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Breeding For Pre-harvest Sprouting (PHS) Tolerance in Greengram

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Abstract

Pre-harvest sprouting (PHS) is the premature germination of seeds on the parent plant, while still in the field before harvest. This phenomenon is triggered by unfavorable weather conditions during the ripening period, leading to significant yield losses and reduced seed quality. The incidence of pre-harvest sprouting is very high in *Vigna* species especially in mungbean. High yielding varieties developed in recent years, despite of their high yield potential, could not increase the yields due to lack of tolerance to pre-harvest sprouting. The primary factors influencing tolerance to pre-harvest sprouting include fresh seed dormancy, hard seededness, pod wall thickness and pod wall epicuticular wax. Among these characters, fresh seed dormancy is found to be the most significant factor contributing to tolerance to pre-harvest sprouting. Hard seededness can increases tolerance to PHS by preventing seeds from germination. Therefore, it is crucial to develop the varieties that are tolerant to pre-harvest sprouting by comprehending the mechanisms and genetics underlying tolerance.

1. Introduction

Greengram, scientifically known as *Vigna radiata*, is a vital legume crop cultivated extensively in Asia. It is cherished not only for its nutritional value but also for its ability to fix atmospheric nitrogen, enriching soil fertility. However, one of the significant challenges faced by greengram farmers is pre-harvest sprouting (PHS), a phenomenon where seeds germinate while still on the plant due to untimely rainfall or high humidity conditions. It leads to considerable yield losses and reduced seed quality. Breeding for PHS resistance is a key strategy to mitigate these losses.

2. Factors Influencing PHS

2.1. Environmental Factors

Pre-harvest sprouting (PHS) is a highly intricate physiological process significantly influenced by external factors such as temperature, light, and moisture. These factors not only affect the extent of PHS but also determine its onset. (He et al., 2000).

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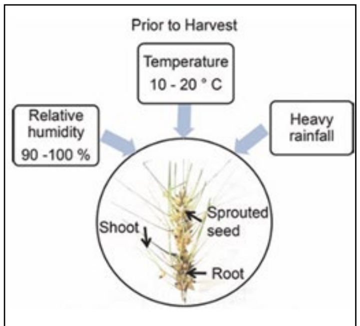
Moisture significantly influences pre-harvest sprouting (PHS) as it is essential for seed germination. Some researchers suggest that excessive moisture in humid environments can trigger PHS by transferring water to the seeds, leading to their germination. (Obroucheva et al., 2017., Chen and Liu, 2017) (Figure 1).



Pre-harvest sprouting in Wheat head; Patwa and Penning (2020)

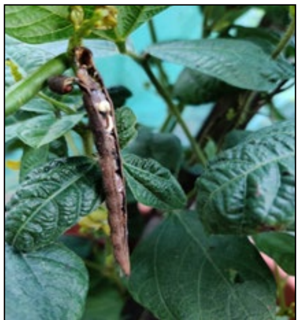

Pre- harvest sprouting in greengram

Figure 1. Environmental factors influencing Pre-harvest sprouting

2.2. Genetic and Physiological Factors

Pre-harvest sprouting (PHS) is regulated not only by abscisic acid (ABA) production but also by ABA signaling. The balance between abscisic acid (ABA) and gibberellins (GA) is critical. ABA promotes dormancy and inhibits germination, while GA promotes germination. Higher ABA levels relative to GA in developing seeds contribute to enhanced dormancy and PHS tolerance.

One of the most extensively studied genes related to PHS in plants is *Viviparous1 (VP1)*, the homolog of ABI3 in rice and maize. VP1, a transcription factor that senses ABA in maize, plays a crucial role in amplifying the hormonal responses specific to seeds (McCarty et al., 1991). Additionally, *ZmVP1* has been shown to inhibit α-starch hydrolysis and specifically disrupt gibberellin (GA) signaling, thereby preventing germination. In wheat and rice, several genes responsible for PHS have been identified and characterized, with their functions mostly involved in the biosynthesis, metabolism, and signaling pathway of ABA and GA.

3. Seed and Pod Characteristics Associated with Pre- Harvest Sprouting Tolerance

3.1. Seed Characteristics

3.1.1. Dormancy

Seed dormancy is the primary factor affecting PHS tolerance. It is controlled by various genes that regulate the seed's ability to remain dormant even under favorable germination conditions. Varieties with strong genetic dormancy are less prone to PHS. Dormancy is considered an undesirable trait, and during the domestication and breeding process, selection has been made against it. However, excessively low seed dormancy can reduce seed quality and induce pre-harvest sprouting (PHS) if sufficient moisture is available. Consequently, wild types, which have not undergone artificial selection, exhibit higher levels of dormancy compared to cultivated varieties.

3.1.2. Seed Coat Properties

Seeds with less permeable coats absorb water more slowly, which helps in delaying germination under humid conditions. The permeability of the seed coat is influenced by its chemical composition, including waxes and phenolic compounds. A thicker seed coat can act as a physical barrier to water and oxygen, which are essential for germination.

3.1.3. Alpha-amylase Activity

The activity of α-amylase is low in dry mungbean seeds, but its activity gets induced rapidly and increases as the germination process proceeds. This enzyme breaks down starch into sugars during germination. Lower levels of alpha-amylase activity in the seed can delay germination, thereby increasing PHS tolerance. The expression of alpha-amylase is tightly regulated by hormonal and environmental signals.

3.1.4. Physiological Maturity

Seeds need to be harvested at optimal physiological maturity when they have reached full dormancy potential but before environmental conditions become conducive to sprouting. Prematurely harvested seeds are more susceptible to PHS due to incomplete dormancy development.

