluble carbohydrate consumption, with a low ATP gain.

6. Molecular Regulation of Major Glycolytic Flux: Amylase Activity with GA Induction

Major glycolytic flux is based on the availability of soluble sugars, which determines the rate of germination and seed growth in rice under submergence. The regulation of α -amylase expression by GA is a major control for soluble sugars as a chief fuel for glycolysis [47]. Hypoxia-induced coleoptile growth is thus matched with GA biosynthesis, catabolism, and the regulation of amylase activity [48]. The expression of the specific α amylase gene is also under activation when embryos deplete sugars from endosperm. This was well established from the expression of α amylase in rice embryos, as derived from suspension cells in aleurone, which becomes a major source of α amylases. Under two sugar-depleted culture sets of genes, α -Amy3 and α -Amy8 [49] are major amylases. This is responsible for starch breakdown below the threshold level for sugar content, which supports the germination of embryos. The sugar repression of AM3 and AM8 is based on the regulation of the transcription rate, as well as the stability of transcripts. Typical TA box (5'-TATCCA-3') is the main sugar response element in rice embryos [50]. Referring to the GA level for the activation of α amylase, three distinct promoter sequences containing GA response elements (GREs) flanked by TA/AMY box are responsible. In another case, GA-induced MYB factor (R2R3 MYB TF on GRE) activates the α -amylase promoter and hydrolyzes rice aleurone cells. Regarding anoxic embryos, it can express several hundred genes activated by GA3. This predominantly encodes transporter proteins, hydrolases, TFs, etc. These proteins are also upregulated in terms of the activity of GA-induced MYB elements [51]. In rice embryos under sugar starvation, an MYBS1 (sugar-responsible R1MYB TF) can activate the α -amylase promoter, which binds with the TA box. Therefore, both GA-induced MYB and sugar-repressible MYB (MYBS1) are two major factors where GA induction and sugar-induced signaling are concerns under submergence. GA signaling can interfere with the sugar repression of the α -amylase promoter in germinated rice embryos by the joint activity of MYB GA-GARE [52]. On the other hand, the MYBS1-TA interaction can regulate the α -amylase promoter through the use of both GA and sugar starvation. Sugar represses the entry of MYBS1 into the nucleus but GA and MYBGA can antagonize sugar repression and accelerate MYBS1 in the same way. MYBS1 and MYBGA are bound in the cytoplasm and co-transported into the nucleus. Therefore, the mechanism is evidently more complex, where TFs are recovered from sugar-starved nuclei depending on the GA concentration in the embryo.

7. Constraints and Remediation of Pre-Harvest Sprouting

Under different environmental stresses, the production of cereal seeds is physiologically affected by grain germination on plants, called pre-harvest sprouting. It results in a significant loss of yield, reduced grain quality, and other physiological traits in a wide coverage of cereals including maize, barley, wheat, rye, oats, jowar, etc. [53]. Pre-harvest sprouting is favored with suitable temperature and moisture for seed germination. This is also associated with the energy exhausted by grains and the breakdown of high-energy-producing starch and lipids. The energy released by metabolites may allow shoots to expand under an environmental condition that supports sprouting [54]. Moreover, ge-

netic and environmental factors independently or by interactive means may influence pre-harvest sprouting. Specific varieties have been investigated for sprouting resistance that minimize the loss of yield and grain quality. A selection procedure for the characteristics that aid sprouting resistance from a rice data base has already been identified. A significant amount of information is aided with transcriptomic and proteomic technologies [55]. At the physiological level, resistance to pre-harvest sprouting is associated with developmental and morphological features of grain, seed dormancy, coat permeability, hormonal balance through ET, ABA, and GA, and hydrolase activities in support of the process [56]. At the morphological level of the seed coat, the presence of specific inhibitors, glumes of grains, pubescence or glabrous seed coat, and other spike morphologies are required for the resistance of pre-harvest sprouting. Moreover, seed dormancy happens to be a significant factor in the control of resistance to pre-harvest sprouting [57]. More in-depth studies are required on the molecular background of seed dormancy allied with pre-harvest sprouting in rice using breeding strategies.

