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Development and Application of Speed Breeding Technologies in Groundnut (*Arachis hypogaea* L.): An Advance Approach

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Authors' contributions

This work was carried out in collaboration among all authors. Author PP designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors NT and AK managed the analyses of the study. Author AK managed the literature searches. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Traditional breeding methods for groundnut have been time-consuming, often taking several years to develop improved varieties. However, recent developments in speed breeding technologies have revolutionized the breeding process, significantly reducing the breeding cycle duration and accelerating genetic gain. This review presents an overview of the key principles and methodologies involved in speed breeding, including the manipulation of light, temperature, and photoperiod conditions to promote rapid growth and development. The application of controlled environments, such as growth chambers and specialized greenhouse setups, enables researchers to simulate ideal conditions for groundnut growth, resulting in shortened generation intervals and

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increased breeding cycles. Furthermore, the review highlights the various genetic and genomic tools employed in speed breeding, such as marker-assisted selection (MAS), genomic selection (GS), and high-throughput genotyping technologies. One promising avenue is the integration of Speed Breeding (SB) with genome editing techniques, such as the widely used CRISPR/Cas9 system. Genome editing enables precise modifications of specific genes, leading to the development of plants with desired traits. These tools aid in the identification and selection of desirable traits, enabling breeders to efficiently incorporate traits related to yield, disease resistance, drought tolerance, and nutritional quality. The adoption of speed breeding techniques in groundnut breeding programs has shown promising results in terms of accelerated varietal development and increased genetic gain. The review also discusses the potential applications and future prospects of speed breeding in groundnut, including the integration of omics technologies, gene editing techniques, and the development of pre-breeding populations. These advancements hold significant promise for groundnut improvement and have the potential to address the challenges posed by climate change and food security.

Keywords: Speed breeding; CRISPR/Cas9 system; marker-assisted selection; genomic selection; genome editing and omics.

1. INTRODUCTION

Groundnut, also known as peanut (*Arachis hypogaea* L. Millsp), is a self-pollinated crop belonging to the Fabaceae family [1]. It is characterized as a disomic allotetraploid, with a chromosome count of 2n = 4x = 40. The species is comprised of two sets of chromosomes, highly diplodized, which means that recombination between the A and B genomes is limited, except in rare cases when a quadrivalent forms. Within the Arachis section, groundnut is found alongside *A. monticola*, which is also a tetraploid species. Additionally, there are approximately 25 diploid species within this section [2]. Groundnut is an important food crop worldwide with an annual production of over was 83.69 lakh tonnes on near 45.53 lakh hectares in *Kharif* 2022-23 according to the last statistics of Food and Agriculture Organisation (FAO). The crop’s cultivation, processing and trade significantly impacts the socio-economic development of a large number of developing and least developed countries [3]. Understanding the genetic composition and ploidy level of groundnut is crucial for breeding programs and genetic studies. The tetraploid nature of groundnut presents unique challenges and opportunities for crop improvement, including the potential for novel gene combinations and traits. Groundnut commonly known as peanut is a globally significant oilseed crop cultivated for its nutritional value, oil content, and versatile applications in various food products [4]. As the demand for groundnut continues to rise, there is a pressing need to develop improved varieties with enhanced yield potential, disease resistance, and adaptability to changing environmental conditions. However, traditional breeding methods often involve long breeding cycles, making it challenging to meet the demand for new and improved groundnut varieties in a timely manner.

To address this challenge, the development and application of speed breeding technologies have emerged as a revolutionary approach to accelerate crop improvement programs, including groundnut breeding. Speed breeding is an innovative technique that utilizes controlled environments and optimized growth conditions to expedite plant growth and development, thereby shortening breeding cycles and increasing the efficiency of genetic gain. The principles and methodologies underlying speed-breeding techniques involve manipulating environmental factors, such as light intensity, temperature, and photoperiod, to promote rapid plant growth and early flowering [5]. This approach allows for multiple generations of crops to be produced within a single year, significantly reducing the time required for variety development.

