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RESEARCH ARTICLE

Study of Okra (Abelmoschus esculentus) Breeding with Cytogenetics, Pan-Genomic Diversity, and Molecular Tools

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ABSTRACT

Okra is one of the nutritious and economically important vegetables found in tropical and subtropical areas. Asia and Africa are the largest producers of okra. Okra has the potential to combat food insecurity, malnutrition, and low-income obstacles, specifically in developing countries. Despite many benefits, it also comes across barriers, such as a narrow genetic basis, polyploid complexity, and stress in the midst of climate change, which lead to shifts in weather patterns. Traditional breeding, pedigree selection, mutation breeding, and heterosis delivered significant results. Now modern technologies, such as genome editing, pangenome, and CRISPR Cas9, give efficient results, and reduce the time. Researchers can also utilize advanced technology to explore the potential of non-model crops, such as okra. It also possesses properties like bioactive compounds, mucilage, and seed proteins. Limitations are the genomic database, interspecific incompatibility, and poor use of wild germplasm. Future improvement will require integrated approaches that combine genomics, biotechnology, and conventional breeding to develop climate-resilient and nutritionally enhanced cultivars, ensuring okra's role in sustainable agriculture and global food security.

Key words: Okra, Genetic diversity, pangenome, Disease Resistance, Molecular breeding CRISPR/Cas, Cytogenetics, and Climate resilience.

INTRODUCTION

Okra (*Abelmoschus esculentus*) is a vital summer vegetable that is extensively cultivated in tropical and subtropical regions worldwide. Globally, okra is cultivated in regions across Asia, Africa, and some parts of America. It belongs to the hibiscus family, *Malvaceae*. Okra is distinguished by its striking and showy flowers. Remarkably, 95% of okra cultivation takes place in developing countries across Asia and Africa, emphasizing its significance in these regions.(Singh et al., 2024) The lifespan of a plant varies by cultivar, lasting between 60 and 120 days, with seeds as its primary method of propagation. It can grow up to 4 meters tall, featuring a sturdy, erect stem with varying degrees of branching. The leaves are palmately five-lobed and alternate along the stem. Single blooms emerge from the axils of these leaves, as the Fig. 2.

In 2023, global okra production exceeded 11 million tons, with significant contributions from countries like India, Nigeria, and other countries that include Mali, Sudan, and Pakistan (FAOSTAT, 2023). Okra is widely recognized as an important component of everyday diets in many parts of the world (Moench, 2025).Its value extends beyond the kitchen, as it supplies essential vitamins and minerals that support overall health. Regular consumption of okra is associated with improved digestive function and a lower likelihood of developing chronic diseases.(Wato et al., 2024).

Okra is thought to have originated and been domesticated in northeastern Africa, with present-day Ethiopia and Sudan considered the primary centers, as *Abelmoschus ficulneus* and *Abelmoschus esculentus* are the wild relatives that validate the theory of African origin. The Egyptian origin of okra is considered as early as 2000 BCE, when people used its immature green pods as food, and the mucilage content due to its nutritional value.(Swamy, 2023).

Other researchers claim that okra also originated from northeast Africa and, gradually, by using the trade routes via the Red Sea in the Arabian Peninsula, prior to the Indian subcontinent, okra was introduced in the

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subcontinent, and cultivated successfully because the conditions were feasible for the crop. Okra started to be used as a food and agricultural cop. It was restricted to European regions of temperate areas due to climatic conditions, but some Arab travelers introduced it into the Mediterranean Sea. In America, it did not directly originate; rather, some Africans who used to be slaves came up with this crop as an agricultural vegetable in the 17th century in the southern United States and the Caribbean Sea.(Sousa & Raizada, 2020) .

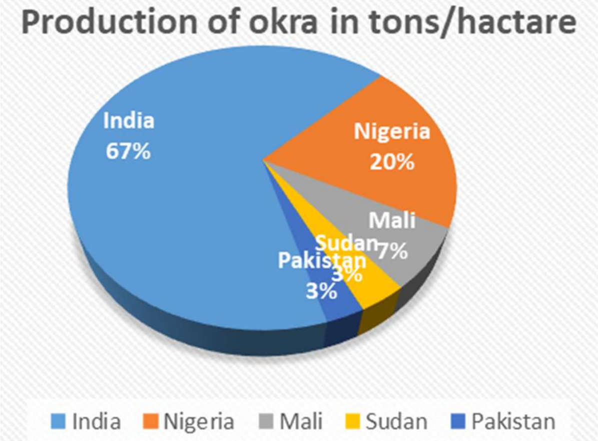

Fig. 1: Top five okra-producing countries.

