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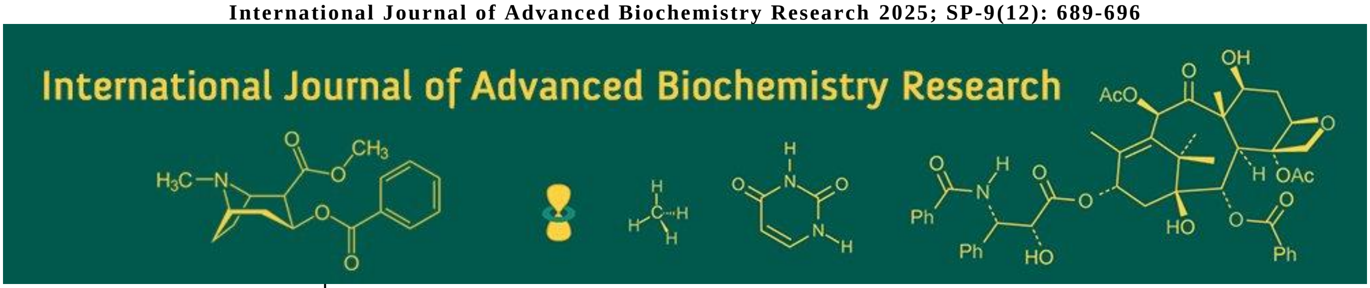

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Effect of Hypoxia during seed germination

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Abstract

Oxygen accessibility is a pre-requisite for the lives of many living beings. In plants, when the oxygen supply is insufficient, most cellular capacities are compromised, which can lead to death. Hypoxia is a condition where oxygen level limits aerobic respiration (usually between 1% and 5%). Oxygen is a major factor of seed germination since it allows resumption of respiration and subsequent metabolism reactivation during seed imbibition, thus leading to the production of reducing power and ATP. It is also demonstrated that the covering structures of seed mainly inhibit germination by limiting oxygen supply to the embryo during imbibition. The pivotal function of oxygen in the molecular networks that govern the processes of seed germination and dormancy via the hormonal balance (ethylene, ABA, and GA), the hormone-signaling pathway, specifically the ABA sensitivity, the growing significance of mitochondria in the generation of reactive oxygen species during hypoxia, and the participation of the N-degron pathway in the turnover of proteins linked to seed tolerance to hypoxia. Due to the slow diffusion of O_2 in water, submerged plant tissues become O_2 deficient. Ethylene response factors (ERFs) are transcription factors that allow rice plants to survive submergence paved the way for the discovery of the ability to sense oxygen in plants (Loreti and Perata, 2020). Therefore, molecular and metabolic reactions are rapid and profound reprogrammed to withstand the stress of O_2 deficiency. The upregulation of the key enzyme Amy 3 is effective in mobilizing starch to produce energy and shifts ATP production from aerobic to anaerobic respiration through signaling cascades and metabolic regulation under low O_2 stress. O_2 deficiency induces the termination of the Krebs cycle and oxidative phosphorylation, diverts ATP production from mitochondrial electron transport chain (mETC) to ethanol fermentation (Taiz *et al*., 2015) [22]. The ability to use "omic" technologies, genetics and micro-measurements using microsensors has made significant progress in understanding the mechanisms behind the development of hypoxia tolerance possible over the years.

Keywords: Hypoxia, oxygen deficiency, seed germination, anaerobic respiration, ethylene response factors, starch mobilization, omics technologies

Introduction

1. Hypoxia and its Effects

Oxygen is supplied to seeds inside the soil through the small air spaces present in the soil. These air spaces are created by the loose structure of the soil, which allows oxygen from the atmosphere to permeate. When a seed is planted, it begins to respire, using the oxygen present in these air pockets for aerobic respiration. This process is crucial for the seed's metabolism and energy production. However, if a seed is buried too deeply or if the soil is waterlogged, the seed may not get enough oxygen, which can inhibit germination. In such cases, seeds can switch to anaerobic respiration, a process that doesn't require oxygen (Corbineau, 2022) [7] such conditions are referred as hypoxic conditions.

Hypoxia, defined as oxygen levels significantly below the atmospheric norm of 20.9% by volume, is a common physiological condition during seed development. Its severity fluctuates with developmental stages and is closely linked to the seed's metabolic activity. The primary consumer of oxygen in seeds is mitochondrial respiration, which drives energy production but also contributes to internal oxygen depletion. Its effects on Metabolic Processes by impairing key functions such as nutrient assimilation, core metabolic pathways, and the accumulation of storage compounds. Disrupting redox Balance, cytosolic $NAD^+/NADH$ ratio, a critical redox indicator, aligns with developmental speed. NAD^+ serves as an essential electron acceptor in numerous reactions, and its regeneration is hindered under hypoxia (Rolletschek *et al*., 2024) [19]. This imbalance may slow development by reducing NAD^+ availability. Suppression of mitochondrial activity leads to delayed cell maturation. A

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slower developmental pace, marked by reduced mitotic activity, may allow more time for DNA replication and repair, potentially minimizing mutation risks.

