

Feature Review

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Current Status of Collection, Evaluation, and Utilization of Sorghum Germplasm Resources

Chunxin Ĉao ^{1,3*}, Nuan Wang ^{2*}, Yubing Liu ^{1,3}, Hongming Huang ², Meijuan Wu ², Xinhua Liu ^{1,3} 🗷

1 Jinhua Academy of Agricultural Sciences, Jinhua, 321000, Zhejiang, China

2 Lanxi Agricultural Technology Extension Center, Lanxi, 321100, Zhejiang, China

3 Key Laboratory for Innovative Utilization of Characteristic Food Crop Resources in Central Zhejiang, Jinhua, 321000, Zhejiang, China

* These authors contributed equally to this work

Corresponding email: jhzjny@163.com

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Abstract Sorghum (*Sorghum bicolor* L.) is one of the most important food crops in the world, especially showing excellent resistance to adverse conditions such as drought and salinity, and has multiple uses. This study discusses the current status of global collections of sorghum germplasm resources, focusing on the main germplasm banks and their role in genetic diversity conservation. The phenotypic and genomic evaluation methods of sorghum germplasm were analyzed, and the application of these evaluations in breeding was demonstrated through specific cases. The discovery of sorghum drought resistance genes and their application in breeding were elaborated in detail, especially the accelerated development of stress-resistant varieties through gene editing technology. This study also looks forward to the impact of global climate change on the utilization of sorghum germplasm resources, and puts forward suggestions for strengthening international cooperation and using new technologies to meet the challenges in future agricultural production.

Keywords Sorghum; Germplasm resources; Phenotypic evaluation; Genomics; Breeding for drought resistance

1 Introduction

Sorghum (*Sorghum bicolor* L.) is a vital cereal crop globally, ranking among the top five in terms of production and planting area. Its significance is particularly pronounced in regions with severe abiotic stresses such as drought and saline-alkali soils, where its excellent stress resistance allows it to thrive. Sorghum is a multipurpose crop, classified into grain sorghum, energy sorghum, and silage sorghum based on its domestication direction and utilization traits. This multifunctionality endows sorghum with broad breeding and economic value, making it an essential crop for food, feed, and industrial applications (Zheng et al., 2023). Sorghum grain is a rich source of nutrients and health-beneficial phenolic compounds, which have potent antioxidant activity and can improve gut health and reduce the risks of chronic diseases. Additionally, sorghum is used in the development of functional foods and beverages, and its phenolic compounds can be isolated for use as natural multifunctional additives in various food applications (Xiong et al., 2019).

Germplasm resources play a crucial role in the genetic improvement and diversification of sorghum. Globally, over 236 000 sorghum germplasm accessions are conserved in genebanks, with significant collections held by institutions such as the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and the USDA-ARS. These germplasm collections include core and mini core collections, as well as genotyping-based reference sets, which provide access to a large diversity of genetic material. The mini core collection at ICRISAT, for instance, is widely used for identifying sources of resistance to various biotic and abiotic stresses, as well as for agronomic and grain nutritional traits (Upadhyaya et al., 2016). The diversity within sorghum germplasm, including wild and weedy relatives, offers valuable genetic resources for crop improvement, enabling the development of more resilient and productive sorghum varieties (Ananda et al., 2020).

This study provides a comprehensive overview of the status, evaluation, and application of sorghum germplasm resources, emphasizing a multidimensional exploration from phenotype to genome, summarizing the phenotypic



and genomic characteristics of current sorghum germplasm, highlighting key findings and advancements, discussing the methods and technologies used in sorghum germplasm evaluation-including phenotyping platforms, genome sequencing, and genome-wide association studies (GWAS) and examining the practical applications of these resources in plant breeding and crop improvement, particularly in developing sorghum varieties with resistance and high yield, aiming to guide future research and breeding efforts to fully utilize the potential of sorghum germplasm resources.

