Advances in Okra Breeding: Integrating Genomics for Enhanced Crop Improvement

Abstract

Okra (Abelmoschus esculentus L.) stands as a crucial vegetable crop, flourishing in tropical and subtropical regions worldwide, particularly prevalent in the warmer expanses of temperate zones. Traditionally, breeding methodologies tailored for self-pollinated crops have been employed to enhance okra's genetic makeup. These include techniques such as plant introduction, pure line selection, hybridization coupled with selection, mutation breeding and heterosis breeding. Notably, in regions like India, pedigree selection and heterosis breeding have yielded numerous varieties and hybrids, contributing significantly to the local agricultural landscape.In contemporary okra breeding, emphasis is placed on traits essential for optimal performance, including high yield potential, resistance to prevalent diseases such as Yellow Vein Mosaic Virus (YVMV) and Okra Enation Leaf Curl Virus (OELCV), tolerance to sucking pests and borers, ease of harvest, deep green fruit coloration, and desirable plant architecture. Public sector institutions have been pivotal in the development and dissemination of improved okra varieties and hybrids, with over 33 such cultivars introduced in recent decades. Some of these varieties have already showcased substantial enhancements in okra production across India.This review provides an overview of the current landscape of okra breeding, encompassing genetic resources, cytogenetic relationships, breeding objectives, varietal development processes, resistance breeding efforts, biotechnological interventions, and future improvement strategies. By synthesizing existing knowledge and highlighting ongoing research endeavors, this article aims to guide and inspire further advancements in okra breeding and genomics, ultimately contributing to the sustainability and productivity of this vital vegetable crop.

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Keywords:*hybridization, cytogenetic, resistance, genomics, mutation and heterosis breeding.*

Introduction

Okra (*Abelmoschus esculentus*L.) holds significant commercial importance as a summer vegetable cultivated primarily in tropical and subtropical regions worldwide. Commonly referred to as lady finger or Bhindi, it is a staple crop during the summer and rainy seasons, particularly prominent in India [1]. Belonging to the Malvaceae family, okra is distinguished by its vibrant and showy flowers. The bulk of okra cultivation, approximately 99%, is concentrated in developing nations across Asia and Africa [2].With an estimated global production of 9.96 million tons, India stands as the leading producer of okra, contributing over 72% to the global output. According to FAOSTAT 2020, India produced 6.35 million tonnes of okra from 0.52 million hectares of land. Notably, around 75% of the okra market is dominated by India, followed by Nigeria at 12% [3]. Okra is cultivated both as a rain-fed and irrigated crop and is highly valued and popular due to its versatility, being consumed in fresh, frozen, and dried forms [4].

In recent years, okra has garnered increasing recognition and acceptance as a global crop, attributed to the growing appreciation for its nutritional benefits among consumers. India's role in the global okra market is further underscored by its export of okra seeds to more than 20 countries worldwide [5]. This export activity signifies the expanding popularity of okra as a global commodity. Moreover, hybrid seeds, accounting for over 80% of the market share, predominantly cover the Indian market for okra seeds.Overall, okra's widespread cultivation, nutritional value, and growing global demand underscore its significance as a commercially important crop, particularly in countries like India where it holds a dominant position in both production and market presence [6].

In the case of okra, understanding the genetic variability is crucial for crop improvement efforts. Crosses between parents with maximum genetic divergence are often the most responsive for genetic improvement. This highlights the importance of genetic diversity in breeding programs to introduce novel traits and enhance desirable characteristics in okra varieties.Heritability estimates, along with genetic advance, provide valuable insights for plant breeders in selecting appropriate breeding methodologies [7]. High heritability and significant genetic advance for specific traits indicate that selection efforts are likely to be effective in improving those traits through breeding programs [8]. These parameters guide breeders in focusing their efforts on traits that have a strong genetic basis and are more likely to respond positively to selection.