8. Metabolomic Approaches for Hypoxia Tolerance in Rice

In the recent past, a detailed comparative multi-omics analysis suggested the involvement of different pathways to tolerate hypoxia for embryos and coleoptiles. Rice seeds, with their in-built tolerance to grow under oxygen deficiency, lead to a conversion to pyruvate following alcohol. From metabolomic studies, a complete set of enzymes for starch mobilization under hypoxic stress remains active [58]. Agronomically, this tolerance demonstrates rice as being a crop for direct seedlings in cultivation. The cell-wall-specific high expression of wall-modifying enzymes like trans-glycosylase as well as proteins for coleoptile growth are important [59]. The turnover of glycolytic pathways induces a higher survival of hypersensitive rice seeds to hypoxia than other intolerant ones. A number of biometabolites are important for carbohydrate metabolism supporting the sucrose immobilization of the embryo. Trehalose-6-phosphate phosphatase for the synthesis of trehalose is the most important in the sucrose conversion to starch, which is over expressed under hypoxia [60]. Low land cultivar-specific promoters like *OsCVL10* can maintain the low-level expression of other genes but improve α -amylase expression. Therefore, *OsCVL10* is a negative regulator against hypoxia tolerance, which has been proven via molecular regulation. Recent developments in metabolomic data suggest the significance of a few key genes in support of elevated nitrogen efficiency, the response of grains to 2,2',4,4'—Tetrabromodiphenyl ether ($C_{12}H_6Br_4O$), and heat tolerance in reproductive phases facing drought stress. Hypoxic germination reveals the scope of multi-omics study where carbohydrate metabolism, ROS turnover, alcoholic fermentation, the interaction of ABA and ET, and the division of coleoptile cells remain important [61]. In most cases, studies are more related to the transcriptomic level, whereas a multi-omics approach has been used less in relation to hypoxic germination. This has been more accomplished with purine, shikimic acid metabolism, nucleotide transformation, cell membrane transporter (ABC transporter), and the biosynthesis of important amino acids (alanine, aspartic acid, glutamate, etc.). The metabolome analysis of different types of seeds that germinate under aerobic conditions and differential metabolite regulation has been observed. In comparative transcriptome profiling through hypoxic seed materials, two distinct domains were identified: amino acid and carbohydrate metabolism. From the expression profile of the rice coleoptile, two rice groups, QSZ (sensitive) and WR (tolerant to hypoxia), were identified. It reveals a number of genes, indicating the differential accumulation of metabolites for germination sensitivity. Experiments were conducted, making comparisons of WR–aerobic against WR–hypoxic and QSZ–aerobic against QSZ–hypoxic. From a huge number of gene pools, most of the genes responsible for carbohydrate and amino acid metabolism were studied. Therefore, coleoptile responses would be targets for the downstream development of seedlings with regard to sugar signaling (Figure 2).