Pakistan is fifth in global okra production with a 2.62% share. Okra is grown on 15.7 thousand hectares in Pakistan, yielding 7.6 tons per hectare. The highest yield of 11.8 tons/ha from an area of 5.9 thousand hectares is recorded in Punjab, followed by Sindh with yield of 3.9 tons/ha over an area of 4.9 thousand hectares. Okra production in KPK and Baluchistan is lower than in Sindh and Punjab. Okra is grown on 2.36 thousand hectares in KPK, yielding 6.4 tons per hectare. In Baluchistan, the average yield is 6.19 tons per hectare from 2.4 thousand hectares. (MNFSR 2018-2019).

Botanical and Cytogenetic Overview of *Abelmoschus esculentus*

The genus Abelmoschus was previously classified within Hibiscus. However, due to distinct morphological and cytological differences, it is now recognized as a separate genus within the family Malvaceae and the tribe Hibiscus (Abdullah Yousuf Akhond et al., 2000; Aflitos et al., 2014)). This genus comprises about 11 to 15 species, including both wild and cultivated types, many of which remain underutilized despite their valuable traits for stress tolerance and disease resistance.

Okra belongs to the family Malvaceae, which also includes economically and ecologically important plants such as cocoa (*Theobroma cacao*), cotton (*Gossypium hirsutum*), lime trees (*Tilia* spp.), mangroves of the genus *Heritiera*, and durian (*Durio zibethinus*). Okra flowers continuously and is primarily self-compatible, although studies have reported cross-pollination levels of up to about 20%. The flowers are typically hermaphroditic, with white to pale yellow perianths composed of five petals and five sepals. At the base of the flower, the calyx, corolla, and stamens are fused, which is a characteristic feature of the species (Nieuwenhuis et al., 2021).

Fig 2: Okra flower with green pods (fruits).

Basic Cytology: Chromosome Number and Polyploidy

Chromosome counts in okra have shown significant variation, ranging from 2n = 72 to 144, with 2n = 130 being the most frequently observed count in various studies(Aflitos et al., 2014; R. Nieuwenhuis et al., 2024). A high-quality genome assembly reveals the presence of 65 basic chromosomes (n = 65 in the diploid complement) in the haploid form, suggesting the existence of two sub genomes, each containing approximately 30 and 35 chromosomes. This observation supports the allopolyploid origin of okra.(R. Nieuwenhuis et al., 2024).

Estimates of genome size based on flow cytometry range from approximately 3.98 pg to 17.67 pg (2C), which indicates considerable cytological plasticity among different genotypes. The plant shows a tolerance for polyploidy, demonstrating its flexibility in the addition or loss of chromosomes.(Swamy, 2023) Conventional breeding approaches such as pedigree selection, mutation breeding, and the use of heterosis have contributed to steady progress in crop improvement. Yet, these strategies are often time-consuming and insufficient when it comes to tackling the rapidly evolving stresses faced by crops. The challenge is even greater in okra, given its highly complex and polyploid genome (2n = 130), which complicates the efficient transfer and stabilization of desirable traits.(Aflitos et al., 2014).

Recent advancements in genomics, including chromosome-scale assemblies of the okra genome (approximately 1.19 Gbp) and high-quality diversity panels, now provide a solid foundation for accelerating breeding efforts.(Wang et al., 2023) Advanced breeding tools, including the creation of high-quality pan-genomes and the use of genomic selection, can greatly accelerate the identification and implementation of beneficial alleles for traits such as drought tolerance, virus resistance, and yield stability. Techniques like speed breeding and genome editing, such as CRISPR/Cas, further enhance this process (Razzaq et al., 2021).

Pangenomics, which encompasses the entire genetic report of a species by integrating both core and variable genes, provides an effective framework for studying structural variations, presence–absence variants (PAVs), and rare alleles related to stress tolerance and agronomic performance. Although pangenomic research has already transformed genomic studies in humans and model crops, its use in okra remains limited. Exploring this diversity in okra could open new avenues for identifying adaptive traits and guiding the development of climate-resilient cultivars (Liu et al., 2024).