Controlled environments, such as growth chambers and specialized greenhouse setups, provide researchers with precise control over environmental conditions, enabling year-round breeding cycles and consistent results [3]. These controlled environments facilitate the simulation of optimal growth conditions, which is particularly advantageous for groundnut breeding programs, as the crop is highly sensitive to climatic variations and has specific photoperiod requirements for flowering and fruiting. The integration of genetic and genomic tools has further enhanced the effectiveness of speed breeding in groundnut improvement. Marker-assisted selection (MAS) and genomic selection (GS) techniques have been successfully employed to identify and select desired traits, allowing breeders to focus on specific traits related to yield, disease resistance, and nutritional quality [6]. High-throughput genotyping technologies have also facilitated the rapid screening of large populations, accelerating the identification of genetically superior individuals for further breeding programs.

While speed breeding offers remarkable opportunities for accelerated groundnut variety development, there are challenges that need to be addressed. Maintaining genetic diversity and stability over rapid breeding cycles is crucial to prevent genetic bottlenecks and ensure the long-term success of groundnut breeding programs [7]. Furthermore, the potential physiological changes associated with accelerated growth and development in speed breeding require careful consideration to ensure the overall quality and performance of the resulting varieties. The integration of omics technologies, such as transcriptomic and proteomics, can provide valuable insights into the underlying molecular mechanisms associated with speed breeding in groundnut [8]. Additionally, emerging techniques like gene editing hold promise for precise trait manipulation, enabling breeders to rapidly incorporate desirable characteristics into groundnut varieties [9].

In conclusion, speed-breeding technologies offer a transformative approach to groundnut breeding by shortening breeding cycles and increasing genetic gain [10]. By leveraging controlled environments and advanced genetic tools, speed breeding enables breeders to expedite the development of improved groundnut varieties with enhanced traits [11]. This review aims to provide a comprehensive analysis of the development and application of speed breeding technologies in groundnut breeding programs, highlighting their potential impact on sustainable agriculture, food security, and the future of groundnut cultivation.

2. SPEED BREEDING

In temperate regions, groundnut breeders typically face limitations in growing only one generation per year, resulting in a lengthy process of 10-15 years from the initial cross to the release of a new cultivar [12]. The use of a winter nursery to increase homozygosity levels through single seed descent can slightly improve the situation by allowing for two generations per year, reducing the development time to around 7-8 years [13]. However, recent advancements in "speed breeding" techniques have demonstrated even greater efficiency in groundnut breeding.

The greenhouse speed breeding system proved to be highly effective in reducing generation times in the peanut breeding program. By implementing controlled environment conditions and continuous high intensity PAR light, similar to the approach used in wheat breeding, two generations of a full season maturity genotype could be progressed within 202 days. This represents a significant improvement compared to the traditional field-based pedigree system, which would have required around 290 days across two full summer cropping seasons (i.e., 17 months). Additionally, the greenhouse system offers advantages such as reduced land requirements and decreased susceptibility to adverse environmental conditions [14].

The greenhouse speed breeding system is particularly valuable for small-scale breeding programs with limited land, machinery, and labor resources [15]. It provides these programs with an alternative approach for rapidly advancing early generation breeding material. However, it should be noted that the system requires intensive monitoring of both biotic and abiotic stresses [16]. The potential for issues, such as the heater malfunction described in the F2 generation, highlights the need for careful management and maintenance [17]. Disease outbreaks have been reported in other controlled environment breeding programs, although, so far, no significant problems have been encountered in the peanut speed breeding system [18]. In conclusion, the greenhouse speed breeding system offers a promising solution for accelerating the breeding process in peanuts [19]. While challenges and risks exist, proper monitoring and mitigation strategies can help ensure the successful implementation of this system in peanut breeding programs [20].

2.1 A Proposed System for Expediting Peanut Variety Development

The potential reduction in development time for new peanut cultivars can be achieved through the implementation of a speed breeding system combined with a single seed descent breeding strategy [14]. This approach differs from conventional methods that typically rely on field-based pedigree breeding strategies [21]. In most private and public peanut breeding programs, initial yield trials (Stage 1) are initiated around the F5 or F6 generation [22]. By this stage, the process of inbreeding has minimized heterozygosity, allowing for more effective selection of complex quantitative traits. To illustrate the time and cost advantages of utilizing speed breeding and single seed descent (SSD) compared to field-based pedigree breeding methods [23], let's consider a scenario where a breeder begins with a reasonable quantity of F2 seeds (e.g., 500) obtained through self-pollination of an F1 hybrid plant [24]. The breeder's goal is to develop stable F5-derived lines for inclusion in preliminary yield trials [25].