2 Current Status of sorghum germplasm Collection

2.1 Current status of germplasm collections in the world and major sorghum growing regions

Sorghum germplasm collections are extensive and globally distributed, with significant repositories in various regions. The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) maintains a substantial collection exceeding 37 000 accessions, from which a core collection and a mini core collection have been developed to facilitate research and utilization (Upadhyaya et al., 2009). Similarly, the USDA National Plant germplasm System (NPGS) holds the largest sorghum germplasm collection worldwide, including 7 217 accessions from Ethiopia, a key center of sorghum diversity (Cuevas et al., 2016). These collections are crucial for preserving genetic diversity and supporting breeding programs aimed at improving sorghum's agronomic traits and stress resistance.

2.2 Important sources of sorghum germplasm

Sorghum germplasm sources are diverse, encompassing both wild and cultivated species. The genus sorghum includes 24 species, with cultivated sorghum (*sorghum bicolor*) derived from the wild progenitor *S. bicolor* subsp. *verticilliflorum*, predominantly found in Africa (Ananda et al., 2020). Wild sorghum species, particularly those native to Australia and Africa, are valuable for their genetic traits, such as drought tolerance and disease resistance, which are crucial for crop improvement. The Ethiopian sorghum germplasm, characterized by high genetic and phenotypic diversity, represents a significant source of adaptive traits and rare alleles (Girma et al., 2020). Additionally, germplasm from West and Central Africa, including Senegal and Burkina Faso, has been identified as a rich source of resistance genes to fungal diseases like anthracnose and grain mold (Cuevas et al., 2016; Cuevas et al., 2018).

2.3 Current collection challenges and potential solutions

The collection and utilization of sorghum germplasm face several challenges. One major issue is the underrepresentation of wild species in germplasm collections, which limits the genetic diversity available for breeding programs (Ananda et al., 2020). Additionally, the large size of some collections, such as the ICRISAT's core collection, can be cumbersome for researchers to manage and utilize effectively. To address these challenges, the development of mini core collections, which represent a smaller, more manageable subset of the larger collections, has been proposed and implemented successfully. Furthermore, extensive genomic characterization and phenotyping of germplasm, as demonstrated in the Ethiopian and Senegalese collections, can enhance the identification and utilization of valuable genetic traits (Cuevas et al., 2018). These efforts, combined with targeted conservation strategies and international collaboration, can improve the efficiency and effectiveness of sorghum germplasm collection and utilization.

3 Phenotypic and Genotypic Evaluation of Sorghum Germplasm Resources

3.1 Evaluation methods for phenotypic diversity of germplasm resources

Phenotypic evaluation of sorghum germplasm resources involves assessing various traits such as morphology, stress resistance, and yield. For instance, the Ethiopian sorghum germplasm was phenotyped for different traits across multiple locations, revealing significant genetic diversity and rare natural variations (Girma et al., 2020). Similarly, the ICRISAT gene bank developed a mini core collection of sorghum, which was evaluated for 11 qualitative and 10 quantitative traits, including resistance to biotic and abiotic stresses (Upadhyaya et al., 2009). These evaluations often use hierarchical cluster analysis and other statistical methods to identify phenotypic diversity and correlations among traits (Enyew et al., 2021).



3.2 Progress in genotypic evaluation

Genotypic evaluation has advanced significantly with the use of molecular markers and genome sequencing technologies. For example, genotyping-by-sequencing (GBS) was used to analyze 1628 Ethiopian sorghum accessions, identifying distinct cluster groups and candidate genes associated with adaptation to abiotic factors (Girma et al., 2020). The USDA-NPGS Ethiopian sorghum germplasm collection was characterized using 148 476 SNP markers, revealing high genetic diversity and unique genetic profiles for each accession (Cuevas et al., 2016). Whole-genome sequencing (WGS) of the sorghum Association Panel (SAP) identified over 43 million variants, enhancing the understanding of genomic diversity and enabling the identification of novel genomic associations for important traits (Figure 1) (Boatwright et al., 2022).