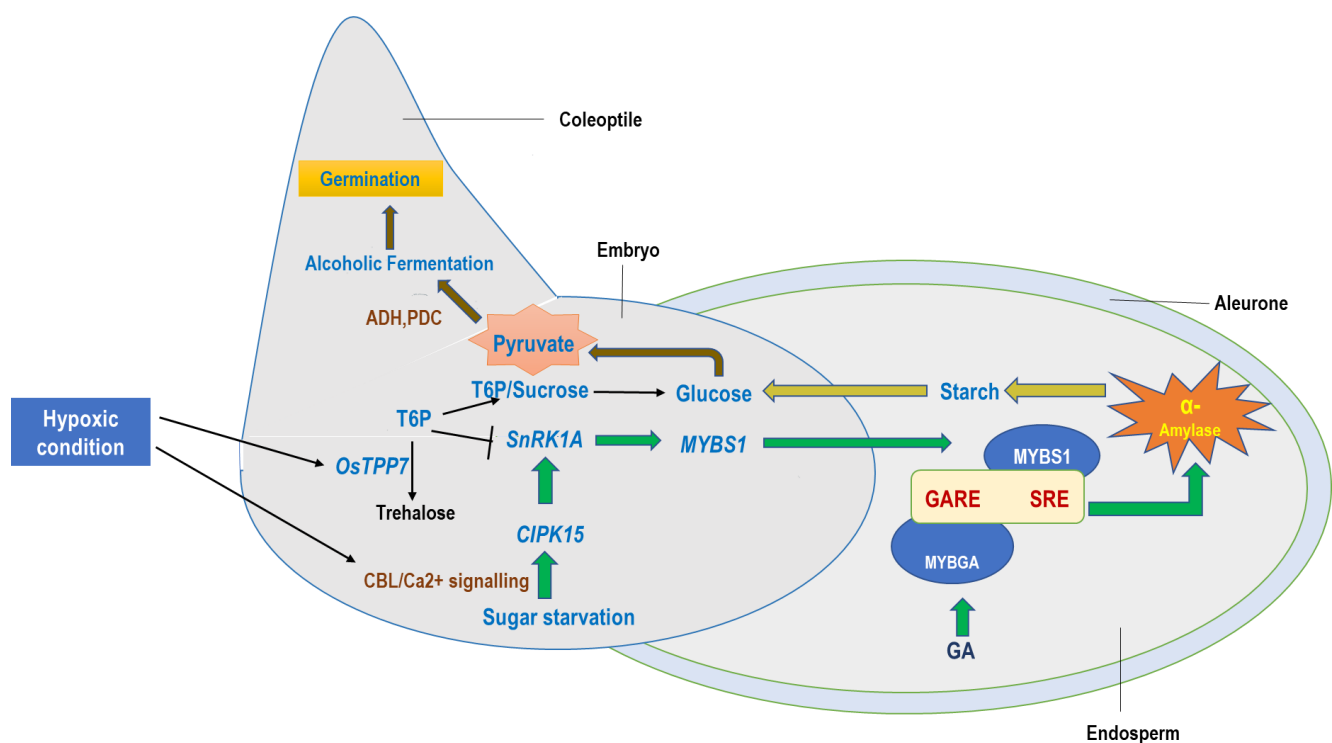


Figure 2. Sugar metabolism and its possible contribution in cell signaling for responses of seeds under hypoxia.

Collectively, it is concluded that hypoxia-tolerant genes mostly downregulate the hypoxic cascade and this induces a shift in amino acids to energy-yielding metabolites. Other facets of gene expression include the synthesis of carbon residue for complex biomolecules in anaplerotic pathways. Altogether, this leads to the self-elongation and division of coleoptiles following germination. This is contrasted by hypoxia seeds, where oxidative-phosphorylation-related genes can induce hexokinase and the CIPK15-SnRK1A-MYBS1 phosphorylation cascade. These promote the expression of the *Amy3D* gene [62,63], as well as genes encoding α -amylase (including *OsAmy3B*, *3C*, *3D*, and *3E*) for sugar catabolism. These are mostly concerned with maintenance respiration in viable coleoptile cells rather than exercising growth respiration [64].