2.2 Opportunities for Combining SB with Modern Breeding and Phenotyping Tools

The field of crop improvement has witnessed significant advancements in the 21st century through the utilization of DNA marker technology, genomics-assisted breeding, and genome editing techniques [26]. These modern tools have revolutionized agricultural practices and have opened up new possibilities for enhancing plant varieties [27]. One promising avenue is the integration of Speed Breeding (SB) with genome editing techniques, such as the widely used CRISPR/Cas9 system [28]. Genome editing enables precise modifications of specific genes, leading to the development of plants with desired traits [29]. By incorporating SB into the process, edited plants can be grown under optimized SB conditions, allowing for the rapid production of edited seeds [30]. This approach accelerates the achievement of genetic uniformity and increases the potential rate of genetic improvement in the edited plants. The combination of SB and genome editing also facilitates efficient screening and selection of desirable plant lines [31]. Edited plants can be preselected at the T1 generation, and rigorous evaluations can be carried out at the T2 generation to eliminate any unintended off-target effects [32]. This integrated approach has already demonstrated success in crops such as Brassica napus, B. oleracea, and soybean.

As genome editing techniques continue to advance and expand to different crop species, the integration of SB with genome editing is expected to gain further traction. The ability to rapidly generate edited seeds and evaluate their performance under SB conditions provides a powerful tool for expediting crop improvement and the development of new plant varieties with enhanced traits [33]. Fig. 1 illustrates the application of Speed Breeding (SB) in conjunction with genome editing, showcasing the preselection of edited plants at the T1 generation and the rigorous evaluation at the T2 generation to ensure the elimination of off-target genotypes.

Speed breeding involves creating a controlled environment with continuous light, optimal temperatures, and humidity, which enables the advancement of lines from the F2 to the F4 generation within a single calendar year [14]. This significantly accelerates the breeding process, reducing the time required to release a new cultivar to 6-7 years. Compared to field-based breeding, speed breeding can decrease the growing period to maturity by approximately 30%, allowing for three generations to be grown per calendar year [5].

Three breeding strategies can be employed to illustrate the timeframes involved in groundnut breeding [14]:

- Strategy 1 (42 months): Pedigree breeding with one generation per year during the summer season.
- Strategy 2 (23 months): Pedigree breeding with two generations per year, involving one generation in the summer and another in a winter nursery.
- Strategy 3 (17 months): Speed breeding combined with single seed descent (SSD), where the F_2 to F_4 generations are grown in a controlled environment with continuous light, optimal temperatures, and humidity.

By adopting speed-breeding techniques, groundnut breeders can significantly expedite the breeding process and achieve more generations per year, leading to faster cultivar development in Fig. 2. Strategy 3, which involves speed breeding combined with single seed descent (SSD), is particularly well suited for breeding programs that employ a backcrossing strategy to introduce a simply inherited trait controlled by one or two genes into a new variety. This approach allows breeders to rapidly develop lines with the desired trait by advancing generations quickly within a controlled environment.

Strategy 3, which involves speed breeding combined with single seed descent (SSD), can significantly reduce the development time of inbreeding from F2 to F5 to approximately 17 months, as opposed to 42 months required by the conventional pedigree breeding approach (Strategy 1). Furthermore, compared to Strategy 2, which incorporates a second winter generation, Strategy 3 offers a substantial time improvement of around 23 months [34]. The cost-effectiveness of using speed breeding techniques with continuous light conditions is also a determining factor when compared to traditional breeding systems [35]. The implementation of the speed breeding system incurs higher monetary costs, necessitating a cost analysis comparing field-based pedigree breeding with speed breeding/SSD breeding systems.