Furthermore, variability parameters such as genetic diversity within germplasm collections serve as valuable resources for breeding programs. Germplasm collections offer a reservoir of genetic variability that can be tapped into to introduce novel traits and enhance the genetic base of cultivated okra varieties [9]. By selecting superior genotypes with desirable traits, breeders can develop improved varieties with enhanced yield, disease resistance, and nutritional quality [10]. Genetic variability parameters such as heritability and genetic advance are essential tools in okra breeding programs. They provide valuable insights into the genetic basis of traits, guide breeding methodologies, and facilitate the selection of superior genotypes for crop improvement efforts. By leveraging genetic variability and employing appropriate breeding strategies, breeders can develop okra varieties that meet the evolving needs of growers and consumers while ensuring the sustainability of okra cultivation [11].

5G breeding approachin crop improvement

In our proposed 5G breeding approach, we aim to bring about disruptive changes in crop improvement by leveraging advancements in genomics and biotechnology. The five key components of this approach are Genome assembly, Germplasm characterization, Gene function identification, Genomic breeding (GB), and Gene editing (GE) [12].

Genome assembly involves the sequencing and assembly of the entire genome of a crop species. This provides a comprehensive understanding of its genetic makeup and enables the identification of key genomic regions associated with important traits [13].Germplasm characterization involves the thorough characterization of a diverse collection of plant germplasm at both sequencing and agronomic levels. This allows for the identification of genetic variation within the germplasm pool and the discovery of marker-trait associations and superior haplotypes.Gene function identification focuses on identifying the functions of specific genes involved in pathways that lead to the expression of desired traits. By understanding the genetic basis of traits, diagnostic markers can be developed for targeted trait improvement [14].

Genomic breeding (GB) and gene editing (GE) methodologies utilize the genomic information obtained from genome assemblies, germplasm characterization, and gene function identification to enhance breeding efforts [15]. GB involves the utilization of genomic data for marker-assisted selection and genomic selection, enabling more precise and efficient breeding strategies. GE, on the other hand, allows for the targeted modification of specific genes to introduce desired traits or enhance existing ones [16].Combining these approaches with a rapid cycle breeding strategy accelerates the breeding process, allowing for the rapid development of improved crop varieties. By integrating genomics and biotechnology into breeding programs, we can revolutionize crop improvement efforts and address the challenges of food security and sustainability in the face of a changing climate and growing global population [17].

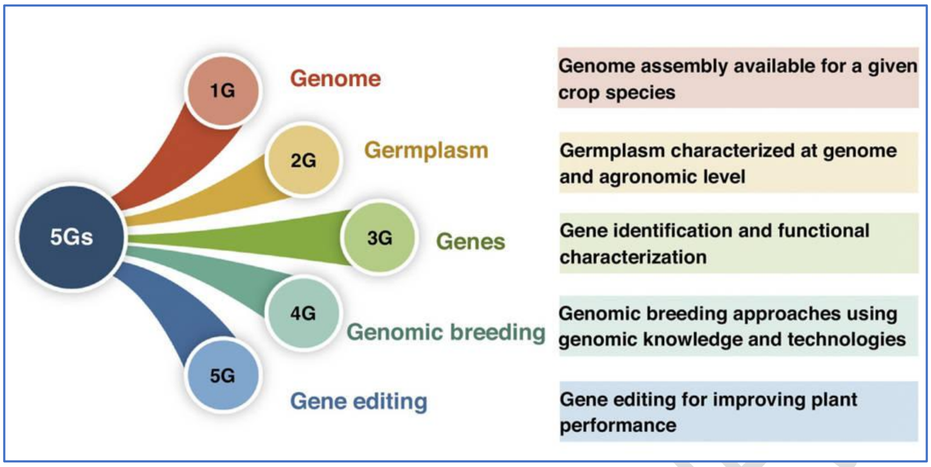

Figure 1: *The 5Gs of genomics are vital to accelerate crop breeding.* ***Source:****Varshney et al.* [12]

Traditional Breeding for Genetic Enhancement

Okra's flower structure predominantly supports self-pollination, yet there remains a potential for outcrossing up to 10-15%, rendering it an intermittently cross-pollinated crop [18]. Given its autogamous breeding system, conventional breeding methods commonly employed for self-pollinated crops are applicable for enhancing its genetic traits. These methodologies encompass plant introduction, pure line selection, intraspecific and interspecific hybridization utilizing backcross techniques, heterosis breeding, mutation, and polyploidy breeding.