Figure 1 Genome-wide associations for plant height (Adopted from Boatwright et al., 2022) Image caption: (a) and tannin content (b) using linear mixed models in GEMMA. Horizontal lines with solid, dotted, and dashed patterns represent the Bonferroni-adjusted threshold of 0.05 for SNPs, indels, and CNVs, respectively. Vertical dotted lines indicate the positions of known genes and loci for height (Dw), maturity (Ma), and tannin (Tan) (Adopted from Boatwright et al., 2022)

3.3 Application cases of evaluation to illustrate the contribution to sorghum germplasm improvement

The evaluation of sorghum germplasm has led to significant improvements in breeding programs. For instance, the development of a core subset of 387 lines from the Ethiopian sorghum germplasm has facilitated the identification of candidate genes for abiotic stress tolerance, which can be exploited for breeding more resilient sorghum varieties (Girma et al., 2020). The mini core collection developed by ICRISAT has been extensively evaluated for agronomic traits, aiding in the identification of accessions with desirable characteristics for crop improvement (Upadhyaya et al., 2009). Additionally, the genomic characterization of the USDA-NPGS Ethiopian sorghum germplasm has provided valuable resources for sorghum breeders, enabling the effective screening and selection of specific populations for breeding programs (Cuevas et al., 2016). These efforts demonstrate the critical role of phenotypic and genotypic evaluations in enhancing the utilization and improvement of sorghum germplasm resources.

4 Classification and Organization of Sorghum Germplasm Resources

4.1 Classification of germplasm resources based on phenotypic and genotypic data

The classification of sorghum germplasm resources involves both phenotypic and genotypic data to ensure a comprehensive understanding of the genetic diversity and potential of the crop. Phenotypic characterization includes the assessment of various morphological and agronomic traits such as plant height, panicle length, grain yield, and resistance to biotic and abiotic stresses. For instance, a study on Ethiopian sorghum germplasm revealed significant phenotypic diversity across different agro-climatic regions, highlighting the association of important traits with specific environments (Girma et al., 2020). Similarly, the evaluation of sorghum germplasm from the Eastern Highlands of Ethiopia showed a wide range of morpho-agronomical diversity, which is crucial for conservation and improvement efforts (Mulima et al., 2018).



Genotypic characterization, on the other hand, involves the use of molecular markers such as single nucleotide polymorphisms (SNPs) and simple sequence repeats (SSRs) to assess genetic diversity and population structure. For example, the USDA-NPGS Ethiopian sorghum germplasm collection was genotyped using SNP markers, revealing high genetic diversity and the presence of unique alleles (Cuevas et al., 2016). Another study utilized SSR markers to develop a sorghum diversity research set, which helped in understanding the genetic relationships among accessions from different geographic regions (Shehzad et al., 2009). These genotypic analyses are essential for identifying distinct cluster groups and sub-populations, which can be used to improve the utilization of germplasm in breeding programs.

4.2 Establishment of classification standards and their application in germplasm banks

The establishment of classification standards is vital for the effective management and utilization of sorghum germplasm resources in genebanks. These standards typically involve the development of core and mini core collections that represent the genetic diversity of the entire germplasm collection. For instance, the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) developed a mini core collection of sorghum, which comprises 1% of the entire collection. This mini core collection has been extensively used for identifying sources of resistance to various stresses and for agronomic and grain nutritional traits (Upadhyaya et al., 2009).

Classification standards also include the use of high-throughput phenotyping platforms and genome-wide association studies (GWAS) to link phenotypic traits with genotypic data. This approach has been employed to identify genomic regions associated with important agronomic traits and resistance to biotic and abiotic stresses (Upadhyaya et al., 2016). Additionally, the use of hierarchical cluster analysis and principal component analysis (PCA) helps in grouping germplasm accessions based on their genetic and phenotypic similarities, which aids in the selection of parents for breeding programs (Mulima et al., 2018).

