



Unravelling the Genetic and Physiological Basis of Flood Tolerance in Rice Germination

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Rice has a remarkable ability to adapt to various hydrological conditions, ranging from well-drained uplands to waterlogged areas. However, prolonged floods and partial submergence still pose significant challenges to rice production, which have been exacerbated by climate change. Nonetheless, the rich genetic diversity of rice provides opportunities for developing flood-tolerant varieties through genetic manipulation and selective breeding. While rice exhibits tolerance to waterlogging during the vegetative stage, it is highly vulnerable to anaerobic conditions during germination and early growth. Anaerobic germination and the establishment of roots and leaves are crucial for direct-seeded rice in flood-prone areas. Insufficient land levelling or waterlogging after seeding can lead to poor crop establishment or complete failure. Cultivating rice varieties capable of germinating in flooded soils offers several advantages for direct-seeded rice systems, including cost reduction, weed suppression, and decreased reliance on manual or chemical weed control methods. Significant progress has been made in understanding the genetics and physiology of flood tolerance during the vegetative stage and germination in rice. Researchers have identified genetic variation and specific landraces that exhibit tolerance to flooded conditions. However, further research is needed to enhance the development of flood-tolerant rice varieties suitable for direct-seeding systems and to address waterlogging challenges in other crops. It is crucial to understand the underlying physiological and molecular mechanisms responsible for the genetic variability in germination tolerance. By identifying relevant traits and pathways, effective breeding strategies can be devised to enhance tolerance and improve crop productivity in flood-prone regions.

Key words: Anaerobic germination, Rice, Phytohormones, Carbohydrates

Response of plant to flooding during germination

When plants germinate under flooded conditions, they face unfavourable situations in the root zone. These include a lack of oxygen, which hinders root growth and respiration, as well as the accumulation of gases like carbon dioxide and ethylene. The low oxygen levels reduce root function, making it difficult for the plant to absorb nutrients and water. Additionally, toxic substances like reduced iron, hydrogen sulphide, manganese and organic acids can reach harmful concentrations, altering the soil pH and further hindering nutrient availability. These conditions can cause damage to the roots and even lead to plant death. Despite the existence of genetic diversity in waterlogging tolerance within certain crops, such as maize, wheat, barley, and rye, this diversity has not been fully utilized in breeding efforts.

Plants adapted to waterlogged or submerged conditions during germination employ various strategies to cope with these challenges. They undergo morphological adaptations like developing specific root types or elongating certain plant parts. For example, some plants

develop mesocotyl roots, while others form adventitious roots or elongate petioles and coleoptiles. Rice genotypes that show better tolerance to flooding during germination have been identified, leading to the study of associated traits. Breeding lines suited for direct-seeded systems have been developed, and genetic loci related to flooding tolerance during germination are being targeted for further research.

One important trait when selecting tolerant rice genotypes for germination under submergence is coleoptile elongation. This trait is easily observed and is achieved through cell elongation rather than cell division. While active cell division occurs in the first 48 hours of submergence, which coincides with high oxygen demand, cell elongation becomes the primary process driving growth under oxygen-deprived conditions. However, when seeds are pre-soaked before submergence, there is no significant difference in growth between tolerant and sensitive genotypes. This suggests that most rice genotypes can initiate germination but face limitations in utilizing energy reserves when oxygen is limited. Coleoptile elongation is influenced by specific proteins called expansins, which loosen cell walls. Different expansins, such as EXPA2, EXPA4, EXPA7, EXPB12, EXPA1, EXPB11, and EXPB17, are expressed or induced under submergence and may contribute to coleoptile elongation.

Phytohormones, including ethylene, abscisic acid (ABA), and gibberellins (GA), are involved in submergence tolerance during germination. The SUB1A gene in rice regulates ethylene production and affects growth under submergence. Ethylene can either promote or inhibit growth depending on its concentration and plant development stage. ABA is a positive regulator of rice germination under submergence, promoting seed dormancy release and enabling germination in anaerobic conditions. GA may inhibit the coleoptile response to submergence and impact elongation.

Different traits, such as seedling survival, enzyme activity, root characteristics, seed longevity, and morphological features, can be used to assess anaerobic germination tolerance. These traits help compare and screen different genotypes for their tolerance to flooding during germination. However, further evaluation is needed to determine the significance and effectiveness of these traits as selectable markers, particularly in terms of survival and coleoptile elongation.

