

## Feature Review

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## Review of Exploration and Evaluation of New Germplasm Resources in Fresh-Eating Maize

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**Abstract** As consumer demand for high-quality, nutrient-rich, and stress-resistant new varieties grows, the exploration and evaluation of diverse germplasm resources have become central to fresh corn improvement. This review summarizes advancements in the exploration and evaluation of new germplasm resources for fresh corn, discusses the application of traditional and modern technologies in resource development, analyzes achievements in the evaluation of traits such as morphology, nutritional quality, and stress resistance, and identifies current challenges and future research directions. The review highlights that traditional germplasm sources include landrace maize varieties and wild relatives, while modern genomic technologies, such as genome-wide association studies (GWAS) and resequencing, have significantly enhanced resource discovery efficiency. Furthermore, marker-assisted selection (MAS), genomic selection (GS), CRISPR/Cas9 gene-editing technology, and integrated multi-omics approaches have accelerated the breeding of superior varieties. The development of high-sugar varieties and waxy corn demonstrates the remarkable achievements in germplasm exploration, with local germplasm playing a pivotal role in stress-resistance breeding globally. Systematic exploration and scientific evaluation of germplasm resources are crucial for improving the yield, quality, and adaptability of fresh corn. This not only drives the development of new varieties but also provides key solutions to address climate change.

**Keywords** Fresh corn; New germplasm resources; Germplasm evaluation; Stress-resistance breeding; Genomic selection

### 1 Introduction

Fresh-eating maize, commonly known as sweet corn, holds a significant place in global agriculture and the food industry due to its high nutritional value and consumer preference. It is a vital source of carbohydrates, vitamins, and minerals, making it a staple food in many regions. The versatility of maize allows it to be used in various forms, including fresh, canned, and frozen, catering to diverse culinary practices worldwide. Additionally, maize's role extends beyond human consumption; it is also a crucial feed crop for livestock and a raw material for the biofuel industry, further emphasizing its economic importance (Ortiz et al., 2010).

The continuous improvement of maize varieties is essential to meet the evolving market demands and address challenges such as climate change, pest resistance, and nutritional deficiencies. Diverse germplasm resources are critical in this context as they provide a broad genetic base for breeding programs. Utilizing genetically diverse germplasm can introduce unique alleles that enhance traits such as yield, stress tolerance, and nutritional quality (Hallauer and Carena, 2013; Njeri et al., 2017; Mubarak et al., 2023). For instance, the adaptation of tropical maize germplasm to temperate environments has shown promising results in improving agronomic performance and expanding the genetic base of breeding programs (Hallauer and Carena, 2013). Similarly, the exploration of local maize accessions has identified resource-efficient germplasm with superior photosynthetic and yield-related traits, which are crucial for sustainable agriculture (Mubarak et al., 2023).

This review will summarize the research progress in the exploration and evaluation of fresh maize germplasm resources, highlight the methodologies and outcomes of various studies aimed at enhancing maize genetic diversity and agronomic traits, and propose future research directions to further improve the utilization of

germplasm resources in breeding programs. This review aims to provide a comprehensive understanding of the current state of research on fresh maize germplasm and offer insights and references for future development strategies.

## 2 Current Status of Fresh Corn Germplasm Resources

### 2.1 Definition and characteristics of fresh corn

Fresh corn, also known as sweet corn, is primarily characterized by its sweetness, tenderness, and flavor. These attributes are crucial for consumer preference and marketability. Sweetness in corn is largely determined by the sugar content, which is influenced by specific genetic markers such as the *shrunken2* (*sh2*) gene that affects starch metabolism in the maize endosperm (Ruanjaichon et al., 2021). Tenderness and flavor are also significant traits, with sensory evaluations often focusing on these aspects to determine the overall eating quality of fresh corn (Dermail et al., 2021). The combination of these characteristics makes fresh corn a desirable crop for both direct consumption and various culinary applications.

### 2.2 Classification of fresh corn types

Fresh corn can be classified into several types based on its genetic and phenotypic traits. The primary types include sweet corn and waxy corn. Sweet corn is known for its high sugar content and is often consumed fresh, while waxy corn is characterized by its sticky texture due to the presence of amylopectin starch. Within these categories, further classifications can be made based on specific traits. For instance, waxy corn can be divided into different germplasm groups, each with unique breeding potentials such as long ear and big seed varieties, or those with high starch content for improved edible quality (Fang, 2005). Additionally, hybrid varieties like sweet-waxy corn combine the characteristics of both types, exhibiting rich genetic diversity in appearance (Figure 1). This diversity offers potential for further varietal optimization while meeting the diverse needs of consumers, growers, and seed producers (Dermail et al., 2021).



