12. Breeding Approaches of Improvement in Millets

Shalini V. Nadipalli¹, Sanjay V. Bennur²

 ¹² M.Sc Scholar,
 Department of Genetics and Plant Breeding, School of Agriculture,
 Lovely Professional University, Phagwara, Punab, India.

Abstract:

Millets, a diverse group of small-seeded grasses, have garnered attention for their resilience in challenging agro ecological conditions and nutritional richness. Despite their significance, millets have often been marginalized in agricultural research. This abstract explores the transformative role of genomic approaches in enhancing millet crops, focusing on species like pearl millet, finger millet, sorghum, and foxtail millet. Genomic tools, including molecular markers, next-generation sequencing, genomic selection, and genome editing, have enabled a profound understanding of the genetic basis underlying crucial traits such as drought tolerance, disease resistance, and nutritional content. Molecular markers facilitate marker-assisted selection (MAS), streamlining breeding efforts by identifying and selecting plants with desired traits. Genomic selection, by utilizing comprehensive genomic information, accelerates the development of high-yielding and resilient varieties. The advent of genome editing technologies, particularly CRISPR-Cas9, has ushered in a new era of precision breeding, allowing targeted modification of specific genes to introduce desired traits. The exploration of millet genomes has unraveled genetic diversity, offering a rich resource for trait improvement. Identifying key genes associated with nutritional traits has paved the way for developing biofortified millet varieties, addressing malnutrition challenges. Despite the transformative potential of genomic tools, challenges like accessibility, infrastructure requirements, and the need for skilled personnel must be addressed for widespread adoption. Genomic approaches are pivotal in unleashing the full potential of millets, contributing to the development of resilient, high-yielding, and nutritionally enriched varieties. As global agriculture faces increasing challenges, the integration of genomics into millet breeding holds promise for ensuring food security, sustainable agriculture, and improved public health.

Keywords:

Next-generation sequencing, genomic selection, genome editing, Molecular markers, marker-assisted selection (MAS), CRISPR-Cas9.

12.1 Introduction:

Millets, a diverse group of small-seeded grasses, have emerged as vital crops with immense potential to address contemporary challenges in agriculture, food security, and nutrition. These resilient grains, including species like pearl millet (*Pennisetum glaucum*), finger

Millets: The Miracle Grains of 21st Century

millet (*Eleusine coracana*), sorghum (*Sorghum bicolor*), foxtail millet (*Setaria italica*), and others, have been cultivated for centuries, particularly in arid and semi-arid regions. Characterized by their adaptability to harsh environmental conditions, millets have gained renewed attention due to their nutritional value, low water requirements, and suitability for sustainable farming practices. In the context of a growing global population and the escalating impacts of climate change, the role of millets in diversifying and fortifying our food systems has become increasingly significant.

Their ability to thrive in conditions where other crops falter positions millets as resilient alternatives, capable of withstanding water scarcity, high temperatures, and challenging soils. As traditional staples in many regions, millets contribute not only to food security but also to the livelihoods of millions of smallholder farmers. However, despite their remarkable attributes, millets have often been overlooked in favor of major cereals like rice, wheat, and maize in mainstream agricultural research and development.

This marginalization has hindered the realization of millets' full potential. Recognizing the need for concerted efforts to enhance millet productivity and nutritional quality, scientists and breeders have turned to genomic approaches to drive targeted improvements in these crops.

Genomic tools have revolutionized the field of plant breeding, offering precision, efficiency, and accelerated progress in developing crops with desired traits. In the case of millets, genomic approaches have become instrumental in unraveling the complex genetic basis of various traits, facilitating the development of improved varieties with enhanced yield, nutritional content, and resilience to environmental stressors. One of the primary focuses of genomic research in millets has been to understand the genetic diversity within different species. This diversity serves as a rich resource for identifying traits that can be harnessed to improve crop performance. Through techniques such as molecular markers and next-generation sequencing, scientists can unravel the genetic variations responsible for traits like drought tolerance, disease resistance, and nutritional quality. The exploration of the millet genome has not only unveiled the intricate genetic architecture of these crops but has also provided a foundation for targeted breeding efforts.

Molecular markers, a key component of genomic research, have proven invaluable in millet improvement strategies. By identifying and tracking specific DNA sequences associated with desirable traits, researchers can streamline the breeding process through markerassisted selection (MAS).

For example, in pearl millet breeding programs, molecular markers linked to traits like drought tolerance and grain quality have enabled more efficient screening and selection of plants with the desired genetic makeup. Genomic selection represents another powerful approach that leverages the entire genomic information of an individual to predict its breeding value (Xu *et al.*, 2020). In millets, this method has been particularly beneficial for complex traits influenced by multiple genes. By analyzing large-scale genomic data from diverse millet populations, researchers can predict the performance of different genotypes, accelerating the development of high-yielding and resilient varieties (Zenda *et al.*, 2021). These tools allow scientists to directly edit specific genes within the millet genome, offering

a targeted and rapid means of introducing desired traits. Genome editing has immense potential for enhancing traits like disease resistance and nutritional content in millets, opening new avenues for crop improvement. Critical aspect of millet genomics is the identification of key genes associated with nutritional traits. Millets are inherently rich in essential nutrients, including iron, zinc, and various vitamins. Unraveling the genetic mechanisms responsible for the accumulation of these nutrients allows breeders to develop biofortified millet varieties that can address malnutrition and contribute to improved public health.