4.3 A Systematic classification method for sorghum germplasm resources in Ethiopia

Ethiopia is a center of diversity for sorghum, and a systematic classification method has been developed to manage its rich germplasm resources. A comprehensive study involving 2010 Ethiopian sorghum accessions was conducted, where phenotypic data were collected for various traits across multiple locations. A subset of 1628 accessions was genotyped using sequencing techniques, which revealed high genetic diversity and the presence of rare natural variations (Girma et al., 2020). The genotypic analysis identified distinct cluster groups and sub-populations, which were used to create a core subset of 387 lines. This core subset was selected based on cluster groups obtained through genotyping-by-sequencing (GBS) analysis, followed by stratified random sampling using quantitative traits.

The classification method also involved genome-environment association analysis, which identified candidate genes associated with adaptation to abiotic factors. This information is crucial for exploiting the adaptive potential of sorghum to different environments (Girma et al., 2020). The systematic classification of Ethiopian sorghum germplasm has provided valuable insights into the diversity and relationship of sorghum collections, facilitating their effective utilization in breeding programs and conservation efforts.

5 Discovery and Breeding Application of Sorghum Drought Resistance Genes

5.1 Selection criteria for case studies

The selection of case studies for the discovery and breeding application of sorghum drought resistance genes was based on several criteria. Firstly, studies that utilized diverse sorghum genotypes under varying environmental conditions were prioritized to ensure a comprehensive understanding of drought resistance mechanisms. Secondly, research that employed advanced genomic and transcriptomic techniques to identify and map drought resistance genes was included. Lastly, studies that demonstrated practical applications of these genes in breeding programs were considered essential to highlight the translational aspect of the research.

5.2 Discovery process of drought resistance genes

The discovery of drought resistance genes in sorghum has been facilitated by various genomic and transcriptomic approaches. For instance, transcriptome analysis of drought-resistant and drought-sensitive sorghum genotypes



under polyethylene glycol (PEG)-induced drought stress revealed that about 180 genes are differentially regulated in response to drought stress, with a significant number being up-regulated in drought-resistant genotypes. These genes include transcription factors, signaling proteins, and stress-related proteins, many of which are novel and uncharacterized (Abdel-Ghany et al., 2020). Additionally, multi-environment field trials of Ethiopian sorghum germplasm identified high phenotypic variation in traits related to drought tolerance, such as chlorophyll content and stay-green, which are crucial for breeding drought-tolerant varieties (Figure 2) (Enyew et al., 2022). Quantitative trait locus (QTL) mapping has also been instrumental in identifying genomic regions associated with drought resistance traits, such as the stay-green trait, which helps in maintaining photosynthetic activity under drought conditions (Sanchez et al., 2002).



Figure 2 Geographical map, constructed using geographic information system (ArcGIS), showing the three testing environments: Mehoni, Mieso and Melkassa in Ethiopia (Adopted from Enyew et al., 2022)

5.3 Application of drought resistance genes in breeding

The application of drought resistance genes in sorghum breeding has shown promising results. For example, the stay-green QTLs identified in sorghum have been consistently associated with drought resistance across multiple field trials, accounting for a significant portion of phenotypic variance. These QTLs have been incorporated into breeding programs to develop drought-resistant sorghum hybrids (Sanchez et al., 2002). Furthermore, the identification of novel drought tolerance traits, such as restricted transpiration and root architecture, has expanded the genetic base for breeding drought-tolerant sorghum varieties. The integration of high-throughput phenomics and genomics in breeding programs has also facilitated the development of genotypes that can withstand both drought and high-temperature stresses, thereby improving yield and resilience (Prasad et al., 2021).