Figure 1 Ear appearances of 24 sweet-waxy corn hybrids excluding reciprocals at physiological maturity stage (Adopted from Dermail et al., 2021)

### 2.3 Global distribution and diversity of germplasm resources

The global distribution and diversity of fresh corn germplasm resources are extensive, with significant variations observed across different regions. For example, in China, fresh sweet-waxy corn is cultivated in various provinces such as Inner Mongolia, Jilin, and Heilongjiang, each exhibiting distinct nutritional profiles and fatty acid compositions (Li et al., 2022a; Li et al., 2022b). This regional diversity is crucial for breeding programs as it provides a wide genetic base for selecting superior traits. The genetic variability among different endosperm mutations and genetic backgrounds further enhances the potential for developing fresh corn varieties with improved eating quality and agronomic performance (Azanza et al., 2004). The continuous exploration and evaluation of these germplasm resources are essential for advancing fresh corn breeding and meeting the diverse needs of global markets.

## 3 Exploration of New Fresh Corn Germplasm Resources

### 3.1 Traditional sources of germplasm resources

Landrace corn varieties are a crucial source of genetic diversity, having been cultivated and adapted over centuries to specific local conditions. For instance, maize landraces from the Aosta Valley in Italy exhibit significant genetic variability and differentiation due to their long-term reproductive isolation and adaptation to mountainous regions (Lezzi et al., 2023). Similarly, landraces from the Emilia Romagna region in Italy have been preserved by farmers in marginal areas, maintaining a rich genetic reservoir that can be utilized for breeding more resilient varieties (Stagnati et al., 2021). In Brazil, the characterization of 25 maize landraces revealed promising genetic potential for breeding, with some landraces showing grain yields comparable to commercial hybrids (Araújo and Nass, 2002).

Wild relatives of maize, such as teosinte, offer valuable genetic traits that can be harnessed for modern agriculture. Teosinte, the wild ancestor of maize, has been found to possess higher protein content and unique zein protein profiles compared to modern inbred lines and landraces, indicating its potential for improving kernel traits and grain quality (Flint-Garcia et al., 2009). The genetic diversity present in teosinte and other wild relatives can provide insights into target traits and allelic variants that are beneficial for crop improvement.

### 3.2 Modern methods for germplasm exploration

Molecular markers, such as simple sequence repeats (SSRs), are widely used to assess genetic diversity in maize germplasm. For example, the evaluation of maize landraces from southwest China using 42 SSR loci revealed a high level of genetic diversity, with 246 alleles detected among the landraces (Chen et al., 2016). Similarly, genetic characterization of local maize accessions from the Emilia Romagna region using SSR markers identified 62 different alleles, highlighting the genetic variability among the landraces (Stagnati et al., 2021). These analyses are essential for identifying unique genetic resources that can be utilized in breeding programs.

Genomic technologies, such as genome-wide association studies (GWAS) and resequencing, have revolutionized the exploration of maize germplasm. For instance, the mapping of haplotype-trait associations in doubled-haploid lines derived from maize landraces has uncovered beneficial haplotypes for early development traits, which can be integrated into elite germplasm for crop improvement. Additionally, the use of high-density SNP genotyping has facilitated the identification of untapped beneficial variation in landraces, providing new opportunities for breeding programs (Mayer et al., 2020).

### 3.3 International collaboration in germplasm sharing and conservation

International collaboration plays a vital role in the sharing and conservation of maize germplasm. Organizations like CIMMYT have been instrumental in providing improved maize breeding material for non-temperate regions, ensuring the preservation and utilization of diverse genetic resources (Warburton et al., 2008; Hou et al., 2024). Collaborative efforts are also essential for the conservation of traditional maize agrobiodiversity, as seen in the case of Mexican landraces, where strategies have been developed to safeguard rare genotypes and promote in situ conservation (Hayano-Kanashiro et al., 2017). These initiatives are crucial for maintaining the genetic diversity necessary for future crop improvement and resilience against environmental challenges.