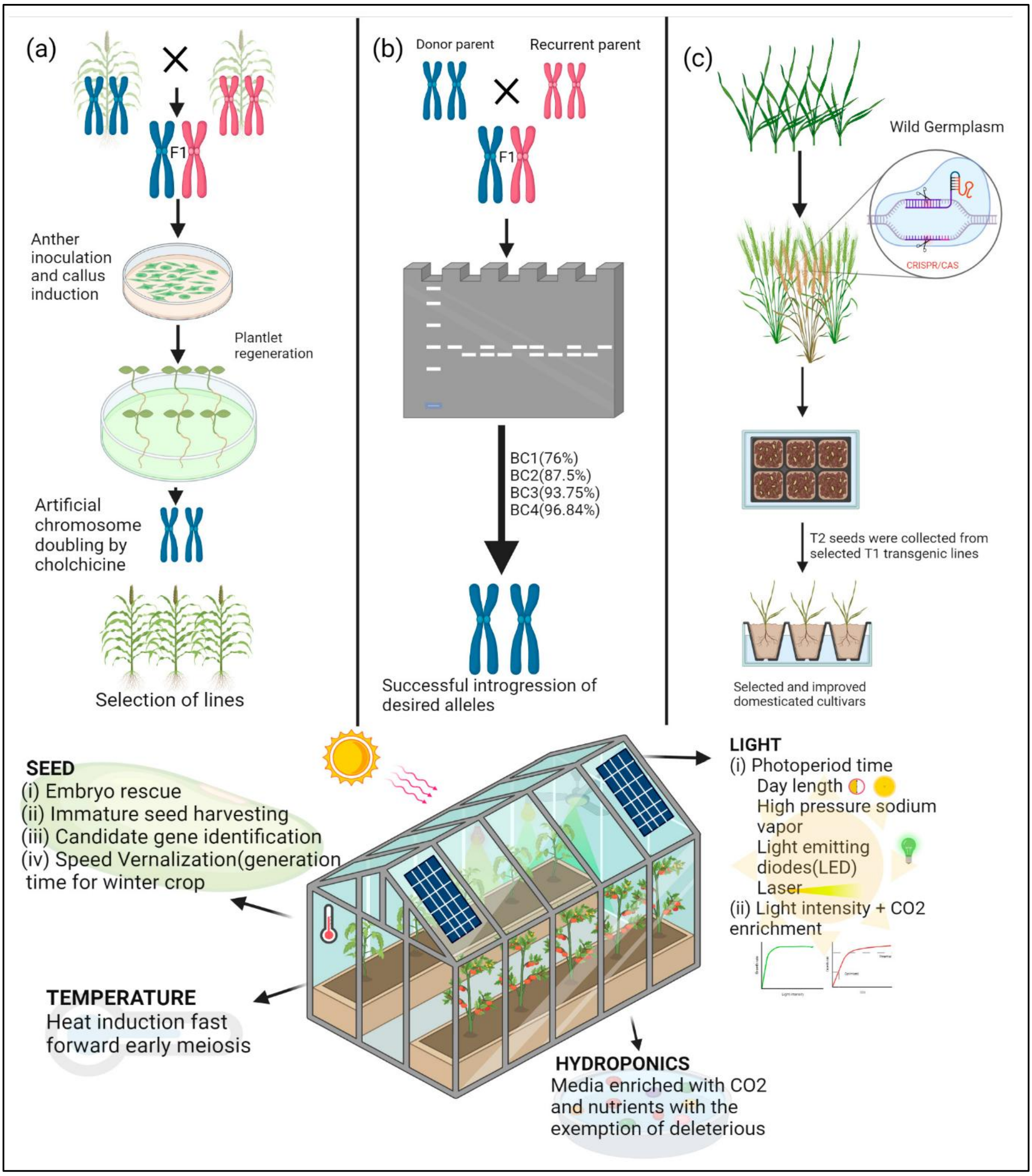

Fig. 1. Integration of SB with other breeding techniques accelerates the rate of progress. The homozygous lines for research and breeding purposes can be obtained expeditiously through hastening procedures aimed at (a) DH production, (b) marker-assisted selection, and (c) gene editing. Source: Chiurugwi et al. [10]