While genomic approaches offer tremendous potential, challenges persist in implementing these technologies for millet improvement. The accessibility of genomic tools, infrastructure requirements, and the need for skilled personnel are obstacles that need to be addressed to ensure the widespread adoption of these methods, especially in resource-limited agricultural settings. Millets, with their remarkable resilience and nutritional benefits, are poised to play a crucial role in shaping the future of global agriculture (Singh *et al.*, 2023). Genomic approaches have become indispensable tools in unlocking the potential of millets, enabling scientists and breeders to navigate the intricate genetic landscape of these crops. As we continue to face challenges posed by climate change, population growth, and malnutrition, the integration of genomics into millet breeding holds promise for developing varieties that are not only high-yielding but also well-adapted to the changing agricultural landscape, ultimately contributing to a more sustainable and food-secure future.

The development of climate-smart cereal crops, with a particular emphasis on millets, has become a critical imperative in the face of changing climatic patterns and growing environmental challenges (Hossain *et al.*, 2021). Integrated multi-genomic approaches have emerged as a powerful strategy to fortify cereal crops against the complexities of a shifting climate. In the context of millets, these approaches involve the synergistic application of various genomic tools, including next-generation sequencing, molecular markers, genomic selection, and genome editing. By integrating data from diverse genomic sources, researchers gain a comprehensive understanding of the genetic architecture governing traits crucial for climate resilience, such as drought tolerance, heat stress adaptation, and disease resistance. Molecular markers play a pivotal role in tracking and selecting specific genes associated with climate-smart traits, enabling more efficient breeding through marker-assisted selection (MAS). Genomic selection, utilizing large-scale genomic data, facilitates the prediction of breeding values for complex traits, expediting the development of varieties with enhanced climate resilience.

The precision offered by genome editing technologies, such as CRISPR-Cas9, allows for targeted modifications of key genes, offering a means to directly enhance the adaptive capacity of millets to environmental stressors. Integrated multi-genomic approaches not only unlock the genetic potential within millets but also contribute to the broader goal of sustainable agriculture. By comprehensively analyzing the genomic landscape, these approaches enable researchers to identify genetic variations associated with improved agronomic performance under adverse climatic conditions. As climate change intensifies, the deployment of such integrated strategies becomes essential for securing food production, ensuring the resilience of millets, and promoting climate-smart agricultural practices in figure 12.1.

Millets: The Miracle Grains of 21st Century



Figure 12.1: Integrated multi genomic approaches for development of climate smart cereal crop especially in millets. (Source: Singh *et al.*, 2021)

Pearl millet (*Pennisetum glaucum*) is a resilient cereal crop that plays a crucial role in ensuring food security, especially in arid and semi-arid regions where water scarcity is a significant challenge. Drought is one of the primary constraints affecting pearl millet production, leading to yield losses and compromising food availability for millions of people. In recent years, advancements in genomics have paved the way for a paradigm shift in plant breeding strategies, offering novel approaches to enhance drought tolerance in crops. This article explores the application of genomic tools in pearl millet breeding and their role in developing varieties with improved drought resilience.

12.2 Understanding the Genetic Basis of Drought Tolerance:

Drought tolerance is a complex trait influenced by multiple genetic factors. Traditional breeding methods, while effective, are time-consuming and often lack precision. Genomic tools, such as next-generation sequencing, marker-assisted selection, and genomic selection, have revolutionized the field of plant breeding by providing a deeper understanding of the genetic architecture underlying drought tolerance in pearl millet. One of the key breakthroughs has been the identification of candidate genes associated with drought tolerance. Through techniques like genome-wide association studies (GWAS) and quantitative trait loci (QTL) mapping, researchers have been able to pinpoint specific regions in the pearl millet genome linked to drought-related traits. This information is invaluable for breeders as it allows for the targeted manipulation of these genes to enhance drought tolerance in new cultivars.

12.3 Molecular Markers and Marker-Assisted Selection:

Molecular markers are DNA sequences associated with particular traits of interest. In pearl millet breeding, molecular markers have become indispensable tools for tracking and selecting desirable traits efficiently. Marker-assisted selection (MAS) involves using these markers to identify individuals with the desired genetic characteristics, thereby expediting the breeding process. For drought tolerance, researchers have identified molecular markers

linked to specific genes or QTLs associated with traits like root architecture, water-use efficiency, and stress response. By incorporating these markers into breeding programs, breeders can screen and select plants with a higher likelihood of drought tolerance at the molecular level, significantly accelerating the development of drought-resistant pearl millet varieties.