For instance, varieties like Perkin's Long Green and Clemson Spineless Louisiana, initially developed in the USA, were introduced to India in the early 1950s for commercial cultivation through plant introduction [19]. Pure line selection led to the breeding of the first improved okra variety, Pusa Makhmali, sourced from germplasm material in West Bengal. Other cultivars such as CO-1 and Gujarat Bhinda 1 were also developed through pure line selection. Notably, the YVMV-tolerant cultivar 'Pusa Sawani' in India resulted from inter-varietal hybridization using the genotype IC-1542, followed by pedigree selection [20].

Furthermore, artificial mutagenesis utilizing physical mutagens (e.g., gamma rays) or chemical mutagens (e.g., EMS) has been instrumental in generating variability. For instance, the mutant EMS-8 (Punjab-8), resistant to YVMV and tolerant to fruit borer, was developed through induced mutation from Pusa Sawani treated with 1% EMS [21]. Additionally, significant yield improvements ranging from 50 to 70% have been reported in okra through heterosis exploitation. Heterosis breeding is extensively utilized not only for yield enhancement but also for sustainable protection against YVMV and OELCV diseases by incorporating resistant genes. Substantial heterosis has been observed for yield and its contributing traits [22].

Breeding Strategies for Viral Disease Resistance

The cultivation of okra faces various biotic stresses, among which viral diseases pose a significant threat, particularly in India. Yellow Vein Mosaic Virus (YVMV) and Okra Enation Leaf Curl Virus (OELCuV), transmitted through whiteflies, are particularly damaging. Yield losses caused by YVMV can range from 49 to 84% if the crop is infected within 50 to 65 days after sowing, while OELCuV can lead to 30 to 100% yield loss, depending on the plant's age at the time of infection [23].

Resistance breeding appears to be a viable solution, but the stability and durability of resistance attributes in the cultivated gene pool remain challenging. Resistance may break down after 5-8 years of development, rendering cultivars susceptible once again. Pusa Sawani, the first resistant variety against YVMV, is a prominent example, bred at IARI, New Delhi, using the genotype IC-1542 from West Bengal [24]. The resistance in this variety is attributed to two recessive alleles at two loci. To introgress resistance genes into cultivated varieties, Crop Wild Relatives (CWRs) such as Abelmoschus manihot ssp. manihot, Abelmoschus caillei, and A. tetraphyllus have been utilized, despite challenges like unilateral incompatibility and sterility problems in interspecific hybrids. Notable okra varieties like Parbhani Kranti, Arka Anamika, Punjab Padmini, and Punjab-7 have resulted from such breeding programs [21, 25].

For OELCuV, plant breeders have looked beyond the cultivated gene pool to wild relatives like A. crinitus, A. ficulneus, A. angulosus, and A. manihot for stable resistance sources. The mode of inheritance of resistance genes plays a crucial role in determining durability and stability against pathogens. YVMV resistance, for instance, has been described with various inheritance patterns ranging from monogenic to polygenic control. Some studies suggest two dominant complementary genes, while others indicate a single dominant gene or additive gene action. Similarly, OELCV resistance involves duplicate recessive genes and both additive and non-additive effects. Given the complexity of viral resistance, a comprehensive approach involving transcriptomics, proteomics, and metabolomics is necessary for thorough understanding and effective breeding strategies [26-27].

Enhancing Okra through Biotechnological Innovations

Despite being a nutritionally rich crop, okra has not extensively benefited from modern biotechnological advancements for genetic enhancement due to a lack of sufficient molecular information and tools [22]. Facing challenges from pests, diseases, and environmental stressors, okra's yield and quality are often compromised. High-throughput biotechnological methods offer swift solutions for okra improvement, including chromosomal engineering, RNA interference (RNAi), genome-wide selection (GWS), targeted gene replacement, next-generation sequencing (NGS), and nano-biotechnology [24]. These technologies provide avenues for precise genetic manipulation to overcome existing challenges.