Process	Major findings	Species: Reference
Assimilate uptake	Low O_2 limits nutrient uptake by seed/embryo; this could be due to constraints in energy-demanding transport mechanisms and/or sink activity.	Soybean: Thorne, 1982 Wheat: van Dongen *et al.*, 2004 [24, 25]
Storage activity	Low O_2 limits biosynthetic fluxes toward storage goods, specifically protein and oil, as opposed to starch	Wheat: Gifford & Bremner, 1981 Rapeseed: Vigeolas *et al*., 2003 Soybean: Rolletschek *et al*., 2005 [10, 26, 20]
Mitochondrial respiration	When oxygen levels are insufficient, the generation of energy is restricted; when the availability of oxygen is increased, the rate of respiration intensifies	Rapeseed: Vigeolas *et al*., 2003 Wheat: van Dongen *et al*., 2004 [26, 25]
Fermentation activity	The activity of fermentative enzymes diminishes as the supply of O_2 increases; changes in internal O_2 during development are inversely correlated with fermentation activity	Pea: Rolletschek *et al*., 2003 Rapeseed: Vigeolas *et al*., 2003 [21, 26]
Assimilate partitioning Shift in energy–consuming processes	The division between the endosperm and the embryo, specifically the transition from invertase to sucrose	Arabidopsis: Gibon *et al*., 2002; Baud & Graham, 2006

Hypoxia induces the transcription of fermentation-related enzymes like alcohol dehydrogenase and lactate dehydrogenase. Acetate Fermentation in Oilseeds: 1. Facilitates NAD^+ regeneration, supporting glycolysis.2. Redirects pyruvate away from the TCA cycle, which is compromised under low oxygen. 3. Provides acetate as a precursor for fatty acid biosynthesis. also act as an epigenetic regulator, promoting lipid synthesis and cell survival under hypoxic conditions. In most cereals struggle to mobilize endosperm reserves under hypoxia due to the oxygen dependence of key enzymatic processes. Rice is an exception, expressing α-amylases that function independently of gibberellin (GA) and are activated by oxygen scarcity. Barley seeds are particularly sensitive to oxygen deficiency during germination, lacking the hypoxia-responsive α-amylase found in rice. This prevents starch breakdown and delays germination until GA-dependent enzymes become active. Hypoxia, whether due to flooding or structural impediments like thick seed coats, glumellae, or pericarps, can induce secondary dormancy. These physical barriers restrict oxygen access to the embryo. Additionally, enzymes like polyphenol oxidase on the seed surface may consume available oxygen, further exacerbating hypoxia (Pucciariello & Perata, 2024; Taranto *et al*., 2017) [8, 23, 18].

2. Hypoxia types and classification of seeds based on tolerance to hypoxia

Oxygen deprivation results in anoxia, whereas hypoxia happens when aerobic respiration is limited by oxygen levels, which are typically between 1% and 5%. Acute and chronic hypoxia are the two different forms of hypoxic situations (Loreti and Perata, 2020) [14]. The phrase "acute hypoxia" refers to a brief decrease in plant oxygen availability that may be brought on by unfavourable environmental circumstances, like flooding, or by atypical, momentary spikes in seed oxygen consumption. Plants experience acute hypoxia as stressful. Conversely, persistent hypoxia is a constitutive and typically non-stressful state in which a specific cell population rather than the entire plant maintains a low oxygen level. In other words, chronic hypoxia can be a physiological condition in specific plant tissues.

The sensitivity of seeds to oxygen is affected by the level of dormancy as well as other environmental parameters such as temperature, light, and water potential of the medium. When most species are deprived of oxygen, their seeds do not germinate, and seedling growth is hampered. Al-Ani *et al*. (1982, 1985) divided seeds into two groups based on their reaction to low oxygen concentration. Group I seed are fatty seeds (cabbage, flax, lettuce, radish, soybean, sunflower, turnip), whose germination is entirely stopped when the oxygen content is close to 2% (Fig. 1). Group II seeds are mostly starchy seeds, such as cereals (barley, maize, oat, rice, sorghum, wheat) and peas, and they did not germinate in an atmosphere with less than 1% oxygen (Fig.1). It also indicates that 50% of the seed population germinate in 1-3 and 7-8% oxygen for groups II and I, respectively.