Interspecific Compatibility and Wild Relatives

Wild species within the genus Abelmoschus, such as *A. manihot*, *A. caillei*, *A. angulosus*, and *A. tetraphyllus*, exhibit valuable traits that contribute to disease resistance (e.g., resistance to YVMV), drought tolerance, and enhanced nutritional qualities. However, there are several barriers to crossing these species, including mismatched ploidy levels, pollen sterility, and reduced seed set in interspecific hybrids.

Despite these challenges, successful directed introgression techniques, such as embryo rescue and the use of "bridge" interspecific hybrids, have been employed. Nevertheless, the development of commercial varieties utilizing wild germplasm remains limited.(Sandeep et al., 2022).

Limitations Due to Narrow Genetic Base

Despite having a high chromosome number, okra exhibits low nucleotide diversity, with approximately one single-nucleotide polymorphism (SNP) for every 2.1 kb. This limited diversity restricts the potential for molecular breeding and the utilization of heterosis. Genetic studies have reported a lack of available markers, such as RAPD, AFLP, and SSR, as well as difficulties in extracting DNA and RNA due to the presence of mucilage and polyphenolics. These factors hinder high-resolution genetic mapping. Most cultivated germplasm shows low diversity, which highlights the need to incorporate diverse wild accessions and landraces into breeding programs to expand genetic variation.(Ronald Nieuwenhuis et al., 2024).

Genetic Architecture and Trait Inheritance in Okra

Breeding techniques that depend on efficient selection and hybridization are primarily used to improve okra crops. It possesses several positive attributes, including its shorter lifespan, photo insensitivity, and ability to adapt to various soil and climate conditions. As a result, breeders can quickly analyze genetic data through crop cultivation several times a year. Okra's hybrid vigor can be commercially exploited due to its high fruit set percentage and ease of heterosis in okra. Genetic research is necessary to measure the genetic influences governing significant traits (Vinay et al., 2021).

Okra breeding primarily focuses on increasing yield and enhancing resistance to yellow vein mosaic virus (YVMV) and enation leaf curl virus (ELCV). Yield is determined by multiple quantitatively inherited traits, which depend on the type and extent of genetic variation. Analyzing these variations clarifies the genetic basis of key traits. Understanding genetic diversity within a population enables breeders to select effective breeding strategies for yield improvement. Most genetic models applied are additive or additive-dominance. Researchers commonly use line x tester analysis or diallel methods to investigate the genetics of growth and yield traits in okra (Arora, 1993).

Combining ability and heterosis analysis, the generation mean analysis was used to test the required results. Okra is a heterogeneous crop that yields good hybrid vigor, with offspring outperforming their parents in terms of yield and other contributing traits. Conversely, yield is a quantitative polygenic trait that exhibits significant environmental influences. By expressing distinct molecular and biochemical genes, hybrids respond differently to different environments due to differences in the micro and macroenvironments. Furthermore, "changing climate," which has reduced the potential of hybrids, is the core issue. Plant breeders' next objective would be to develop climate-resilient hybrids that would yield reliably on marginal and cultivable land. To identify stable hybrids, one must understand the gene-environment interaction. (Patel et al., 2023).

In okra, dominance (non-additive) gene action, rather than pure additive effects, governs the majority of fruit-yield traits. Breeders have intentionally used hybridization to increase pod yield and introduce virus-resistance genes (against YVMV and OELCV) into hybrids, which is consistent with the fact that exploitation of heterosis has produced huge yield boosts, typically 50–70% over mid-parent levels. This heterotic approach has made a significant number of commercial okra hybrids, according to the review; in India alone, 33 improved varieties/hybrids with high yield and multi-trait resistance have been released.(Singh et al., 2023).

Fig. 3: Role of genetic resources and climate resilience in okra breeding.

Genomic Resources and Current Advances

Several studies have reported on the use of RAPD (Random Amplified Polymorphic DNA), AFLP (Amplified Fragment Length Polymorphism), and SSR (Simple Sequence Repeat) markers to study genetic diversity in okra (lata et al., 2021). linkage maps, and reference genome, this has further hindered genome and transcriptome studies, which further hinders advanced breeding. The presence of significant levels of polyphenolic and mucilaginous compounds in various tissues has also complicated molecular studies and interfered with the preparation of genetic materials (lata et al., 2021). Furthermore, because of the highly polyploid nature of the genome and the anticipated large size of the transcriptome and genome, proper de novo assembly is assumed to be complex.