Marker-assisted breeding emerges as a powerful tool in accelerating the development of improved okra varieties. By identifying molecular markers linked to specific genes associated with desirable traits, breeders can efficiently select plants with enhanced resistance to diseases like Fusarium wilt, Okra Enation Leaf Curl Virus (OELCV), and Yellow Vein Mosaic Virus (YVMV) [24, 25, 28]. This approach expedites the breeding process, reducing time and resources required for developing disease-resistant okra varieties. Additionally, genetic engineering has been instrumental in enhancing okra's nutritional profile. Efforts to elevate levels of essential nutrients such as vitamin A and iron have addressed prevalent nutritional deficiencies in regions where okra is a staple food [29].

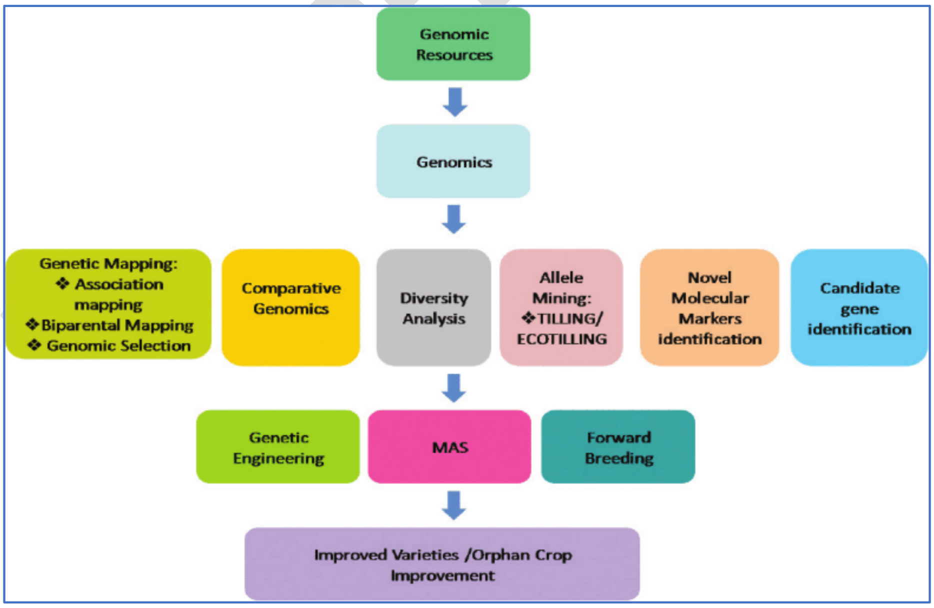

Figure 2:*Workflow of crop improvement through genomics*

Integrating nonconventional breeding methods with biotechnological techniques offers a comprehensive approach to combat the incidence of YVMV and OELCV in cultivated okra lines [21]. To maximize the impact of biotechnology, it is crucial to ensure its proper incorporation and utilization at the grassroots level. This concerted effort promises to bolster okra resilience, improve its nutritional value, and ultimately enhance food security and livelihoods.

Advancing Okra Improvement with CRISPR/Cas9 Technology

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated protein 9 (Cas9) technologies have ushered in a new era in genetic engineering, offering a precise and efficient tool for targeted gene editing [30]. In the realm of okra improvement, CRISPR/Cas9 technology has been harnessed to introduce specific genetic modifications with unprecedented accuracy. Researchers have successfully employed CRISPR/Cas9 to develop okra varieties with enhanced traits, including heightened resistance to specific pathogens or improved drought tolerance. The unparalleled precision of CRISPR/Cas9 enables the modification of individual genes without the introduction of foreign DNA, mitigating concerns associated with genetically modified organisms (GMOs) [31].

CRISPR/Cas9 technology unlocks novel avenues for tailoring okra varieties to suit diverse environmental conditions, addressing specific needs and challenges encountered in cultivation. With its ability to precisely edit the okra genome, CRISPR/Cas9 stands as a powerful tool in advancing the genetic enhancement of this valuable crop [32].