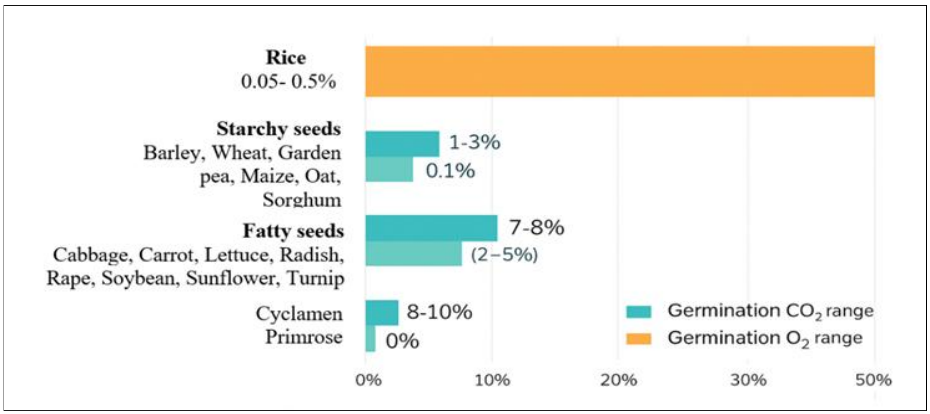

Fig 1: Oxygen concentration of the atmosphere below which no seed germinated

3. Effect of seed coat and mucilage on O_2 requirement for germination

Oxygen is essential for respiration but its requirement for seed germination depends on the species, depth of dormancy and temperature. Under hypoxic conditions, the seeds' ability to maintain EC is made possible by fermentation's generation of ATP (Come and Corbineau, 1989) [5]. On the other hand, complete oxygen deprivation can cause certain seeds to come out of dormancy, while partial or total oxygen deprivation may help a variety of aquatic species or latent seeds germinate. Thus, the transfer of oxygen from the seed coat to the embryo is the primary factor in seed germination.

In certain species, the embryo is covered by certain structures like nucellus, testa and endosperm. In the case of caryopses, the pericarp is joined to the seed testa; in the case of achenes, it is not fused to the testa. They are linked to a number of other structures, such as dead perianth tissues, as in the case of beets, and hulls made of glumes or glumellae, depending on the Poaceae species. By limiting the embryo's access to oxygen and water during germination, they prevent seeds from sprouting (Bewley and Black, 1994) [3]. Depending on their thickness and biochemical makeup, surrounding structures have an effect and may reduce the amount of oxygen that reaches the embryo. During seed imbibition, oxygen passes through the seed coat by dissolving in the water they contain; however, because of the poor oxygen solubility, not much oxygen reaches the embryo. Since ingrained coatings form an uninterrupted moist layer around the embryo, they subsequently act as a barrier to the diffusion of oxygen.

In cereals, the covering structures result in the inability of the grains to germinate at temperature higher than 10-15 °C. In barley, the glumellae have the main role in dormancy (Corbineau and Come, 1998) [6], while in oat both glumellae with pericarp, and probably testa participate to dormancy. The surrounding structures inhibit germination by reducing oxygen supply to the embryo due to enzymatic and/or non-enzymatic oxidation of the phenolic compounds.

Table 1: Effects of oxygen tensions on the germination of seeds (Come and Corbineau, 1998) [6]

Oxygen levels (%)	Germination (%) at 20 °C of isolated embryos of seeds			Germination (%) at 20 °C of seeds		
	Melon	Oat	Sunflower	Melon	Oat	Sunflower
0.5	0	0	68.2	0	0	0
1	0	33.3	76.2	0	25.7	0
2	69.1	84.1	83.3	23.1	55.8	0
3	91.3	96.8	100	55.3	75.7	0
5	95.2	100	100	80.7	95	12.8
10	100	100	100	95.7	100	90.2
21	100	100	100	100	100	100

Because the fixation of oxygen in the surrounding structures increases with temperature, the inhibitory effect of the covering structures increases. According to Table 1, 2-3% oxygen in the environment is adequate for the germination of separated embryos. Isolated embryos from non-dormant seeds require less oxygen to germinate than complete seeds, showing that seed coverings restrict germination even in non-dormant seeds by lowering oxygen delivery to the embryos (Come and Corbineau, 1998) [6].

A key characteristic of the seed coat is the production of pectin-rich mucilage within the epidermal cells, a process known as myxospermy. When a dry seed encounters water, it triggers a swift release of a clear, gelatinous mucilage that envelops the seed. Most research on seed coats and mucilage production has focused on the Brassicaceae family, with *Arabidopsis thaliana* being the most extensively studied species in terms of seed coat development and mucilage generation. Observations during germination showed that mucilage aids in germination under both well-watered and water-logged conditions, but not during water stress. This suggests that mucilage may serve an ecological function in *L. perfoliatum* by helping it adapt to environments with abundant or excessive water.

It has been observed that the production of mucilage in seeds is an evolutionary adaptation of plants to dry conditions. Due to its high capacity and efficiency for water absorption, seed mucilage has long been believed to support seed germination and seedling establishment. Research demonstrated a significant difference in germination patterns between intact seeds and those from which mucilage had been removed, indicating that mucilage is vital for successful seed germination of L. *perfoliatum* in both well-watered and water-logged settings (Haung *et al.*, 2015). Additionally, results showed that mucilage is essential for quick seed germination and strong seedling growth.

4. Effect of hypoxia on seed dormancy

Dormancy in seeds can be influenced by the embryo, the seed coat, or a combination of both, depending on the species. Secondary dormancy is primarily triggered by elevated expression of ABA biosynthesis genes within the embryo. Additionally, genes involved in GA metabolism are significantly regulated and respond earlier to hypoxic conditions, suggesting that dormancy induced by high temperature or hypoxia at 15 °C follows distinct signalling mechanisms, as demonstrated by hoang *et al.* (2013) [11] in their study on primary dormant barley seeds. Prior research indicates that low-temperature hypoxia can effectively induce secondary dormancy in barley, sunflower (Dominguez *et al.*, 2019) [8]. Under hypoxic conditions, the decline in embryo ABA levels occurs more gradually. This dormancy induction is associated with reduced GA synthesis and inactivation, likely resulting in lower levels of active GA. The mechanisms by which hypoxia and high temperature induce secondary dormancy differ: hypoxia appears to rely more heavily on GA regulation and less on ABA, whereas high temperature shows the opposite trend. Notably, three days of hypoxia (5% oxygen at 15 °C) produced a dormancy response similar to that observed after three days at 30 °C, where the embryo was also subjected to hypoxia within the grain envelopes. In both cases, only 30% of the grains successfully germinated within seven days at 15 °C in air.