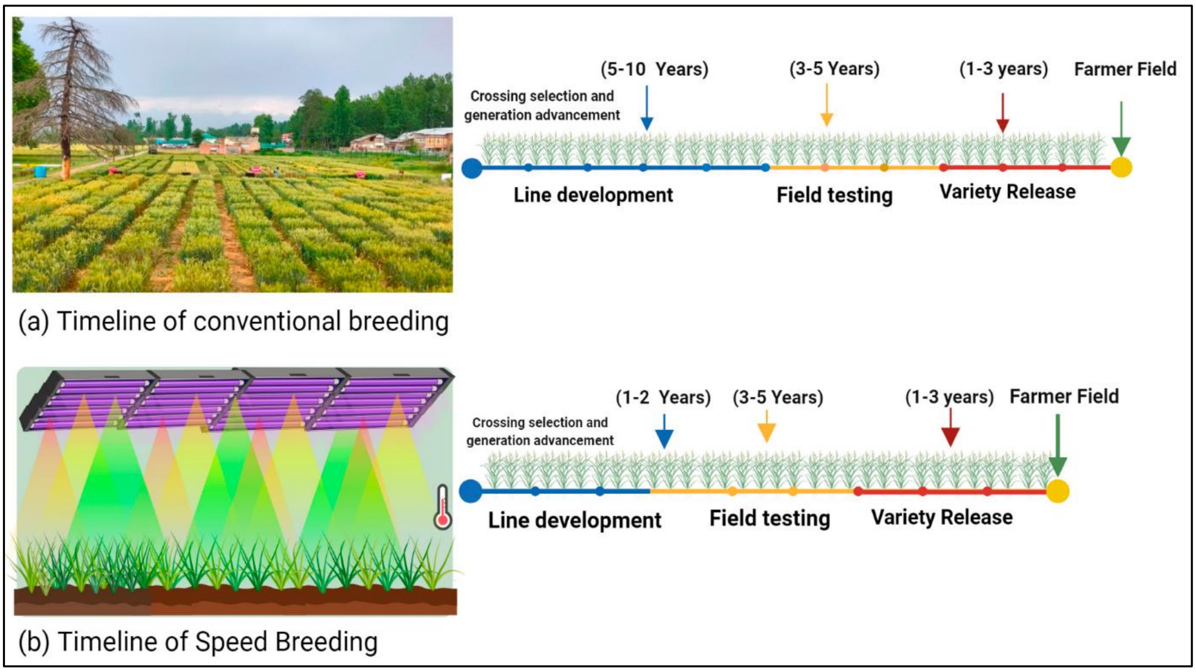

Fig. 2. Timelines of varietal development with (a) conventional breeding and (b) speed breeding. Source: Samantara et al. [8]

Additionally, Strategy 3 is also suitable for the rapid development of Recombinant Inbred Lines (RILs). RILs are valuable tools for genetic studies and molecular marker discovery, as they provide a stable and reproducible population with fixed genetic characteristics. By using speed breeding and SSD, breeders can efficiently generate a large number of RILs in a shorter period, facilitating genetic analyses and the identification of molecular markers associated with specific traits [36]. Overall, Strategy 3, combining speed breeding and SSD, offers an effective approach for accelerating the development of lines with desired traits and for creating populations such as RILs that are instrumental in genetic studies and molecular marker discovery.

The utilization of marker-assisted selection (MAS) is increasingly common in cereal plant breeding programs worldwide, effectively expediting the process of selecting desired traits [37]. However, a challenge arises in efficiently implementing MAS due to the collection of dependable phenotypic data for the vast amount of genomic data generated through next-generation sequencing (NGS) technologies [38]. The speed breeding system discussed in this context holds promise for the rapid development of recombinant inbred lines (RILs), which are crucial for the discovery of molecular markers.

3. METHODS USED IN SPEED BREEDING

Speed breeding methods refer to a set of techniques used to accelerate the generation turnover and shorten the breeding cycle of plants. These methods aim to expedite the process of developing new crop varieties by reducing the time it takes to obtain successive generations. Here are some commonly employed speed breeding methods illustrated in Fig. 3:

- ***Continuous Light:*** By providing plants with extended periods of light, typically 24 hours of continuous light, their growth and development can be accelerated. This method eliminates the need for a dark period and promotes rapid flowering and seed production.
- ***Controlled Environment Chambers:*** Plants are grown in controlled environment chambers, such as growth chambers or greenhouses, where environmental conditions, including temperature, humidity, and photoperiod, can be precisely regulated. This allows for optimal growth conditions and enables researchers to manipulate the environment to speed up plant development.
- ***Artificial Lighting:*** Supplemental lighting using high-intensity artificial light sources, such as LEDs, can enhance

photosynthesis and promote faster growth and development. By providing specific light spectra and intensities, researchers can tailor the lighting conditions to maximize plant productivity.