When genome sequencing is not available for non-model crops like okra, RNA sequencing (RNA-Seq) is a helpful technique for creating SSR markers(Strickler et al., 2012). Only restricted There are reports of transcriptomic and genomic investigations, and not many EST-SSRs have been created for okra (Schafleitner et al., 2013) documented the first transcriptome assembly in okra, wherein 161 polymorphic SSR markers were created, 19 markers were used for diversity analysis, and 935 non-redundant SSR motifs were found from unigenes data. In their initial report on the creation of genomic SSR markers in okra, (Ravishankar et al., 2018) used 50 randomly chosen SSR primers to amplify okra DNA from 2708 contigs that contained microsatellites.

A study conducted by (El-Afifi et al., 2018) in which transcriptome sequencing data to develop SSR molecular markers related to fruit color in A. esculentus. Furthermore, the SSR properties of genes linked to fruit color were examined. Using the unigenes derived from the transcriptome sequencing data of A. esculentus, we conducted an SSR analysis. An earlier study examined the genetic variation among six okra varieties using SSR, ISSR, and SRAP markers. According to the findings of the sequence enrichment analysis, the unigenes that contain SSRs may primarily be linked to signaling pathways in plants. The EST-SSR primers created in this work may therefore mainly anneal to genes linked to plant signaling. It is possible to evaluate the genetic diversity of an A. esculentus population and the variation in particular genes and associated characteristics among A. esculentus germplasm resources using the EST-SSR markers.

Pangenomics and Germplasm Diversity

The pangenome of okra is not fully established, but assemblies are present. The large size of the okra genome, polyploidy, and high repetitive content make it challenging to assemble; however, several draft assemblies have now established a strong basis for functional genomics. For example, (Ronald Nieuwenhuis et al., 2024) provided an even more refined assembly anchored by HiFi reads and incorporating structural repeat insights essential for accurately annotating gene families and subgenome structure, while (Wang et al., 2023) created a chromosome-scale assembly (~1.19 Gb) using PacBio long reads.

Transcriptomic resources have expanded considerably in recent years. (Yadav et al., 2023)used RNA-Seq to develop over 106,000 EST-SSR markers from multiple cultivars, supporting diversity and expression studies. A full-length transcriptome, published using Illumina and SMRT technologies under various stress conditions (Zhan et al., 2021), has enhanced gene model annotation and facilitated the identification of stress response genes.

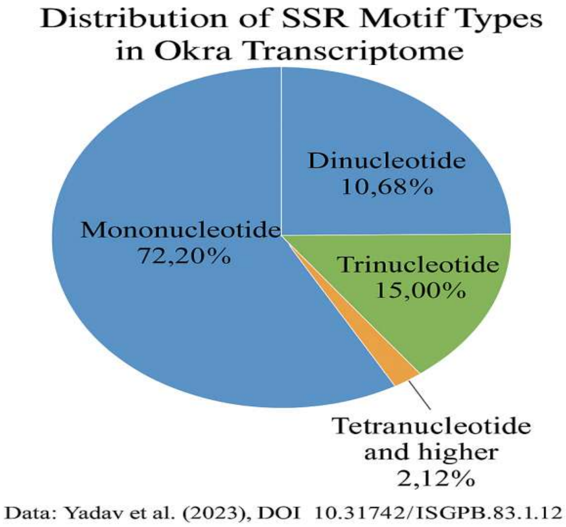

Fig. 4: Types of SSR motifs in okra.

The genetic and genomic variation required for developing resilient crop varieties cannot be fully captured through a single reference genome. Unlike individual genomes, a pangenome provides a comprehensive view of the genetic diversity within a species by integrating multiple genomes. It categorizes genes into *core genes*, which are consistently present across all accessions, and *variable (dispensable) genes*, which may be absent in some accessions. In recent years, pangenomes have been assembled for several important crops, including soybean, tomato, cotton, rice, chickpea, sorghum, sunflower, maize, and canola. These genomic resources have significantly advanced crop improvement by enabling the identification of genes and alleles associated with climate resilience. For example, pangenomic studies have helped identify key traits such as fruit size, uniform seed maturity, seed compactness, retention ability, larger seed dimensions, and rapid germination, all of which are highly relevant for breeding strategies designed to address the impacts of climate change.(Petereit et al., 2022).