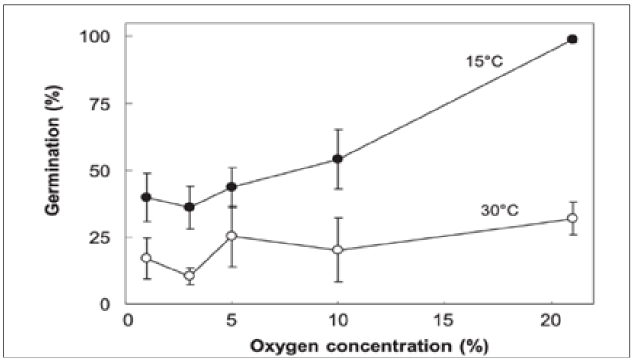

Fig 2: Effect of Oxygen tension on germination of primary dormant barley seeds (Hoang *et al*., 2013) [11]

5. Effect of hypoxia on metabolites during seed germination

Hypoxia also leads to proteolysis in seeds which result in protein turn over through N-End pathway. In response to hypoxia transcription factors like ERF VII were released which are acted upon by enzymes methionine amino peptidases and converted into cysteine -ERF VII, in absence of O_2 C- ERF VII gets stabilized showing responses like seed dormancy, open stomata, elongated hypocotyl etc. where in the presence of O_2 cysteine gets oxidized in the presence of plant cysteine oxidases and get arginylated in the presence of arginyl T- RNA transferases which gets acted upon by Ubiquitin molecules leading to proteolysis and hence germination of seeds (Gibbs *et al*., 2015) [9].

In order to keep it in a nut shell the network of events occur in seed under hypoxia, mitochondria act as a sensor organelle which reduces ATP production but also releases ROS and NO with the stabilization of ERF VII factor which migrates to nucleus to activate hypoxia responsive genes which provide tolerance to hypoxic conditions prevailed in seeds.

When comes to right panel it indicates the effect of hypoxia on hormonal balance where, C_2H_4 improves germination of dormant seeds with decrease in ABA level where the production of ROS and NO by mitochondria also decrease ABA level by regulating the production of ABA hydroxylase expression leading to seed germination (Corbineau, 2022) [7].

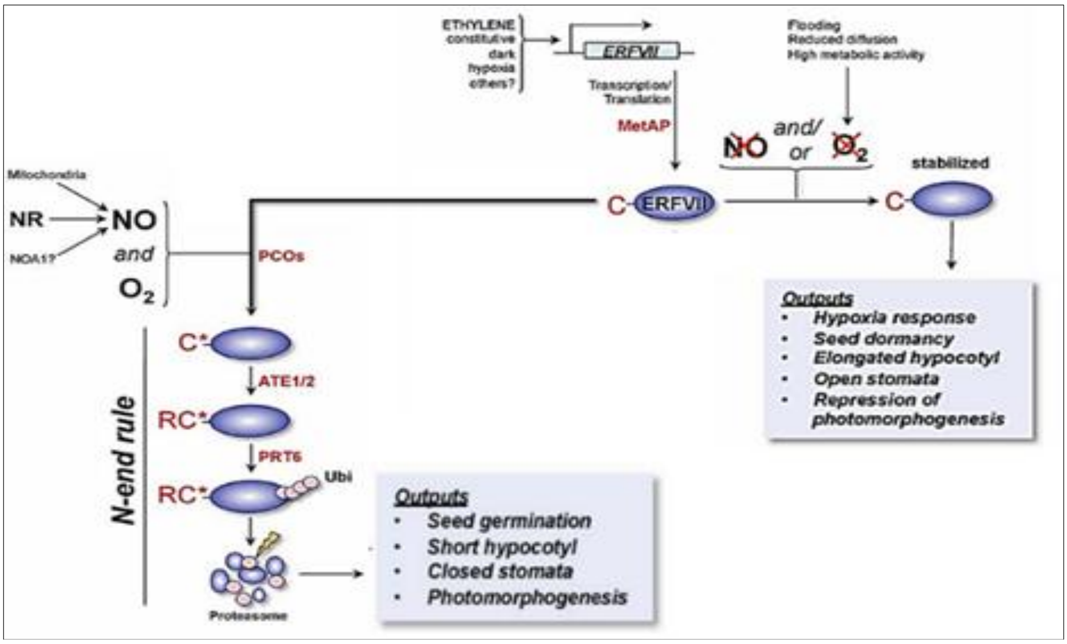

Fig 3: N-end rule pathway of Protein turns over under hypoxia (Gibbs *et al*., 2015) [19]