## 4 Evaluation of Fresh Corn Germplasm Resources

### 4.1 Morphological and agronomic traits evaluation

The evaluation of fresh corn germplasm resources involves assessing key morphological and agronomic traits such as yield, plant height, and flowering time. Yield is a critical trait, often influenced by various genetic and environmental factors. For instance, studies have shown that grain yield in maize can be significantly affected by plant density and environmental stress levels, with certain hybrids demonstrating substantial tolerance to high plant densities (Mansfield and Mumm, 2014). Plant height and flowering time are also essential traits, with plant height showing a positive linear relationship to crude fiber content and flowering time correlating with other nutritional traits such as crude protein and starch (Alves and Filho, 2017). Additionally, the use of advanced phenotyping methods, such as UAV-based hyperspectral data, has proven effective in estimating grain yield and flowering time with high accuracy, thus aiding in the efficient selection of desirable traits (Fan et al., 2022).

Ear characteristics and grain quality are pivotal in the evaluation of fresh corn germplasm. Traits such as kernel rows per ear, kernel length, and the number of ears per plant are crucial for determining overall grain quality and yield potential. Research has identified quantitative trait loci (QTLs) associated with these traits, highlighting the importance of genetic factors in improving ear characteristics. Moreover, the stability of these QTLs across different environments underscores the potential for breeding programs to develop maize varieties with superior grain quality and yield (Ragot et al., 1995).

### 4.2 Nutritional and quality traits evaluation

The nutritional and quality traits of fresh corn germplasm are evaluated based on sugar content, texture, and flavor compounds. These traits are essential for consumer acceptance and marketability. The sugar content in maize, for example, is a key determinant of its sweetness and overall flavor profile. Advanced phenotyping technologies, such as hyperspectral imaging, enable monitoring of key growth stages in maize canopies, with a particular focus on the distribution and growth dynamics of tassels (Figure 2). By analyzing images from different growth stages, tassel regions and their spectral reflectance characteristics can be effectively identified. This provides data support for evaluating maize growth, optimizing fertilizer application, and predicting harvests, assisting breeders in selecting varieties with optimal sugar content and ideal texture (Fan et al., 2022). Additionally, the texture and flavor compounds of maize are influenced by its genetic makeup and environmental conditions, necessitating comprehensive evaluation across different growing environments (Mansfield and Mumm, 2014).

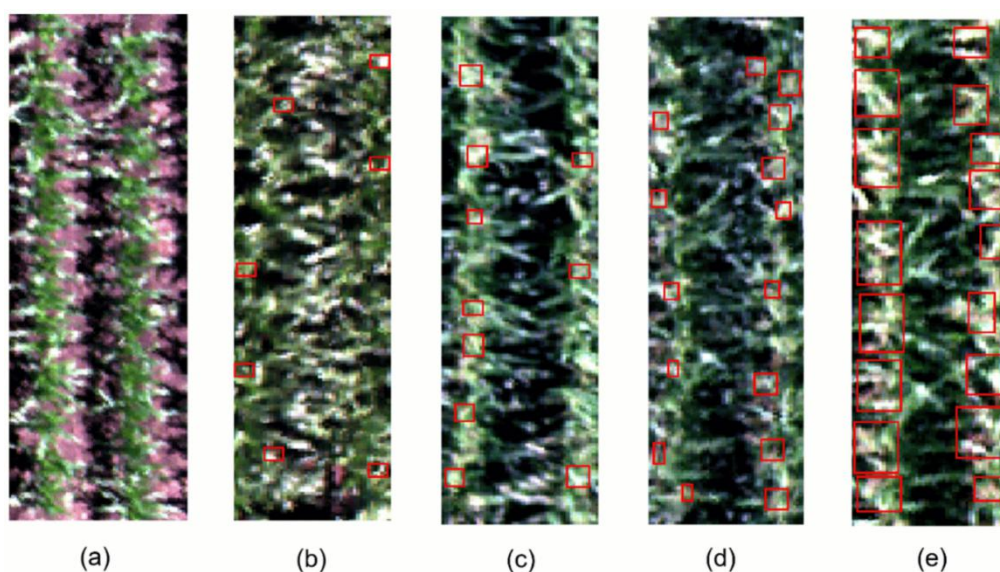


Figure 2 Monitoring maize canopy growth stages based on hyperspectral imaging (Adopted from Fan et al., 2022)