9. Regulation of Transcripts in Seed Germination under Submergence

As already reported, rice seeds favor some sort of anoxia which is moderated by the adoption of a quiescence strategy. A number of bio-metabolites and hormonal influences are pre-requisites for quiescence strategy under the regulation of some genes from the whole transcriptome [65]. These genes include the enzyme system for the fermentative mechanism, where key enzymes for glycolysis are pyruvate decarboxylase (PDC), alcohol dehydrogenase (ADH), etc. [66]. At the metabolome level, alcoholic fermentation can provide adequate energy for seed germination, coleoptile growth, and ATP synthesis to facilitate the quiescence mechanism. In alcoholic fermentation, pyruvate is catalyzed into acetaldehyde by PDC. This is followed by ADH and other constitutive genes responsible for alcoholic fermentation [67]. ADH becomes a key gene for submergence tolerance, which is regulated in a feedback manner with *slender rice 1* (*SLR1*) and *slender rice like 1* (*SLRL1*). However, for quiescence strategies, ADH is inhibited or less active for the *adh1* mutant associated with a decrease in the $\text{NADP}^+/\text{NADPH}$ ratio [68]. In this mutant, coleoptile growth and its elongation are hindered in the molecular regulation of anaerobic genes. A class of TFs is important, where basic leucine zippers (bZIPs) happen to be major DNA-binding proteins. For coleoptile growth and elongation under submergence, bZIPs are bound with the DNA-binding motif and leucine zipper region which forms dimers. The whole

complex acts as a transcription activator for seeds grown under inundation. In addition, the DNA-binding domain and leucine zipper play other roles in submergence tolerance related to TFs within families of NAC, MYB, and WRKY. This was reported with regard to well-known salt tolerance and land races like *Porteresia coarctata* (L.) [69]. bZIPs in fact act as positive regulators for ABA-mediated signaling for the inhibition of seed germination under anaerobic conditions. This is exemplified in *bZIP23* mutants where any significant seed germination becomes speedy under ABA treatment. Therefore, ABA must have some roles in seed viability and germination, at least when submergence is overcome by plants. For the germination phenomenon, bZIP over expression controls ABA sensitivity, which must be considered for anaerobic conditions. Other TFs (TRAB1, WRKY, and OsbZIP66) have homology with bZIPs and would be exercised for ABA signaling, sensitivity, and the modulation of seed germination [70]. Still, the roles of bZIPs in seed germination and coleoptile elongation under submergence stress remain unelusive. Notwithstanding this obscurity, in almost all cases, the bZIP promoter remains available for anaerobic respiration and fermentation reactions. TFs activating *ADH1* and *PDC* which are recognized as key genes for direct seeding under submergence are used for developing expressed sequence tags (ESTs) in the mapping of anaerobic genes.

10. Biochemical Implications of Anoxic Seed Germination

Rice seeds, due to their anaerobic germination, show two distinct modalities for biotechnology implications, particularly in anaerobic conditions. Primarily, they have minimum or basal oxygen requirements for seed germination for dormancy-related pathways. Secondly, the use of specific devices to maintain minimum oxygen tension and its diffusion for developing embryos is important [71]. A number of chemical elicitors to influence the metabolic expression from developing embryos have been well addressed. Seed-coat-residing phenolics and their oxidation into other residues are related with the removal of dormancy under anoxic conditions. For aerobic rice, where oxygen concentration is not a constraint, seed coat phenolic residues are also affected. In some cases, established physiological and biochemical pathways are clear, but the signaling mechanism for the soil moisture deficit, ROS, and growth regulators is not explained. Therefore, the biotechnological implication of anaerobic seed germination requires a consolidated background with plant physiology, biochemistry, ecology, and cellular and molecular biology of seeds in the plasticity of environmental constraints. With other omics furnished with microsensor- and software-based advancements, the prediction of seed and environmental interaction might be a possible approach. This has also been established earlier in those dormant endogenous substances like nitrogen di-oxide (NO₂), and ROS production and different signaling components are important. Additionally, biotechnological implications and the modification of different substances like 1- methyl cyclopropane (1-MCP) may influence ET biosynthesis as well as signaling under low oxygen tension for anaerobic germination. ET inducing dormancy was also found to be responsive in hypoxia tolerance via the stability of specific TFs and the ethylene binding proteins (ERBPs), which also control mitochondria retrograde signaling [72]. In rice seeds, hypoxia reduces ATP synthesis when the ethylene response factor (ERVII) that activates the nucleus and other TFs shows a few hypoxia-responding genes [73]. Therefore, complicated cascades are involved in the mechanism of ROS-mediated oxidative responses, and thereby a better understanding is required for proteomic approaches. More insights into the role of ROS in dormancy, reversible ROS dynamics, their generation, subcellular distribution, sensing, and auto reduction are crucially important. These finally become cumulative approaches in a better understanding of dormancy under anoxic conditions (Figure 3).