- ***Tissue Culture and Micropropagation:*** In vitro tissue culture techniques enable the rapid propagation of plants from small plant tissue samples. This method involves the use of growth regulators and sterile culture conditions to stimulate the development of plantlets, allowing for rapid multiplication and generation turnover.
- ***Early Generation Selection:*** Instead of waiting for multiple generations to select desired traits, early generation selection techniques expedite the process by identifying desirable traits in the early stages of plant development. This allows breeders to focus on promising individuals and accelerate the development of improved varieties.
- ***Marker-Assisted Selection (MAS):*** Molecular markers linked to specific traits of interest are used to identify and select plants with desired characteristics. This technique allows breeders to efficiently screen large populations and select plants carrying the desired genes, reducing the time required for traditional phenotypic selection [39,40].
- ***Genomic Selection:*** Genomic selection involves using high-throughput genotyping techniques and statistical models to predict the performance of plants based on their genomic information. This method enables breeders to select individuals with favorable genomic profiles, accelerating the breeding process [41,42].

4. MERITS OF SPEED BREEDING

Speed breeding methods are continuously evolving and being refined to expedite crop improvement programs. These techniques offer the potential to shorten the breeding cycle, rapidly introduce new genetic variation, and enhance the efficiency of plant breeding efforts.

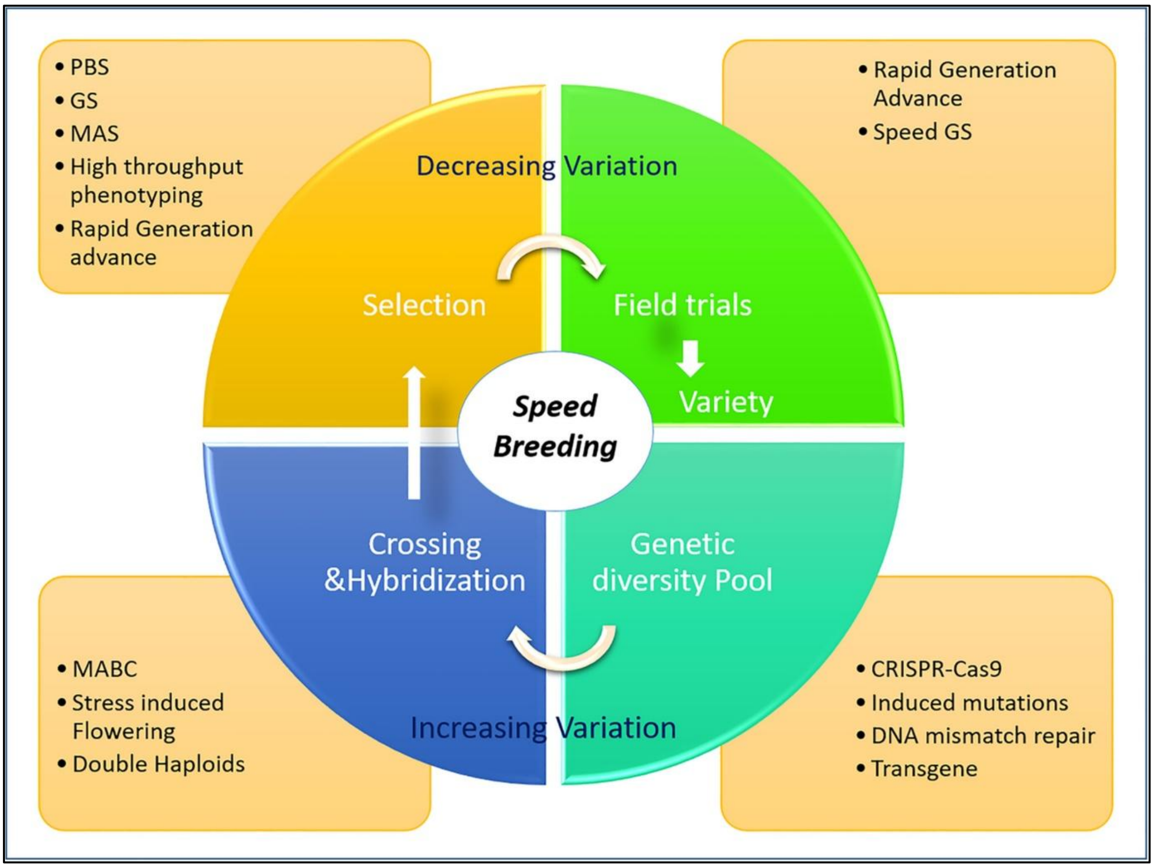

Fig. 3. Combining speed breeding with traditional and genomics-assisted breeding for crop improvement. Source: Pandey et al. [6]