The excellent chromosome-scale assemblies currently available on the market provide a strong basis for building an okra pangenome, although no formal one has been published as of yet. Global germplasm characterization has advanced significantly at the same time. A core collection of about 20% (166 accessions) from the World Vegetable Center has been shown to retain up to 87% of quantitative variation and over 90% of SSR-based diversity, making it an excellent starting panel for trait mining and pangenome representation. Studies of complementary molecular diversity, such as transcriptome-based SSR mining and newly developed SNP-based association mapping, highlight potential for integrating wild.

Trait Dissection Using Genomics

Mucilage content

Okra mucilage, a complex polysaccharide mostly composed of galacturonic acid, L-rhamnose, and D-galactose, has attracted increasing interest due to its potential as a therapeutic and nutritional supplement. In addition to its significance as a source of dietary fiber, its viscous nature makes it a valuable candidate for the management of type 2 diabetes since it improves glucose binding and lowers intestinal sugar absorption (Chukwuma et al., 2018) Its anti-inflammatory, anti-microbial, antidiabetic, and antioxidant qualities are mostly due to bioactive polysaccharide fractions such as rhamnogalacturonan-rich pectic structures that enhance lipid composition and reduce oxidative stress (Dantas et al., 2021). Because of these biochemical characteristics, okra mucilage stands out among natural plant-based substances with a wide range of pharmacological and preventative health advantages, making it a prospective adjuvant in nutraceutical formulations as well as a functional food ingredient.

Table 1: Bioactive composition of okra and its derivatives. https://link.springer.com/article/10.1007/s44187-025-00492-6

Components	Okra fruit	Okra seed	Okra flowers	Okra leaf
Total phenolics (mg GAE/g)	18.21–25.51	2.5–2.85	40.7–44.2	4.33–7.51
Protocatechuic acid	0.021–0.116	-	-	-
Total flavonoids (mg RE/g)	1.88–2.95	0.07–4.54	3.28–3.42	8.36–28.27
Quercetin-3-O-gentiobioside	0.65–1.70	27.4–29.3	-	-
Quercetin	0.001–0.011	0.0003–0.003	-	-
Iso-quercitrin	0.38–1.07	20.64–21.11	-	-
Total tannin (%)	0.21–0.33	0.00091–0.00378	-	0.27–3.39
Polysaccharide (%)	7.68–20.15	5.3–14.8	15.33–20	13.0–15.2
dietary fiber	3.5–8.2	-	-	2.14-9.12
Saponin (%)	0.284–0.612	-	-	0.569–3.13

Disease resistance (YVMV, powdery mildew)

Yellow vein mosaic virus (YVMV), spread by the whitefly (*Bemisia tabaci*), remains the most serious biotic challenge to okra cultivation, with infection capable of reducing yields by 50–94% depending on when the crop is attacked (Sheikh et al., 2013). Infected plants typically show yellowing of veins, chlorosis, dwarfing, and small, distorted fruits (Venkataravanappa et al., 2022). Since large-scale chemical management is impractical and uneconomical, breeding for host resistance has become the most reliable control measure. Notably, the cultivar Pusa Sawani, introduced in the 1960s as resistant, was later found to be highly susceptible in Indian growing environments.(Sastry & Singh, 1974).

To identify sources of resistance against powdery mildew, a total of 37 genotypes, along with a susceptible local check, were evaluated under natural field conditions. Observations were recorded at 15-day intervals, and the percent disease index (PDI), area under the disease progress curve (AUDPC), and apparent infection rate were calculated for each genotype. Based on the disease response, 14 genotypes were classified as moderately resistant, 13 as moderately susceptible, and 10 as susceptible to powdery mildew. None of the evaluated genotypes exhibited complete immunity or a highly resistant reaction.(Diwakar, 2020)

Research on the genetic diversity of okra through molecular markers has been relatively scarce. Existing studies typically rely on a small number of markers and germplasm collections with limited genetic backgrounds. Future investigations should focus on broader germplasm sources and apply high-throughput marker platforms to generate a more reliable diversity profile.