Image Caption: Canopy images of a maize plot at (a) the V6–V8 stage (41 DAS), (b) the VT stage (61 DAS), (c) the R1 stage (75 DAS), (d) the R3 stage (90 DAS), and (e) the R4 stage (103 DAS); The images were taken by the hyperspectral camera at a 42 m height and composited using the red (620 nm), green (530 nm), and blue (465 nm) bands from the hyperspectral imagery. Red boxes highlight the tassel parts on the maize canopy (Adopted from Fan et al., 2022)

Nutritional components such as proteins, vitamins, and anthocyanins are critical for the health benefits of fresh corn. Studies have shown that agronomic traits like flowering time and plant height can have positive linear relationships with nutritional traits such as crude protein and starch content (Alves and Filho, 2017). The presence of favorable alleles at specific QTLs can enhance these nutritional components, making certain maize varieties more nutritious (Ragot et al., 1995). Furthermore, the evaluation of these traits across various environments helps in identifying stable and high-quality germplasm resources that can be used in breeding programs to improve the nutritional value of fresh corn (Ragot et al., 1995; Mansfield and Mumm, 2014).

#### **4.3 Stress resistance evaluation**

Resistance to diseases such as rust and leaf spot is a critical factor in evaluating fresh corn germplasm resources. For instance, southern corn rust (SCR) caused by *Puccinia polysora* is a significant disease affecting maize yields. A study identified several quantitative trait nucleotides (QTNs) associated with SCR resistance in Chinese summer maize germplasm. These QTNs were linked to various genetic loci and were validated through post-GWAS analysis, highlighting their potential in breeding programs aimed at enhancing disease resistance (Shu et al., 2023). Additionally, resistance to mycotoxin contamination, such as aflatoxins and fumonisins, has been evaluated under different stress conditions. Aflatoxin-resistant germplasm lines showed significantly lower contamination levels compared to commercial hybrids, especially under high heat stress, indicating their superior resistance to fungal diseases (Abbas et al., 2002).

Abiotic stresses such as drought, salinity, and high temperatures significantly impact maize productivity. The relationship between aflatoxin contamination and physiological responses of corn plants under drought and heat stress has been studied extensively. For example, certain germplasm lines like PI 639055 demonstrated low aflatoxin contamination despite being highly stressed, suggesting inherent tolerance mechanisms (Kebede et al., 2012). This tolerance is crucial for maintaining yield and quality in environments prone to abiotic stresses.

#### **4.4 Molecular-level evaluation**

Genetic diversity analysis using molecular markers is essential for understanding the genetic basis of stress resistance in maize. Multi-locus genome-wide association studies (GWAS) have been employed to identify QTNs associated with disease resistance traits. For instance, in the study of SCR resistance, 13 QTNs were identified across multiple chromosomes, providing insights into the genetic diversity and potential for breeding resistant varieties (Shu et al., 2023).

The identification of QTLs associated with target traits such as disease resistance and stress tolerance is a pivotal aspect of molecular-level evaluation. In the case of SCR resistance, the identified QTNs were linked to candidate genes involved in transcriptional regulation, phosphorylation, and temperature stress response. These findings underscore the importance of QTL mapping in developing germplasm with enhanced resistance to both biotic and abiotic stresses (Shu et al., 2023).

### **5 Advances in Fresh Corn Breeding Techniques**

#### **5.1 Traditional breeding methods**

Traditional breeding methods in fresh-eating maize primarily involve hybridization and backcrossing to improve target traits. Hybridization combines desirable traits from two parent plants to produce offspring with enhanced characteristics, such as increased sweetness or disease resistance. Backcrossing, on the other hand, involves crossing a hybrid with one of its parent plants to reinforce specific traits. These methods have been fundamental in developing new maize varieties with improved yield, taste, and resilience to environmental stresses.

#### **5.2 Marker-assisted selection (MAS) and genomic selection (GS)**

Marker-assisted selection (MAS) and genomic selection (GS) have revolutionized the breeding process by accelerating the development of superior maize varieties. MAS utilizes molecular markers linked to desirable traits to assist in the selection process, significantly speeding up the breeding cycle. For instance, MAS has been effectively used to improve drought adaptation in maize by introgressing favorable alleles at target regions, resulting in hybrids with higher grain yield under water-limited conditions (Francia et al., 2005; Ribaut and Ragot,

2006). Genomic selection (GS) goes a step further by using genome-wide marker data to predict the breeding values of plants, thus enabling the selection of superior genotypes with greater accuracy and efficiency. GS has shown promising results in improving quantitative traits such as yield and stress tolerance in maize (Crossa et al., 2017; Rice and Lipka, 2021; Budhlakoti et al., 2022).