Speed breeding in groundnut offers several merits that can significantly benefit crop improvement programs. Some of the key advantages include:

- ***Faster Generation Turnover:*** Speed breeding techniques allow for accelerated generation turnover in groundnut, reducing the time required to develop new varieties. By manipulating environmental conditions and growth factors, such as light, temperature, and photoperiod, the time taken to reach successive generations can be shortened, enabling breeders to make genetic progress at a faster pace.
- ***Rapid Trait Selection:*** With speed breeding, breeders can expedite the process of trait selection in groundnut. By implementing early generation selection techniques and marker-assisted selection (MAS), desirable traits can be identified and selected in the early stages of plant development. This speeds up the breeding process and enables breeders to focus on individuals with the desired traits.
- ***Increased Genetic Variation:*** Speed breeding methods can facilitate the introduction and incorporation of new genetic variation into groundnut breeding programs. By rapidly cycling through generations, breeders have more opportunities to create and select diverse populations, leading to increased genetic variation. This can enhance the potential for finding novel traits and improving crop performance.
- ***Optimal Environmental Control:*** Speed breeding techniques allow precise control over environmental conditions, providing optimal growth conditions for groundnut plants. By creating controlled environments, breeders can manipulate factors like temperature, humidity, and light intensity to maximize plant growth and development. This control enables year-round breeding activities and reduces the reliance on seasonal variations.
- ***Time and Cost Efficiency:*** Speed breeding methods can improve the efficiency of groundnut breeding programs, saving both time and costs. By reducing the generation turnover time, breeders can achieve their breeding objectives more quickly. This efficiency can translate into cost savings in terms of resources, labor, and infrastructure required for conventional breeding methods.
- ***Accelerated Crop Improvement:*** Overall, speed breeding in groundnut can expedite the crop improvement process. By combining the benefits of faster generation turnover, rapid trait selection, increased genetic variation, and controlled environmental conditions, breeders can accelerate the development of improved groundnut varieties. This can help address challenges such as disease resistance, yield improvement, and adaptation to changing environmental conditions [43-45].

It is important to note that the specific merits of speed breeding may vary depending on the specific goals, resources, and constraints of each breeding program. Additionally, the successful implementation of speed breeding requires careful experimental design, collaboration with stakeholders, and continuous optimization of techniques to maximize its benefits.

5. DEMERITS OF SPEED BREEDING

While speed breeding techniques offer numerous advantages, there are also some demerits or limitations to consider. Here are a few demerits of speed breeding:

Cost: Implementing speed breeding methods can be expensive, particularly when advanced technologies and specialized equipment are required. The costs associated with maintaining controlled environments, artificial lighting, and other necessary resources can be a significant barrier for smaller breeding programs or resource-limited settings.

- ***Genetic Constraints:*** Speed breeding techniques often prioritize the rapid development of desired traits and generation turnover. However, this emphasis on speed may limit the ability to capture the full genetic diversity present in a crop. It may result in reduced genetic variability, potentially leading to a narrower genetic base and increased vulnerability to pests, diseases, and environmental stresses [46].
- ***Environmental Adaptation:*** Speed breeding methods primarily focus on optimizing growth conditions in controlled environments. While this allows for accelerated plant growth, it may not fully capture the complexities of real-world field conditions. Traits related to environmental adaptation, such as drought tolerance or

heat resistance, may not be adequately assessed or selected for in speed breeding programs [46].

- ***Potential Trade-Offs:*** The emphasis on speed in breeding may lead to trade-offs between different desirable traits. For example, prioritizing rapid generation turnover and early selection may result in reduced selection pressure for complex traits or those that manifest later in the plant's life cycle. Balancing the need for speed with the comprehensive evaluation of multiple traits can be challenging [47].
- ***Limited Scale:*** Speed breeding techniques are often implemented on a smaller scale, typically in controlled environments such as growth chambers or greenhouses. This limits the number of plants that can be grown simultaneously and may not be suitable for large-scale breeding programs or the evaluation of genetic materials across diverse environments [48].
- ***Regulatory Considerations:*** The adoption of speed breeding methods may raise regulatory considerations, particularly when it comes to the release and commercialization of genetically modified organisms (GMOs) or plants developed using novel breeding techniques. Compliance with relevant regulations and public acceptance can pose challenges for the widespread adoption of speed breeding in some regions [49].