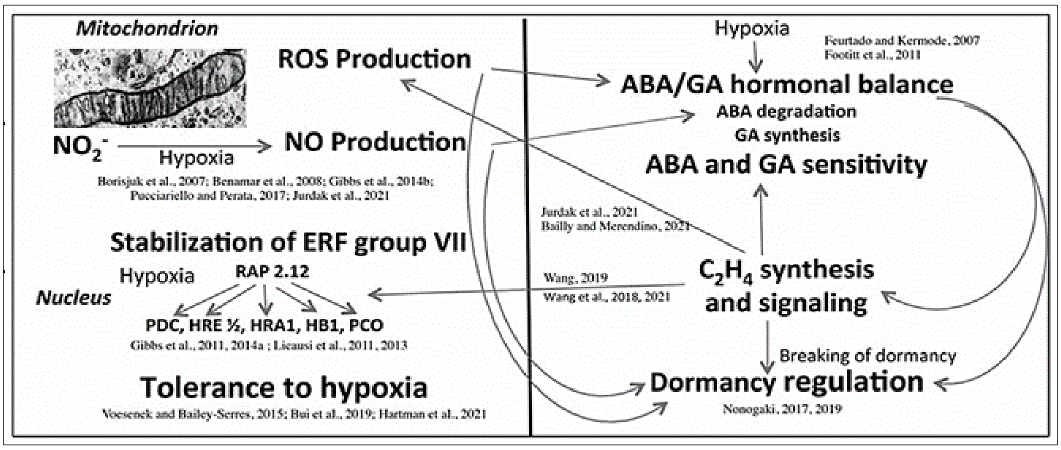

Fig 4: The network between hormones and ROS production involved in the dormancy regulation, and the involvement of hypoxia in stabilization of ERF group VII and tolerance to oxygen deprivation (Corbineau, 2022) [7]

6. Mechanism regulating anaerobic metabolism:

Some of the mechanisms involved for tolerance to hypoxia in plants as follows

A. Morphological adaptation for anaerobic growth

Under submergence, limited oxygen availability from the atmosphere restricts gas exchange, triggering ethylene production through the action of enzymes ACS and ACC (1-aminocyclopropane-1-carboxylic acid). This ethylene enhances the expression of the RBOHH gene (Respiratory Burst Oxidase Homolog), which converts oxygen into superoxide, leading to peroxide formation. Concurrently, calcium ion influx and reactive oxygen species (ROS) accumulation initiate programmed cell death, resulting in aerenchyma development in hypoxia-affected tissues. Consequently, submergence or hypoxia inhibits coleoptile maturation and promotes cell death. Upon re-exposure to air, submerged coleoptiles undergo three distinct death-related events: hydrogen peroxide burst, tissue splitting, aerenchyma formation, and eventual senescence (Yamauchi *et al*., 2018) [27].

Ethylene Signalling in Dicots

When ethylene levels are low in air, it is detected in the endoplasmic reticulum (ER) by a family of receptors. These active receptors interact with the N-terminal region of CTR1 to form a complex. CTR1 then phosphorylates the C-terminal domain of EIN2, leading to its degradation through F-box proteins ETP1/2, thereby suppressing downstream signaling. In the nucleus, EIN3/EIL1 are further degraded via the ubiquitin-proteasome pathway mediated by F-box proteins EBF1/2. However, when ethylene is present, binding to its receptors inactivates both the receptors and CTR1. This inactivation causes EIN2 to be dephosphorylated and cleaved. The resulting EIN2 C-terminal fragment (CEND) moves into the nucleus, where it stabilizes and promotes the accumulation of EIN3/EIL1. This, in turn, activates the transcription of ERF1 and other ethylene-responsive genes (Yang *et al*., 2015) [28], supporting seedling growth under stress conditions.

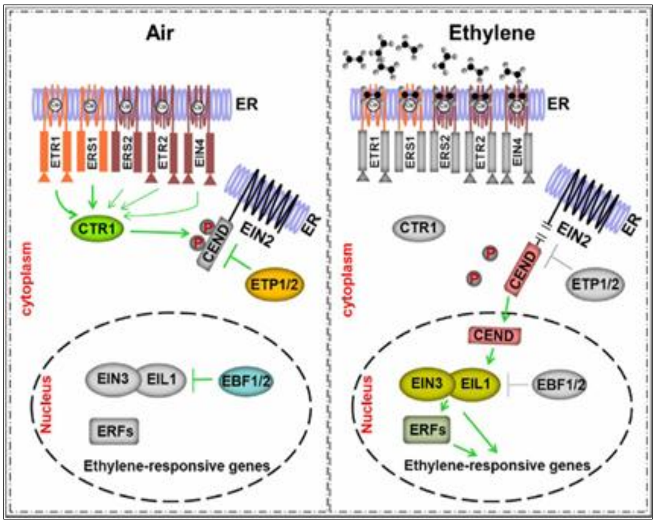

Fig 5: Model of Ethylene Signalling Pathway in Arabidopsis (Yang *et al*., 2015) [28]