Genetic Variability in Okra

Genetic variability, the cornerstone of evolution and adaptation, plays a pivotal role in shaping the characteristics and resilience of plant species. Among these, okra (*Abelmoschus esculentus*) stands as a prime example of botanical diversity, harbouring a rich genetic reservoir that offers a spectrum of traits crucial for its survival and utility in agriculture [33]. In this discourse, we delve into the intricate tapestry of genetic variability within okra, exploring its sources, implications, and avenues for utilization in breeding programs.

Genetic variability encompasses the range of genetic differences that exist within a population or species. It is the raw material upon which natural selection acts, enabling organisms to adapt to changing environments and ensuring their long-term survival. In plants like okra, genetic variability manifests in diverse morphological, physiological, and biochemical traits, influencing traits such as yield potential, disease resistance, and nutritional content [34].

Sources of Genetic Variability in Okra

1. Wild Relatives: Wild relatives of okra, such as Abelmoschus tuberculatus and Abelmoschus manihot, serve as valuable reservoirs of genetic diversity. These species often exhibit traits not present in cultivated varieties, such as drought tolerance or resistance to specific pests and diseases. By introgressing genes from wild relatives into cultivated okra, breeders can enhance the crop's resilience and adaptability.

2. Landraces and Traditional Varieties: Indigenous landraces and traditional varieties of okra, cultivated by farmers over generations, harbor unique genetic traits honed by natural and human selection. These varieties often exhibit adaptability to local environmental conditions and may possess valuable traits for breeding programs, such as heat tolerance or flavor profiles suited to regional cuisines.

3. Genetic Mutations: Spontaneous genetic mutations can arise within okra populations, leading to the emergence of novel traits or variations in existing ones. While some mutations may be detrimental, others can confer beneficial characteristics, such as disease resistance or improved yield potential. Harnessing these naturally occurring mutations through selective breeding can accelerate the development of improved okra varieties.

4. Genetic Recombination: Sexual reproduction in okra facilitates genetic recombination, creating new combinations of alleles and contributing to genetic diversity within populations. Cross-pollination between different okra varieties or accessions allows for the exchange of genetic material, leading to the generation of offspring with unique genetic profiles.

Implications of Genetic Variability

The genetic variability present within okra populations has profound implications for both natural ecosystems and agricultural systems.

1. Adaptation to Environmental Stress: Okra's genetic diversity enables the species to adapt to diverse environmental conditions, ranging from arid regions to humid tropical climates. Varieties with traits such as drought tolerance or resistance to specific pathogens can thrive in challenging environments, contributing to food security and agricultural sustainability.

2. Resilience to Pests and Diseases: Genetic variability provides a defense mechanism against pests and diseases that threaten okra cultivation. Varieties with inherent resistance or tolerance to common pests and pathogens can reduce the reliance on chemical pesticides, promoting environmentally friendly farming practices and reducing production costs.

3. Crop Improvement and Breeding: The wealth of genetic variability within okra populations serves as a valuable resource for breeding programs aimed at developing improved varieties. By systematically evaluating and harnessing genetic diversity, breeders can introgress desirable traits into elite cultivars, enhancing their performance, nutritional quality, and marketability.

4. Conservation of Genetic Resources: Preserving the genetic variability present in okra germplasm collections is essential for safeguarding against future challenges, such as climate change or emerging pests and diseases. Conservation efforts, including the establishment of gene banks and ex-situ conservation initiatives, help maintain the genetic diversity needed for future breeding endeavors.

Utilizing Genetic Variability in Okra Breeding

Breeding programs aimed at enhancing okra's genetic potential rely on the systematic exploration and utilization of genetic variability. Several strategies are employed to leverage this diversity and develop improved varieties tailored to specific needs and preferences.

1. Genetic Screening and Characterization: Comprehensive genetic screening and characterization of okra germplasm collections are essential for identifying valuable traits and genetic resources. Molecular markers, such as SSRs (Simple Sequence Repeats) or SNPs (Single Nucleotide Polymorphisms), facilitate the assessment of genetic diversity and the identification of markers linked to target traits.