5.4 Inspiration from the case and future application prospects

The case studies provide valuable insights into the complex genetic and physiological mechanisms underlying drought resistance in sorghum. The discovery of novel genes and QTLs associated with drought tolerance not only enhances our understanding of plant stress responses but also offers new avenues for breeding resilient crop varieties. The successful application of these genes in breeding programs underscores the potential of integrating advanced genomic tools with traditional breeding techniques to achieve sustainable agricultural production. Future prospects include the exploration of gene editing technologies, such as CRISPR/Cas9, to precisely modify drought resistance genes and the development of climate-resilient sorghum varieties tailored to specific agro-ecological zones. Additionally, expanding the genetic diversity of breeding populations by incorporating wild relatives and landraces could further enhance the drought tolerance of sorghum (Fracasso et al., 2016; Harris-Shultz et al., 2019; Varoquaux et al., 2019).



6 Innovative Use of Sorghum germplasm Resources

6.1 New applications of germplasm resources in genetic engineering and molecular breeding

The utilization of sorghum germplasm resources in genetic engineering and molecular breeding has opened new avenues for crop improvement. For instance, genome-wide association studies (GWAS) have been instrumental in identifying genetic signals associated with key traits such as plant architecture and bioenergy traits. These studies have revealed significant single-nucleotide polymorphisms (SNPs) linked to traits like plant height, tiller number, and reducing sugar content, which are crucial for enhancing sorghum's utility in food, feed, and biofuel production (Luo et al., 2020). Additionally, the development of a mini core collection of sorghum germplasm has facilitated the identification of accessions with desirable characteristics, thereby streamlining the breeding process (Upadhyaya et al., 2009). The integration of these genetic resources into breeding programs has the potential to significantly enhance the genetic diversity and adaptability of sorghum.

6.2 Rapid improvement of specific traits through gene editing technology

Gene editing technologies, such as CRISPR/Cas9, have revolutionized the rapid improvement of specific traits in sorghum. These technologies allow for precise modifications at the genetic level, enabling the enhancement of traits such as grain yield, nutritional content, and stress resistance. For example, the assessment of grain protein in tropical sorghum accessions has identified high-protein germplasm that can be targeted for gene editing to develop nutritionally superior varieties (Luo et al., 2020). Furthermore, the genomic characterization of Ethiopian sorghum germplasm has revealed a wealth of genetic diversity and rare alleles that can be harnessed through gene editing to improve traits like abiotic stress tolerance and agronomic performance (Cuevas et al., 2016; Girma et al., 2020). These advancements underscore the potential of gene editing to accelerate the breeding of sorghum varieties with enhanced traits.

6.3 Functional gene mining and application based on germplasm resources

Functional gene mining from sorghum germplasm resources has led to the discovery of genes associated with important agronomic traits. For instance, a comprehensive phenotypic and genomic characterization of Ethiopian sorghum germplasm has identified candidate genes linked to adaptation to different environments, which are crucial for breeding programs aimed at improving stress tolerance (Girma et al., 2020). Additionally, GWAS has been used to identify loci associated with traits such as plant height, panicle compactness, and smut resistance, providing valuable markers for molecular breeding (Girma et al., 2019). The identification of yield-related genes controlling multiple traits further exemplifies the potential of functional gene mining to enhance sorghum breeding efforts (Zhang et al., 2023). These discoveries highlight the importance of leveraging germplasm resources for the identification and application of functional genes in crop improvement.

7 Germplasm Resource Protection and Future Prospects

7.1 Impact of global climate change on the collection and utilization of sorghum germplasm resources

Global climate change poses significant challenges to the collection and utilization of sorghum germplasm resources. sorghum, known for its resilience to harsh conditions, is increasingly seen as a critical crop for food security in the face of climate change. However, the changing climate could push the growing conditions beyond the tolerance limits of even this resilient crop, jeopardizing food security for millions (Chadalavada et al., 2021; Khalifa and Eltahir, 2023). The development of new sorghum varieties that can withstand the new range of biotic and abiotic stresses caused by climate change is essential. This requires access to novel genetic variants, often found in crop wild relatives, which are difficult to work with and require substantial investment in pre-breeding (Mace et al., 2020). Therefore, the impact of climate change necessitates a more robust approach to the collection, evaluation, and utilization of sorghum germplasm to ensure the sustainability of this vital crop.