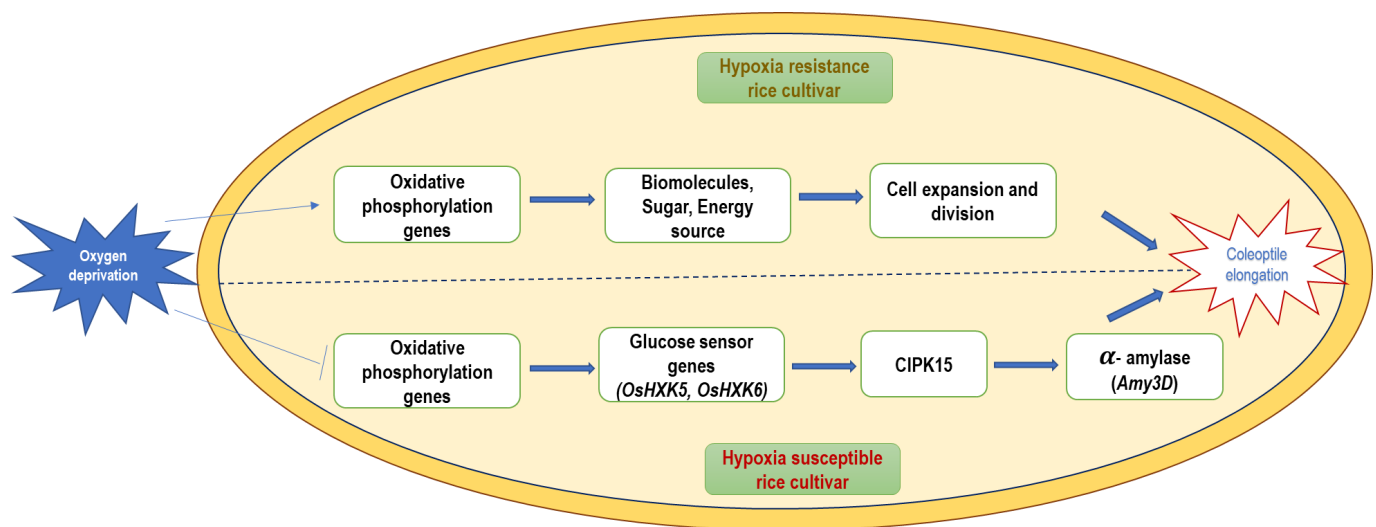


Figure 3. Possible paths for coleoptile responses in seeds of rice under hypoxic-stress-inducing redox perturbation.

11. Conclusions

Rapid seed germination and coleoptile elongation are primary traits of anoxia and hypoxia tolerance. Seeds can overcome anerobic stress when oxidative phosphorylation can provide adequate ATPs. These are required for energy conservation when seeds are to sustain their viability by arresting its growth. This is physiologically manifested into quiescence strategies, where growth is regulated under submergence. Sugar and amino acid metabolism were features of hypoxia-tolerant cultivars that support tissue development in coleoptiles. Multi-omics research suggests that genes functioning in a differential manner for potential transcript(s) may provide tolerance. These genes are genetic resources for the mobilization of nutrients under oxygen deficits in seeds. From existing land races of rice tolerant to submergence, significant functional gene(s) are recovered using the reference genome of FR13A, etc. Therefore, hypoxic germination shares the same background of gene pools concerning oxidative redox, and these must be employed in breeding programs for direct seeding in rice.

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