2. Hybridization and Crossbreeding: Controlled hybridization and crossbreeding between diverse okra accessions, landraces, and wild relatives enable the introgression of desirable traits into elite breeding lines. By combining complementary genetic backgrounds, breeders can generate offspring with improved vigor, yield potential, and stress tolerance.

3. Marker-Assisted Selection (MAS): MAS allows breeders to expedite the development of improved okra varieties by selecting plants with desired traits at the molecular level. By using molecular markers linked to target genes or traits, breeders can identify promising candidates for further evaluation and breeding advancement.

4. Genome Editing Technologies: Emerging genome editing technologies, such as CRISPR/Cas9, offer unprecedented precision and efficiency in targeted gene modification. By precisely editing specific genes associated with desirable traits, researchers can accelerate the development of improved okra varieties with enhanced resilience, productivity, and nutritional quality.

Understanding Heritability and Genetic Advance in Okra Breeding

In the realm of plant breeding, the concepts of heritability and genetic advance are instrumental in evaluating the genetic basis of traits and guiding breeding programs towards the development of improved varieties. When applied to okra (*Abelmoschus esculentus*), these concepts offer valuable insights into the inheritance patterns and potential for genetic improvement within the species.

Heritability

Heritability is a measure of the proportion of phenotypic variation in a trait that can be attributed to genetic factors. It provides an estimate of the degree to which offspring resemble their parents for a particular trait. In okra breeding, heritability plays a crucial role in determining the efficacy of selection for desired traits. Genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) are measures used to assess the extent of genetic and environmental variability, respectively, within a population [35]. GCV represents the variation among individuals due to genetic factors, while PCV encompasses both genetic and environmental influences [36].

Genetic Advance

Genetic advance is a measure of the potential improvement in a trait that can be achieved through selection. It quantifies the expected increase in mean performance resulting from selecting the top-performing individuals within a population. Genetic advance is influenced by both the heritability of the trait and the selection intensity applied.

In okra breeding programs, heritability, GCV, PCV, and genetic advance are utilized to assess the genetic basis of important traits and prioritize breeding objectives. For example, suppose a breeding program aims to improve okra yield. By conducting field trials and phenotypic evaluations across different okra genotypes, breeders can estimate the PCV and GCV for yield-related traits, such as fruit weight or number of fruits per plant [41]. High PCV and GCV values indicate substantial variability within the population, suggesting a strong genetic component for these traits [35]. Furthermore, by calculating the heritability of yield-related traits, breeders can determine the proportion of phenotypic variation attributable to genetic factors. High heritability values indicate that selection based on these traits is likely to be effective in improving yield in subsequent generations [40].

Using the estimated heritability and genetic advance, breeders can design selection strategies to target individuals with superior performance for yield-related traits. By selecting and crossing individuals with the highest estimated breeding values (EBVs) for these traits, breeders can accelerate genetic gain and develop improved okra varieties with enhanced yield potential. Heritability, GCV, PCV, and genetic advance are indispensable tools in okra breeding, providing valuable insights into the genetic basis of traits and guiding selection decisions [35-36]. By leveraging these concepts effectively, breeders can accelerate the development of improved okra varieties tailored to meet the evolving needs of growers and consumers alike.

Unlocking Potential: Heterosis Breeding in Okra

Heterosis, commonly known as hybrid vigor, is a phenomenon observed when offspring resulting from crosses between genetically diverse parents display superior traits compared to either parent. In okra breeding, heterosis offers promising opportunities to enhance yield, quality, and resilience [37]. Through the combination of favorable alleles from genetically distinct parental lines, heterosis can lead to significant improvements in yield potential, disease resistance, and quality traits [38-39]. The success of heterosis breeding in okra hinges on careful selection of genetically diverse parental lines, followed by controlled crosses to produce hybrid progeny with heterotic vigour. These hybrids are subjected to rigorous field evaluations to assess performance across different environments, with superior hybrids selected for further advancement and commercialization. While heterosis breeding holds immense promise for okra improvement, challenges such as genetic erosion and market acceptance must be addressed to ensure long-term breeding sustainability and widespread adoption of heterosis-based varieties.