B. Mechanism regulating anaerobic metabolism

Yu *et al*. (2019) [29] reported that rice adopts a “metabolic and escape strategy” to enable rapid germination and coleoptile elongation under stagnant water. This strategy operates through three interconnected levels: signaling and gene regulation, physiological adjustments, and morphological adaptations. During anaerobic germination or seedling development (AG/ASD), CALCINEURIN B-LIKE (CBL) PROTEIN-INTERACTING Ser/Thr PROTEIN KINASE connects oxygen deficiency signals to the SUCROSE NON-FERMENTING1 RELATED PROTEIN KINASE 1A (SnRK1A)-MYB SUCROSE1 (MYBS1) pathway, which regulates the expression of α-AMYLASE (αAmy). The enzyme αAmy breaks down seed starch into sugars, fueling fermentation-based energy production that supports underwater growth. Under submergence, SnRK1A-INTERACTING NEGATIVE REGULATOR1/2 (SKIN1/2) suppresses SnRK1A activity, while trehalose-6-phosphate phosphatase (TPP7) counteracts trehalose-6-phosphate (T6P)-mediated inhibition of SnRK1A. Additionally, MYB SUCROSE2 (MYBS2) competes with MYBS1 for promoter binding, thereby repressing αAmy expression under low-oxygen conditions. Key enzymes involved in this metabolic adaptation include Alcohol Dehydrogenase 1/2 (ADH1/2) and Pyruvate Decarboxylase (PDC).

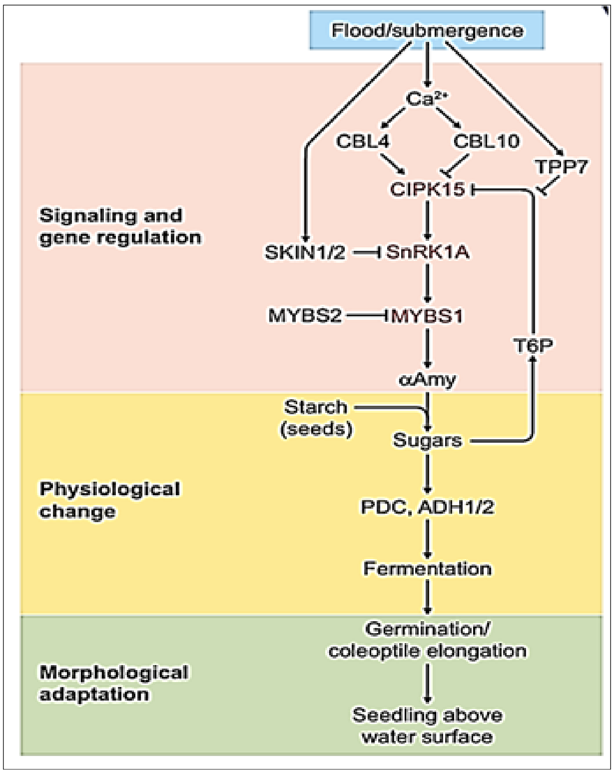

Fig 6: Mechanism regulating αAmy expression in response to O_2 deficiency in rice (Yu *et al*., 2019) [29]

Hypoxia also leads to sugar starvation which leads to phosphorylation of S53 (serine 53) nucleotide which attaches to Myeloblastosis2 (MYB2) sugar to become inactive which paves way for the attachment of MYB1 sugar to transcription activator proceeding the transcription process leading to the production of proteins which increases α Amylase in the cytoplasm leading to its action on starch stored in the seeds which is converted to sugars leading to the growth of seedlings under submerged conditions of low oxygen supply to plants as mentioned in fig 7 (Chen *et al*., 2019) [4].

In order to confirm the activity of CIPK15 and fermentation enzymes can provide tolerance to submerged plants lee and his co-workers (2009) have conducted an experiment in rice cultivars Wt and cipk which are submerged for 6 weeks in water depth of 5-10 cm. In WT embryos the experiment of

CIPK15 was induced by sucrose fermentation regardless of O_2 availability. The expression of 2 ADH genes induced by hypoxia in a CIPK dependent manner as it is clearly expressed in WT and totally abolished in cipk embryos and the transcriptional activity of Adh in the presence of sucrose and hypoxia confirms that the production of sugars and induction of Adh under hypoxic conditions appear to be co-ordinately regulated by CIPK15.

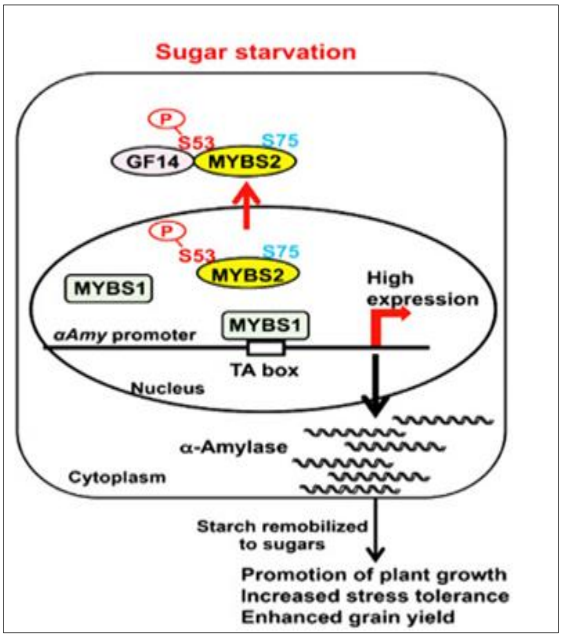

Fig 7: Sugar starvation flooding adaptation in rice (Chen *et al*., 2019) [4]