3.2. Pod Characteristics

3.2.1. Resistance to Shattering

Pods that resist shattering during maturity help in retaining seeds within a dry environment, preventing exposure to moisture that could induce sprouting. Well-sealed pods protect seeds from moisture. Pod integrity is maintained by structural components such as lignin and cuticular waxes, which provide a barrier against water.

3.2.2. Wax Content on Pod Wall

Wax layer on pods acts as a barrier to moisture, reducing the likelihood of seeds becoming exposed to water. Presence of high density of waxy material embedded in the epidermis were associated with low water absorption. This is particularly important in humid or rainy conditions.

3.2.3. Pod Architecture

The orientation and position of pods on the plant affect their exposure to environmental moisture. Upright pods are less likely to collect water compared to horizontal or downward-facing pods. Dense pod arrangements can trap humidity, while more open structures can enhance airflow and drying, reducing the risk of PHS.

3.2.4. Synchronous Maturity

Uniform ripening of pods ensures that seeds reach maturity at the same time, allowing for a more consistent and timely harvest. This uniformity helps in avoiding of early sprouting seeds. Genes that control the timing and uniformity of pod ripening are critical. Breeding efforts often focus on selecting varieties that exhibit uniform maturity.

3.2.5. Pod Wall Thickness

A thicker pod wall acts as a more effective physical barrier against external moisture. During periods of high humidity or rainfall before harvest, thicker pod walls are better at preventing water from penetrating the pod and reaching the seeds. This helps in reducing the chances of seed germination within the pod. Thicker pod walls help in keeping the pods intact until harvest, thereby protecting the seeds from premature exposure. help in maintaining a more stable internal environment within the pod, reducing the likelihood of temperature fluctuations that can trigger germination processes

4. Breeding Strategies for PHS Tolerance

4.1. Screening Germplasm for Tolerance

Collect a wide range of germplasm from different geographical regions, including wild relatives and landraces, which may harbor PHS tolerance traits. Conduct field and controlled environment tests to identify PHS-tolerant lines.

4.2. Conventional Breeding Approaches

4.2.1. Hybridization

Cross PHS-resistant varieties with high-yielding, susceptible varieties to combine desirable traits. Develop segregating populations (F_2, F_3, etc.) and select individuals exhibiting PHS tolerance and agronomic superiority.

4.2.2. Backcross Breeding

Use a recurrent parent with good agronomic traits and backcross it with a donor parent possessing PHS tolerance. Select for PHS tolerance and desired agronomic traits over successive backcross generations. Select and evaluate progeny over multiple generations under field conditions to confirm resistance.

4.3. Molecular Breeding Techniques

4.3.1. Identification of QTLs and Genes

Identification of Quantitative Trait Loci (QTLs) and genes associated with pre-harvest sprouting (PHS) tolerance is crucial for developing improved crop varieties with enhanced resistance to premature seed germination

4.3.2. Genome-wide Association Studies (GWAS)

GWAS takes full advantage of ancient recombination events to identify the genetic loci underlying traits at a relatively high-resolution (Zargar et al., 2015). Helps in Identification of genomic regions associated with PHS tolerance by analysing genetic variation across diverse lines (Figure 2).

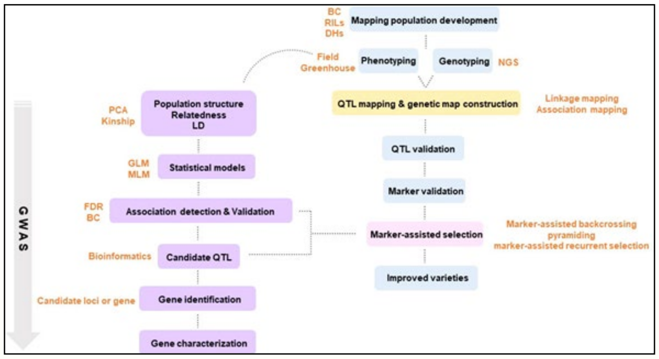


Figure 2: Schematic diagram of Molecular breeding techniques in plants, (Jeon et al., 2023)

4.3.3. QTL Mapping, Marker Development and Validation

Quantitative Trait Loci (QTL) mapping is a powerful technique used to identify specific regions of the genome associated PHS tolerance. These are typically controlled by multiple genes and influenced by environmental factors. Once QTLs and markers associated with PHS tolerance are identified, validation in different genetic backgrounds is crucial to confirm their reliability and consistency.

4.3.4. Marker-assisted Selection (MAS)

Incorporate marker-assisted selection in breeding programs to accelerate the introgression of PHS tolerance traits into elite cultivars. Use molecular markers linked to PHS QTLs to select for tolerant traits in breeding programs.

4.3.5. CRISPR/Cas9 and Genetic Engineering

Use advanced genome editing tools to introduce or enhance PHS resistance genes.

5. Challenges and Future Directions

5.1. Genetic Complexity

PHS resistance is a complex trait influenced by multiple genes and environmental interactions.

5.2. Trait integration

Combining PHS resistance with other desirable traits like high yield, disease resistance, and stress tolerance can be challenging.

5.3. Environmental Variability

Consistent screening under variable environmental conditions is necessary to ensure the stability of PHS resistance.

6. Conclusion

Breeding for PHS resistance in greengram is crucial for improving yield stability and seed quality. By combining traditional breeding methods with advanced molecular techniques, breeders can develop varieties that are resistant to PHS while maintaining high yields and other agronomically important traits. This integrated approach will help mitigate the adverse effects of PHS and support the sustainable production of greengram.

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