It is important to note that these demerits can vary depending on the specific crop, breeding objectives, available resources, and the implementation of speed breeding techniques.

6. FUTURE ASPECTS OF SPEED BREEDING

The future of speed breeding in groundnut holds promising potential for further advancements and applications. Here are some future aspects and areas of development in speed breeding for groundnut:

- ***Optimization of Protocols:*** Continued research efforts will focus on fine-tuning and optimizing speed breeding protocols specifically tailored for groundnut. This includes refining growth conditions, lighting regimes, nutrient management, and other factors to maximize the efficiency and effectiveness of the technique [50].
- ***Incorporation of Omics Technologies:*** Integration of omics technologies, such as genomics, transcriptomics, and metabolomics, can enhance speed breeding by providing a comprehensive understanding of the genetic and molecular basis of important traits in groundnut. This information can aid in the identification of key genes, markers, and pathways associated with desired agronomic traits [51].
- ***Trait-Specific Speed Breeding:*** Speed breeding can be tailored to focus on specific traits of interest in groundnut, such as disease resistance, drought tolerance, and high yield. By streamlining the breeding process and accelerating the development of targeted traits, speed breeding can contribute to the rapid release of improved groundnut varieties [39].
- ***Integration with Marker-Assisted Selection (MAS):*** The combination of speed breeding with marker-assisted selection techniques can expedite the identification and introgression of desirable genes or markers in groundnut breeding programs. This integration can enhance the efficiency and accuracy of trait selection and reduce the time required for breeding cycles [40].
- ***Incorporation of Climate Resilience:*** Groundnut faces challenges from climate change, including increased temperatures, drought, and changing pest and disease dynamics. Future developments in speed breeding may focus on incorporating traits for climate resilience, such as heat tolerance, water-use efficiency, and disease resistance, to develop groundnut varieties better suited to changing environmental conditions [52-53].
- ***Scaling Up and Collaboration:*** As speed-breeding techniques continue to mature, efforts will be made to scale up the technology to larger production systems, including field-scale trials. Collaboration between researchers, breeders, and industry stakeholders will be crucial to exchange knowledge, share resources, and standardize protocols, enabling the widespread adoption of speed breeding in groundnut improvement programs [54].
- ***Automation and Robotics:*** Automation and robotics technologies have the

potential to revolutionize speed breeding by increasing throughput and reducing labor-intensive tasks. Robotics-assisted plant phenotyping, automated imaging, and high-throughput data analysis can accelerate data collection, analysis, and decision-making in groundnut breeding [55].

The future of speed breeding in groundnut will involve the integration of cutting-edge technologies, improved understanding of the groundnut genome, and collaboration among researchers, breeders, and industry stakeholders [56]. These advancements will contribute to the development of high-performing groundnut varieties with improved traits, addressing the challenges faced by groundnut production and contributing to global food security [57].

7. CONCLUSION

The use of speed breeding techniques has emerged as a promising approach to accelerate the development of high-performing cultivars with desired traits in crop improvement programs. By significantly reducing the time, space, and resource requirements for selecting and advancing superior crop varieties, speed breeding enables plant breeders to deliver improved varieties more rapidly. Moreover, the integration of speed breeding with other breeding approaches, such as marker-assisted selection (MAS) and genetic engineering (GE), can further enhance the effectiveness of variety development. Speed breeding facilitates the rapid screening and selection of genotypes with desired traits, including higher yield, improved nutritional qualities, and tolerance to biotic and abiotic stresses. Several selection methods are compatible with speed breeding, including single seed descent (SSD), single plant descent (SPD), and single plant selection (SPS). These methods enable breeders to efficiently evaluate and advance promising lines through successive generations, accelerating the breeding cycle and genetic gain. The successful implementation of speed breeding requires the availability of skilled personnel and adequate facilities to support the technique. However, to fully realize the benefits of speed breeding, it is crucial to address challenges related to training, infrastructure, and government support, particularly in developing countries with limited resources for plant breeding programs.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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