C. Metabolic pathways and genesis in rice paved way for hypoxia tolerance

Mainly in rice during seed germination it undergoes two strategies under submergence low O_2 escape strategy takes place in hypoxia sensitive cultivars there is a slow germination thereby inhibition of germination and coleoptile elongation under prolonged conditions of submergence and low O_2 escape strategy seen in tolerant cultivars these phenotypes show faster coleoptile elongation to reach the water surface for air exchange. The molecular regulatory pathways and metabolic pathways of rice under hypoxic germination and early seedling growth. Sugar starvation caused by low O_2 stress under the submergence condition is the key upstream signal acting metabolic regulation pathways. The Ca^{2+} signal acts as a secondary messenger to mediate the downstream responses. The calcineurin B-like (CBL) proteins bind to Ca^{2+} and then interact with CIPK15, leading to the activation of its kinase activity. Subsequently, the activated CIPK15 physically interacts with SnRK1A, an upstream protein kinase of the transcription factor MYBS1, and activates its activity, consequently elevating the activity of Amy for seed stored starch degradation. SKIN1/2 and MYBS2 negatively regulate SnRK1A and MYBS1, respectively, thereby repressing Amy Figure 15. The molecular regulatory pathways and metabolic pathways of rice under hypoxic germination and early seedling growth. Sugar starvation caused by low O_2 stress under the submergence condition is the key upstream signal affecting metabolic regulation pathways.

The Ca^{2+} signal acts as a secondary messenger to mediate the downstream responses. The calcineurin B-like (CBL) proteins bind to Ca^{2+} and then interact with CIPK15, leading to the activation of its kinase activity. Subsequently, the activated CIPK15 physically interacts with SnRK1A, an upstream protein kinase of the transcription factor MYBS1, and activates its activity, consequently elevating the activity of αAmy for seed stored starch degradation. SKIN1/2 and MYBS2 negatively regulate SnRK1A and MYBS1, respectively, thereby repressing αAmy expression. Additionally, seed imbibition also results in the biosynthesis of gibberellin (GA) in the embryo. GA induces the expression of MYBGA in cereal aleurone cells, thereby upregulating the expression of the αAmy gene. Meanwhile, low O_2-induced OsTPP7 de suppresses the SnRK1A activity that is inhibited by trehalose 6-phosphate (T6P). OsTPP7 increases the sink strength of the embryo axis-coleoptile by the perception of low sugar availability by enhancing the conversion of T6P to trehalose and leads to a decrease in the T6P/sucrose ratio, thus enhancing starch mobilization for energy production to promote coleoptile elongation. In addition, low O_2 results in the shift of aerobic to anaerobic fermentation inducing the expression of PDC and ADH. They have also identified different QTL's and candidate genes from the land race aus of cultivar landrace Kharsu 80A for AG tolerance they are as follows

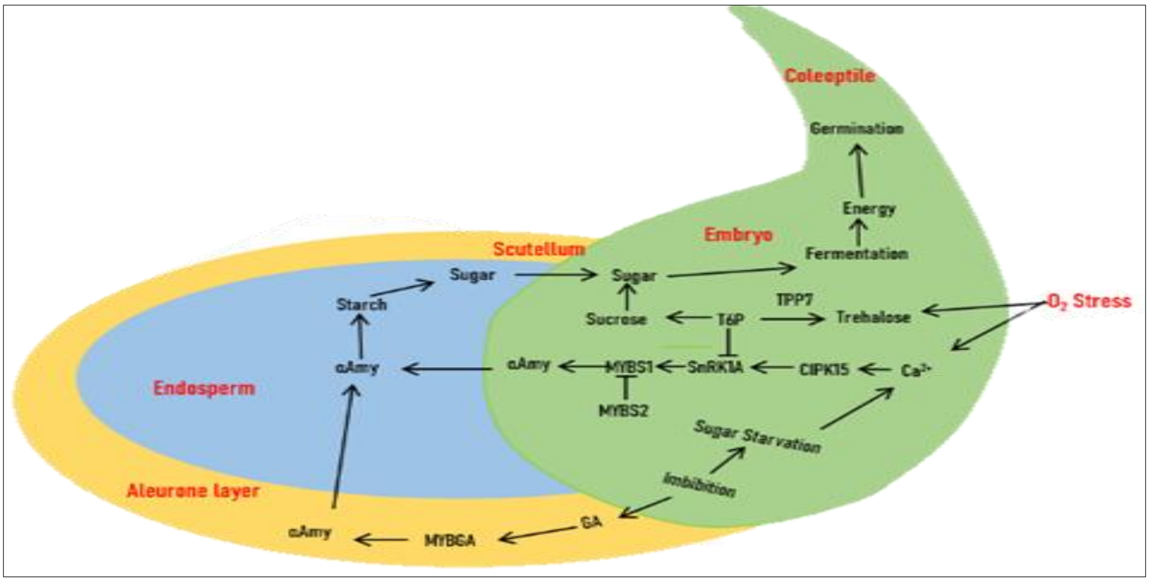

Fig 8: Metabolic pathways of rice under hypoxic germination (Mingquing *et al*., 2020)

Table2: The identified quantitative trait loci (QTLs)/candidate genes for anaerobic germination (AG) tolerance (Mingquing *et al*., 2020)