### **5.3 CRISPR/Cas9 and gene-editing technologies**

CRISPR/Cas9 and other gene-editing technologies offer precise tools for enhancing specific traits in fresh-eating maize. These technologies allow for the targeted modification of genes responsible for sweetness, nutritional value, and other desirable characteristics. By directly editing the maize genome, breeders can achieve improvements that would be difficult or time-consuming with traditional methods. Gene-editing technologies hold great potential for creating maize varieties with enhanced taste, nutritional content, and resistance to pests and diseases (Zhou and Hong, 2024).

### **5.4 Application of multi-omics approaches**

The integration of multi-omics approaches, including genomics, transcriptomics, and metabolomics, has significantly improved breeding efficiency in fresh-eating maize. These approaches provide comprehensive insights into the genetic and molecular mechanisms underlying important traits. For example, genotyping-by-sequencing (GBS) has been used to discover and genotype single nucleotide polymorphisms (SNPs) in maize, facilitating genome-wide association studies and genomic selection (He et al., 2014). By combining data from different omics layers, breeders can better understand the complex interactions between genes and traits, leading to more informed selection decisions and faster development of superior maize varieties.

## **6 Case Studies**

### **6.1 Development of high-sugar fresh corn varieties**

The development of high-sugar fresh corn varieties has been significantly advanced through modern breeding techniques. CRISPR-Cas technology, for instance, has been utilized to create new maize varieties with improved grain quality, including higher sugar content. This technology allows for precise modification of key genes involved in specific traits, making it a powerful tool for enhancing the sweetness of fresh-eating maize (Wang et al., 2022). Additionally, the integration of doubled haploidy and genomics-assisted breeding has further accelerated the development of these high-sugar varieties, ensuring they meet the desired quality standards for fresh consumption (Prasanna et al., 2020; Prasanna et al., 2021).

### **6.2 Utilization and global recognition of waxy corn in niche markets**

Waxy corn, known for its unique starch properties, has gained global recognition and is increasingly utilized in niche markets. The U.S. National Plant Germplasm System (NPGS) has played a crucial role in providing access to diverse maize germplasm, including waxy corn, which has facilitated its breeding and utilization worldwide. The availability of these germplasm resources has enabled breeders to develop waxy corn varieties that cater to specific market demands, such as those in the food industry where waxy corn is valued for its texture and cooking properties. The global distribution of these varieties underscores their growing importance and acceptance in various niche markets (Kurtz et al., 2016).

### **6.3 Successful breeding of stress-resistant varieties using local germplasm in Africa and Asia**

In Africa and Asia, significant progress has been made in developing stress-tolerant maize varieties using local germplasm. Institutions such as the International Maize and Wheat Improvement Center (CIMMYT) have conducted intensive breeding efforts to produce elite tropical maize germplasm with resistance to key abiotic and biotic stresses (Prasanna et al., 2020; Prasanna et al., 2021). CIMMYT collaborates with National Agricultural Research Systems (NARS) and the private sector to establish maize germplasm phenotyping and testing networks in sub-Saharan Africa (ESA), Latin America, and tropical Asia (Figure 3). By integrating international resources and technical support, they precisely select maize germplasm tailored to regional climate, soil, and biological stresses, ensuring that the developed varieties are well-suited to the specific conditions of these regions, thereby enhancing their stress tolerance and productivity (Setimela et al., 2018; Prasanna et al., 2020; Prasanna et al.,

2021). Moreover, public-private partnerships have played a crucial role in the dissemination of these stress-tolerant varieties, ensuring they reach the farmers who need them most efficiently (Prasanna et al., 2020; Prasanna et al., 2021).