Overall, heterosis breeding represents a powerful approach to unlock the genetic potential of okra, offering opportunities for sustainable production and food security in the face of global challenges. Heterosis breeding represents a powerful approach to unlock the genetic potential of okra, offering opportunities to enhance yield, quality [39]. By leveraging the principles of heterosis, breeders can develop high-performing hybrids tailored to meet the evolving needs of growers, consumers, and agricultural systems. Through strategic selection, hybridization, and commercialization efforts, heterosis breeding holds promise for sustainable okra production and food security in the face of global challenges.

Mutation breeding in okra

Mutation breeding in okra involves deliberately inducing genetic mutations in the plant's DNA to create new variations or traits that may be beneficial for cultivation. This method is aimed at enhancing traits such as yield, disease resistance, and nutritional content. In okra, mutation breeding typically begins with exposing seeds or plant tissues to mutagenic agents like radiation or chemicals [42]. These agents cause random changes in the DNA sequence, leading to the creation of mutant plants with altered characteristics. After mutagenesis, the mutant plants are carefully screened to identify individuals exhibiting desirable traits. These traits may include increased yield, improved fruit quality, or resistance to pests and diseases. Selected mutants are then further evaluated through field trials and other phenotypic assessments to confirm their performance and stability. Successful mutants with desirable traits can be incorporated into breeding programs to develop new okra varieties that offer improved performance and resilience [43]. Despite its potential benefits, mutation breeding in okra also comes with challenges, such as the need for extensive screening and the unpredictable nature of induced mutations [44]. However, it remains a valuable tool in the genetic improvement of okra, offering opportunities to develop varieties that meet the evolving needs of growers and consumers [45].

Challenges and Future Directions

While the genetic variability within okra holds immense potential for crop improvement, several challenges must be addressed to fully harness this diversity.

1. Genetic Erosion: The loss of traditional landraces and heirloom varieties threatens to diminish the genetic diversity within okra populations. Efforts to conserve and promote the cultivation of diverse okra varieties are essential for safeguarding against genetic erosion and preserving valuable genetic resources.

2. Limited Genomic Resources: Despite recent advancements, the availability of genomic resources and molecular tools for okra breeding remains limited. Continued investment in genomic research and the development of genomic resources, such as reference genomes and high-density genetic maps, will facilitate more efficient breeding efforts.

3. Regulatory and Ethical Considerations: The adoption of genome editing technologies, such as CRISPR/Cas9, in okra breeding raises regulatory and ethical considerations regarding the safety, environmental impact, and consumer acceptance of genetically modified crops. Transparent communication, stakeholder engagement, and adherence to regulatory guidelines are essential for responsible innovation in genetic engineering.

4. Climate Change Resilience: As climate change poses increasing challenges to agricultural production, breeding resilient okra varieties capable of withstanding extreme weather events, water scarcity, and emerging pests and diseases becomes paramount. Integrating climate-resilient traits into breeding programs will be crucial for ensuring the long-term sustainability of okra cultivation.

Conclusion

Genetic variability within okra represents a vast and untapped resource for crop improvement and adaptation. The diverse genetic makeup of okra offers numerous opportunities for breeders to develop varieties with enhanced resilience, productivity, and nutritional quality. By systematically exploring and harnessing this genetic diversity, breeders can address the evolving challenges faced by okra cultivation, including climate change, pests, and diseases.Over the course of our discussions, we have explored various aspects of okra breeding and genomics, highlighting the importance of genetic variability in driving crop improvement efforts. Through traditional breeding methods such as heterosis breeding, marker-assisted selection, and mutation breeding, as well as cutting-edge biotechnological interventions like CRISPR/Cas9 technology, breeders can introduce novel traits and enhance the performance of okra varieties.Additionally, the global significance of okra as a commercially important crop cannot be overstated. As we look towards the future, it is imperative that we continue to invest in research and development efforts aimed at unlocking the full potential of okra. By leveraging genetic variability, genomic research, and biotechnological innovation, we can ensure the continued success and sustainability of okra cultivation, meeting the needs of growers, consumers, and the environment alike. With strategic breeding efforts and collaborative partnerships, we can chart a course towards a brighter and more resilient future for okra agriculture.

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