QTLs	Chromosome	Trait	Description
qAG-1-2	1	survival rate	Seed germination
qAG-3-1	3	survival rate	Seed germination
qAG-7-2	7	survival rate	Seed germination
qAG-9-1, qAG-9-2	9	survival rate	Seed germination
Candidate Genes	**Chromosome**	**Trait**	**Description**
OsTPP7	9	coleoptile length	Enhancing germination and coleoptile elongation
LOC_Os06g03520	6	coleoptile length	DUF domain containing protein

They have concluded that CIPK15-mediated O_2-deficiency signaling regulatory pathway plays a key role in controlling sugar and energy production during AG. CIPK15 regulates the SnRK1A-dependent sugar sensing, thereby regulating the abundance of αAmy and ADH for the metabolic shift and energy production to adapt to submergence conditions. The upregulation of the key enzyme αAmy3 is effective in mobilizing starch to produce energy and shifts ATP production from aerobic to anaerobic respiration. Detected QTLs and their candidate genes could provide promising perspectives for molecular genetic characterization of AG tolerance and for the rapid development of cultivars with enhanced submergence tolerance.

Cultivar difference imparting hypoxia tolerance

Nakajima *et al*. (2015) [17] investigations on germination percentages under low oxygen conditions at different temperatures, focusing on pre-germination flooding tolerance in six soybean cultivars. In flooding-susceptible cultivars, the seed coat lacked sufficient mechanical strength to withstand the turgor pressure generated by embryo expansion during hypoxia, indicating that higher oxygen levels are required to initiate radicle growth. By contrast, in more susceptible cultivars, the radicle elongated more easily under low oxygen concentrations, but this often led to abnormal seed coat dehiscence as the embryo swelled particularly at $20\,\mathrm{mL\,L^{-1}}$ O_2, where radicle growth did not exceed 2 mm. These findings suggest that variations in germination behaviour under hypoxia between tolerant and susceptible cultivars may be linked to differences in the seed coat's oxygen permeability.

In rice, flooding tolerance is largely regulated by SUB1A, a transcription factor that interacts with ethylene-responsive elements. SUB1A activates Slender-rice-like 1 (SLRL1), which suppresses gibberellic acid induced internode elongation, conserving energy for post-submergence recovery. Alternatively, some rice varieties adopt an escape strategy by elongating internodes to raise leaves above the water surface. This process involves ethylene-responsive factors such as Snorkel1 and Snorkel2, which influence EIN3 and modulate gibberellin signalling, leading to enhanced tissue sensitivity and elongation. Transcription factors including SUB1C, SUB1A, Snorkel1, and Snorkel2 are activated during flooding stress, showing functional divergence from related family members and suggesting specialized roles. As highlighted by Nagai *et al*. (2010), SUB1A, Snorkel1, and Snorkel2 also contribute to hypoxia tolerance in Arabidopsis.

Conclusion

Hypoxia profoundly influences seed germination and plant survival by reshaping metabolic, structural, and hormonal processes. Oxygen is essential for respiration, yet its requirement varies across species, dormancy depth, and seed type, with fatty seeds being more sensitive than starchy ones. Under low oxygen, nutrient uptake, storage activity, and mitochondrial respiration decline, forcing reliance on fermentation to regenerate NAD^+, which slows development but may aid DNA repair. Structural barriers such as seed coats, pericarps, and glumellae restrict oxygen diffusion, delaying germination, while mucilage enhances water uptake and supports seedling establishment. Hypoxia also regulates dormancy through GA/ABA balance, ERF VII stabilization, mitochondrial ROS/NO signaling, and ethylene-mediated ABA reduction, ultimately determining whether seeds remain dormant or germinate. Species-specific adaptations further shape responses such as rice employs hypoxia-responsive α-amylases to mobilize starch reserves, whereas barley and other cereals lack this mechanism, delaying germination. Under submergence, plants integrate ethylene signaling, ROS-driven cell death, and metabolic reprogramming particularly α-amylase-driven starch mobilization and fermentation to survive oxygen-deficient environments. In rice, tolerance is mediated by the CIPK15-SnRK1A pathway, which regulates sugar sensing and energy production, alongside transcription factors such as SUB1A and Snorkel1or2 that balance quiescence and escape strategies. Identified QTLs and candidate genes, including OsTPP7, provide promising targets for breeding submergence-tolerant cultivars, highlighting how oxygen availability critically shapes seed metabolism, dormancy, and adaptive strategies across species.

Hypoxia, the concentration of oxygen limits mitochondrial ATP production (oxidative phosphorylation). The establishment of hypoxic conditions can affect the developmental pattern of gene expression, *in vivo* enzymatic activity, metabolite pool sizes and metabolic fluxes. The identification of quantitative trait loci, gene mapping and marker-assisted breeding programs may help develop plant species for the oxygen-deficient environment. Taking advantage of the latest advancements in the field of metabolomics, proteomics, transcriptomics and genomics techniques, a complete profile of metabolites, proteins and genes can be developed that will elucidate different mechanisms of a hypoxia tolerance in different seeds species. The complexity of oxygen deprivation cannot be tackled without a holistic approach. Therefore, the integrated use of agronomic practices, conventional breeding and biochemical and molecular manipulations is needed for improving hypoxia tolerance in seed.

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