Nutritional value of okra

Okra contains a wide range of bioactive compounds, including phenolics, vitamin C, carotenoids, B-vitamins, amino acids, and essential minerals such as K, Ca, P, and Mg, while being low in cholesterol and saturated fat (Habtemariam, 2019). Its polysaccharides and flavonoids have also gained interest in the pharmaceutical sector (Gaur et al., 2025; Romdhane et al., 2020). In addition to serving as a sustainable alternative protein source through its nutrient-rich seeds, several in-vitro and in-vivo studies highlight okra's diverse health-promoting effects, including cardiovascular, renal, gastric, and neuroprotection, antioxidant, anti-diabetic, and anti-hyperlipidemic actions (Durazzo et al., 2019), as well as anti-fatigue, antibacterial (Petropoulos et al., 2017), anti-inflammatory, and analgesic activities (Alves et al., 2018). Applied research has also tested okra powder in enhancing meat quality in broiler storage (Ashour et al., 2020). Meanwhile, patents document diverse approaches to creating okra-based tea products.

Climate Change and Stress-Resilient Breeding

Salt tolerance in okra is a complicated trait involving biochemical adjustments, ionic balance, and molecular responses. Strategies such as osmolyte synthesis, Na^+/K^+ homeostasis, ion exclusion, antioxidant defense, and water retention underpin the variation in tolerance among species and varieties (Munns & Tester, 2008;

Flowers & Colmer, 2008). Exogenous treatments like gibberellic acid and potassium silicate enhance antioxidant activity and improve resilience (Ijaz Hussain & Khan, 2018), while inoculation with plant growth–promoting rhizobacteria significantly boosts salt tolerance in seedlings (Habib et al., 2016). Biotechnological tools enable the crop to be tolerant to salinity by using techniques and methods that can affect the gene causing salinity or same like against diseases (Haq et al., 2023). Okra is also affected heavily in Pakistan; there is must need for sustainable approaches to fill the gap in vegetable production.

Drought stress is also one of the concerning problems of crops, leading to dysfunction of morphology, Physiology, and biochemistry(Munns et al., 2006). It includes the imbalance of water, poor growth, loss of membrane, and waste of ions and electrolytes. Moreover, heat stress works similarly by disrupting the balance of water and nutrients, ultimately impairing the function of photosynthesis. All the issues impact the overall development and yield of the crop. Additionally, changes in plant water relations reduce turgor pressure, restricting cell expansion and overall growth (Kaur et al., 2018)

The best lines of okra can be identified by screening a large number of genetically diverse germplasm, having wide adaptability. These genotypes are employed as the best surviving lines and also give the best genetic material for the breeding programme. Under drought conditions, good-performing lines give a morphological response to drought. By identifying these lines, breeders can evaluate them for wider adaptability.(Mkhabela et al., 2022)

Studies show that okra respond to heat and drought stresses by changing the expression of genes, osmotic accumulation, and initiation of the antioxidant system. In this procedure, the release of enzymatic and non-enzymatic compounds occurred. Despite these changes, plants still need a more practical solution to defend against diseases and pests.(Hussain et al., 2021)

Practical Implications

Studies on combining ability and heterosis have emerged as essential tools in okra breeding in recent years. They enable breeders to choose effective parental combinations and more precisely predicted hybrid performance. This reduces costs and while accelerating the creation of new cultivars with enhanced yield and quality traits. By maximizing resource use, superior hybrids not only increase farm productivity but also support sustainable agriculture. Simultaneously, hybrids that meet quality standards domestic and international markets. consumer preferences improve the crop's ability to stand in both in local and international market.(Ranga et al., 2024)

Okra pest control has also benefited from biotechnological advancements. Transgenic lines that express insecticidal proteins like Bt toxins have demonstrated strong resistance to fruit borers and other pests. These genetically modified plants serve as an environmentally friendly crop protection method that improves human health and the environment by reducing dependency on chemical pesticides.

Future Perspectives and Conclusions

As technology continues to develop, researchers and breeders are making efforts to make crops more productive than ever before. Okra is now one of the emerging and potentially beneficial vegetables, and its importance cannot be denied in terms of nutrition, cytogenetics, and as a potential source of revenue. Both the modern and classical methods will help to maintain sustainable and climate-resilient agriculture.

Collaboration with the private sector can help translate breeding outputs into widely adopted okra varieties by improving investment, seed production, and market reach. Stronger public–private linkages allow research institutions to move beyond experimental success toward consistent on-farm performance. At the same time, stable funding for breeding activities remains necessary to maintain progress in developing high-yielding and climate-resilient cultivars.

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