7.2 Establish a global collaboration mechanism to strengthen the sharing and protection of germplasm resources

To effectively address the challenges posed by climate change and ensure the sustainable use of sorghum germplasm resources, establishing a global collaboration mechanism is crucial. Such a mechanism would facilitate the sharing and protection of germplasm resources across different regions and institutions. The



International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) has already made strides in this direction by developing a mini core collection of sorghum germplasm, which allows for more efficient evaluation and utilization of genetic resources (Upadhyaya et al., 2009). Additionally, the USDA National Plant germplasm System (NPGS) has characterized a core set of Ethiopian sorghum germplasm, highlighting the importance of international cooperation in germplasm conservation and utilization (Cuevas et al., 2019; Cuevas et al., 2023). By fostering global collaboration, researchers can pool resources, share knowledge, and develop strategies to protect and utilize sorghum germplasm more effectively.

7.3 The potential of new technologies in germplasm resource preservation

New technologies offer promising avenues for the preservation of sorghum germplasm resources. Cryopreservation, for instance, allows for the long-term storage of genetic material at ultra-low temperatures, ensuring the preservation of genetic diversity for future use. Digital germplasm banks, which involve the digitization of genetic information, can also play a significant role in germplasm conservation. These technologies enable the efficient storage, retrieval, and sharing of genetic data, facilitating global collaboration and research (Boyles et al., 2018). The integration of genomic resources and high-throughput phenotyping platforms further enhances the ability to connect genotype with phenotype, aiding in the identification and utilization of valuable genetic traits. By leveraging these advanced technologies, the preservation and utilization of sorghum germplasm resources can be significantly improved, ensuring their availability for future crop improvement efforts.

8 Conclusion

The global collection of sorghum germplasm is extensive, with over 236 000 accessions conserved in various genebanks worldwide. Notably, the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and the USDA-ARS hold significant portions of these collections, with ICRISAT alone maintaining over 37 000 accessions. These collections are crucial for preserving genetic diversity and providing resources for crop improvement.

Efforts have been made to develop core and mini core collections to facilitate easier access and more efficient utilization of these vast germplasm resources. For instance, ICRISAT developed a mini core collection comprising 242 accessions, representing 1% of the entire collection, which has been extensively evaluated for various agronomic traits. Similarly, the USDA-NPGS has characterized a core set of Ethiopian sorghum germplasm, revealing high genetic and phenotypic diversity, which is essential for breeding programs.

The evaluation of these collections has focused on identifying traits related to resistance to biotic and abiotic stresses, agronomic performance, and nutritional quality. Advanced genomic tools and high-throughput phenotyping technologies have been employed to map important traits and understand the genetic basis of these traits. This has led to the identification of valuable germplasm with superior traits, which are being utilized in breeding programs to develop improved sorghum varieties.

To enhance the efficiency of utilizing germplasm resources and to innovate breeding strategies, future research needs to conduct comprehensive genomic characterization of the entire germplasm collection. This includes sequencing diverse samples to discover rare alleles and novel genetic variations that can be harnessed for crop improvement. Genomic tools such as genome-wide association studies (GWAS) and genomic selection should be integrated into breeding programs to accelerate the identification and utilization of beneficial traits. At the same time, precise phenotypic analysis is crucial for linking genotype data with phenotypic traits. Developing and deploying high-throughput phenotyping platforms will aid in efficiently screening large germplasm collections for desirable traits, thereby improving the selection process in breeding programs. Core and mini-core germplasm collections should be further refined and utilized to effectively represent the genetic diversity of the entire germplasm collection. These subsets can serve as valuable resources for detailed evaluation and trait mapping, facilitating the discovery of new sources of variation and their incorporation into breeding programs. Additionally, research should explore non-traditional uses of sorghum, such as its potential in the health food market and bioenergy production. Screening germplasm for traits such as high antioxidant levels, gluten-free properties, and suitability for renewable fuel production can open new avenues for the utilization of sorghum.



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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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