Figure 3 Maize germplasm phenotyping/testing network of CIMMYT and partners in the tropics of ESA, Latin America, and Asia (Adopted from Prasanna et al., 2021)

## 7 Challenges in the Exploration and Utilization of Fresh Corn Germplasm Resources

### 7.1 Limited access to germplasm resources due to conservation policies and management restrictions

Access to germplasm resources is often restricted by conservation policies and management practices, which can limit the availability of diverse genetic materials for breeding programs. The conservation of genetic variability is crucial, but the high costs and long-term nature of these activities pose significant challenges. This issue is not confined to a single region but affects many developing countries, where the utilization of germplasm banks remains low (Nass and Paterniani, 2000). Additionally, the proprietary nature of many inbred lines, protected by patents and plant variety protection acts, further restricts access until these protections expire (Mikel and Dudley, 2006).

### 7.2 Over-reliance on specific germplasm resources, leading to genetic bottlenecks

The over-reliance on a narrow genetic base in maize breeding has led to genetic bottlenecks, which limit the potential for genetic improvement and adaptation. Much of the current maize germplasm originates from a few progenitor lines, resulting in a limited genetic pool (Mikel and Dudley, 2006). This narrow genetic base restricts the capacity to increase genetic gain through conventional breeding methods, making it essential to integrate a broader range of genetic resources into breeding programs (He and Li, 2020).

### 7.3 Technological and resource constraints in developing countries

Developing countries face significant technological and resource constraints that hinder the effective utilization of germplasm resources. The adoption of advanced breeding technologies, such as doubled haploid (DH) technology, is often limited by the high costs and technical requirements associated with artificial chromosome duplication (Kleiber et al., 2012). Moreover, the lack of infrastructure and expertise in these regions further exacerbates the challenges, making it difficult to implement efficient germplasm conservation and breeding strategies (Nass and Paterniani, 2000).

### 7.4 Impact of climate change on the adaptability of germplasm resources

Climate change poses a significant threat to the adaptability of germplasm resources. The changing environmental conditions can affect the viability and performance of maize germplasm, necessitating the development of cultivars with enhanced resistance and tolerance to biotic and abiotic stresses (He and Li, 2020). The long-term conservation of seeds under varying storage conditions also impacts seed viability, with significant differences



observed in seed longevity among different accessions (Guzzon et al., 2021). Therefore, it is crucial to integrate genomic selection and emerging technologies to accelerate the development of climate-resilient cultivars (He and Li, 2020).

## **8 Future Directions**

### **8.1 Enhancing germplasm diversity through international collaboration and conservation programs**

International collaboration and conservation programs are pivotal in enhancing germplasm diversity. The Germplasm Enhancement of Maize (GEM) project exemplifies a successful public-private partnership aimed at increasing the genetic diversity of U.S. maize by incorporating exotic germplasm. Similarly, the Latin American Maize Project (LAMP) has demonstrated the benefits of coordinated national and international efforts in evaluating and utilizing diverse maize accessions. These initiatives underscore the importance of sustained, collaborative efforts in germplasm conservation and enhancement, which can be further bolstered by global partnerships and resource sharing.

### **8.2 Integrating artificial intelligence and machine learning for germplasm evaluation and selection**

The integration of artificial intelligence (AI) and machine learning (ML) in germplasm evaluation and selection holds significant promise. Genomic prediction models, as utilized in the GEM project, have shown substantial improvements in prediction accuracy for traits such as grain yield and moisture. These models can accelerate the breeding process by identifying promising genetic combinations more efficiently than traditional methods. Collaborative diversity panels and genomic predictions have also been effective in identifying genetic resources for enriching elite germplasm, highlighting the potential of AI and ML in optimizing breeding strategies.

### **8.3 Developing climate-resilient, stress-tolerant fresh corn varieties**

Developing climate-resilient and stress-tolerant maize varieties is crucial in the face of changing environmental conditions. The adaptation of tropical maize germplasm to temperate environments has been a focus of breeding programs, resulting in the successful integration of unique alleles that enhance stress tolerance and agronomic performance. The use of stratified mass selection and other advanced breeding techniques has facilitated the development of maize varieties that can thrive in diverse climatic conditions, ensuring food security and agricultural sustainability.

### **8.4 Strengthening partnerships among research institutions, governments, and commercial breeders**

Strengthening partnerships among research institutions, governments, and commercial breeders is essential for the successful development and dissemination of improved maize varieties. The GEM project serves as a model for such collaborations, involving USDA-ARS, public and private research scientists, and commercial breeders to enhance maize germplasm diversity. These partnerships enable the pooling of resources, expertise, and data, thereby accelerating the breeding process and ensuring that new varieties meet the needs of producers and consumers alike.

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## **Conflict of Interest Disclosure**

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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