Carbohydrate metabolism adjustment under low oxygen conditions

When oxygen levels are low, plants undergo significant changes in their carbohydrate metabolism. The breakdown of starch into simple sugars, which is crucial for energy production, is inhibited due to reduced enzyme activity in low oxygen conditions. However, certain enzymes, such as α -amylases (especially RAm3D), sucrose synthase, and aldolase, remain active in rice genotypes tolerant to hypoxia during germination, while they are inhibited in sensitive genotypes. Another important aspect affected by low oxygen is the generation of energy through the tricarboxylic acid (TCA) cycle. Without oxygen as the terminal electron acceptor, the TCA cycle is inhibited. To compensate for this, plants, including rice, activate alternative pathways to sustain energy production through glycolysis. Fermentative metabolism or anaerobic respiration takes place in submerged coleoptiles, involving pathways such as alcohol, lactate, and alanine fermentation to regenerate NAD⁺ for glycolysis.

Alcoholic fermentation plays a crucial role in rice germination under oxygen deprivation. A large portion of the pyruvate generated through glycolysis is directed towards ethanol production, while a smaller portion goes into lactate fermentation and alanine pathways. Key enzymes involved in alcoholic fermentation, such as pyruvate decarboxylase (PDC), alcohol dehydrogenase (ADH), and aldehyde dehydrogenase (ALDH), are activated in rice during germination under low oxygen conditions. The adjustments in carbohydrate metabolism observed in different rice genotypes germinating under anaerobic conditions

involve the specific activation of enzymes in critical steps where oxygen availability is limited. The metabolism of alanine is also relevant, particularly in rice, as it contributes to the synthesis of amino acids like glutamine and glutamate under anoxic conditions.

Bottle necks in carbohydrate metabolism

The first challenge arises when breaking down starch and complex carbohydrates into soluble sugars, especially for intolerant rice varieties and other cereals like wheat. The enzymes sucrose synthase and α -amylases are key players in carbohydrate breakdown during low oxygen conditions in germinating rice seeds. While sucrose synthase works similarly in both submerged and aerial conditions in rice, it is inactive in wheat and barley seedlings. In contrast, α -amylases show higher activity in tolerant genotypes germinating under submergence, aiding in starch degradation. The activation of the α -amylase gene *RAmy3D* is positively associated with coleoptile elongation and seedling survival in tolerant genotypes, highlighting its significance in anaerobic germination.

The second hurdle involves the metabolism of pyruvate, where three main pathways come into play: alcoholic fermentation, lactate fermentation, and alanine fermentation. Alcoholic fermentation, which avoids lowering cell pH, is considered the primary alternative pathway. This process starts with the conversion of pyruvate to acetaldehyde through the enzyme pyruvate decarboxylase (PDC). Acetaldehyde is then further metabolized to either ethanol by alcohol dehydrogenase (ADH) or acetate by mitochondrial aldehyde dehydrogenase (mALDH). Under low oxygen conditions, aerobic respiration is inhibited, and plants adapt to maintain energy levels and neutralize reactive oxygen species. ADH, the most highly up-regulated enzyme in the fermentative pathway, efficiently metabolizes acetaldehyde to mitigate its toxicity and generate NAD⁺ for glycolysis. The activity of ADH is significantly higher in tolerant genotypes compared to sensitive ones.

In summary, these adaptations in carbohydrate metabolism are critical for the tolerance of anaerobic conditions during germination in rice and play vital roles in providing energy and preserving cell viability.

Conclusion

In summary, there is significant variation in the ability of rice to tolerate submergence during germination. While progress has been made in understanding the traits associated with tolerance, there is still a need for further research to uncover the regulatory and signaling mechanisms involved. Functional studies of key enzymes, such as *RAmy3D* and *ALDH2*, can provide valuable insights. Identifying functional alleles associated with tolerance in major anaerobic pathways will aid in breeding programs for developing tolerant rice varieties and other waterlogged-tolerant crops. However, challenges remain in developing improved tolerant varieties due to inconsistent data and the need for standardized experimental conditions. Future research should focus on well-selected genotypes and field-relevant protocols to enhance our understanding of tolerance mechanisms during germination under submergence.