12.3.1 Genomic Selection:

Genomic selection represents a more advanced approach, utilizing the entire genomic information of an individual to predict its breeding value for a particular trait. This method is particularly advantageous in crops like pearl millet, where multiple genes contribute to complex traits such as drought tolerance. Through the analysis of large-scale genomic data from diverse pearl millet populations, genomic selection models can be trained to predict the performance of different genotypes under drought conditions. This allows breeders to select individuals with superior drought tolerance potential, even before the plants are fully grown. Genomic selection not only accelerates the breeding cycle but also improves the accuracy of trait predictions, leading to more successful development of drought-tolerant cultivars.

12.3.2 Genome Editing Technologies:

The advent of genome editing technologies, such as CRISPR-Cas9, has provided unprecedented precision in manipulating specific genes within the pearl millet genome. This revolutionary tool enables breeders to directly edit the DNA sequences responsible for drought tolerance, offering a level of control and specificity not achievable through traditional breeding methods. By targeting and modifying key genes associated with drought response pathways, researchers can create pearl millet varieties with enhanced drought tolerance. Genome editing also allows for the introduction of beneficial traits from wild or closely related species, further expanding the genetic diversity available for breeding programs.

12.3.3 Challenges and Future Directions:

While genomic tools have significantly accelerated pearl millet breeding for drought tolerance, challenges remain. The implementation of these technologies requires substantial infrastructure, technical expertise, and financial investment. Additionally, ethical considerations surrounding genome editing and the release of genetically modified organisms (GMOs) must be carefully addressed to ensure widespread acceptance and adoption of improved varieties by farmers. In the future, ongoing research may uncover additional genetic factors influencing drought tolerance in pearl millet. Continued advancements in genomic technologies, coupled with collaborative efforts between researchers, breeders, and policymakers, will be essential for overcoming existing challenges and maximizing the potential of genomic tools in pearl millet breeding.

The integration of genomic tools into pearl millet breeding programs has ushered in a new era of precision and efficiency. By unraveling the genetic basis of drought tolerance and leveraging molecular markers, genomic selection, and genome editing technologies,

Millets: The Miracle Grains of 21st Century

researchers are making significant strides in developing cultivars that can withstand the challenges posed by water scarcity. As we move forward, the continued refinement and widespread adoption of these genomic tools hold the promise of not only ensuring food security in arid and semi-arid regions but also contributing to the global effort to address the impacts of climate change on agriculture. The success of genomic tools in pearl millet breeding for drought tolerance serves as a beacon of hope for sustainable agriculture, demonstrating the transformative potential of cutting-edge technologies in securing the future of food production in a changing climate.



Figure 12.2: Next Generation Sequencing and Computational Resources for Improving Millet

Millets are a crucial group of cereal crops that are well known for its high nutritional value. In a world, that is experiencing population growth and large climatic uncertainties, millets are becoming increasingly significant. Millets is a staple diet for around 500 million individuals in over 30 countries, while over 90 million people in Africa and Asia depend on millets as their staple diet. Millets have a long history as a food source and it believed to be one of the earliest cereal grains used for human consumption. The millet group consists of several varieties, including pearl millet (Pennisetum glaucum), small millets such as finger millet (*Eleusine coracana*), Italian or foxtail millet (*Setaria italica*), common or proso millet (Panicum miliaceum), kodo millet (Paspalum scrobiculatum), little millet (Panicum miliare), and barnyard millet (Echinochloa frumentacea). Millets are highly resilient and climate-smart crops that are suitable for environments that are susceptible to drought and extreme heat. They can thrive in various temperatures, moisture conditions, and input conditions, providing food and feed to millions of dryland farmers, primarily in developing countries. Millets are the principal crops cultivated in dry regions where delicate cereals such as wheat and rice are unsuitable for cultivation. Millets are highly valued for their ability to withstand and survive in conditions of continuous or intermittent drought periods caused by low or unpredictable rainfall. Millets, in particular, are one of the only cereal

crops that can thrive in arid lands, requiring as little as 350-400 mm of rainfall annually. Millets have been cultivated for a long time by smallholder farmers in many countries, leading to the development of numerous common and vernacular names for these crops (see Table 12.1).

Crop	Common Names	Place of Origin	Scientific Name	Chromosome No.
Pearl millet	Cumbu, spiked millet, bajra, bulrush millet, candle millet, dark millet	West Africa	Pennisetum glaucum	2n=14 (2x)
Proso millet	common millet, hog millet, broomcorn millet, Russian millet, brown corn	Central and eastern Asia	Panicum miliaceum	2n=36 (4x)
Foxtail millet	Italian millet, German millet, Hungarian millet, Siberian millet	Eastern Asia	Setaria italica	2n=18 (2x)
Finger millet	African millet, koracan, ragi, wimbi, bulo, telebun	East Africa, India	Eleusine coracana	2n=36 (4x)
Barnyard millet	Indian barnyard millet, sawa millet, Japanese barnyard millet	India Japan	Echinochloa utilis	2n=54 (6x)
Little millet	Little millet	Southeast Asia	Panicum sumatrense	2n=36 (4x)
Kodo millet	Kodo millet	India	Paspalum scrobiculatum	2n=40 (4x)

 Table 12.1: Numerous Common and Vernacular Names for Millets

Source: Millets in human nutrition, FAO

12.4 Different Crops with Different Approaches of Improvements:

• Rice:

Rice, a staple food for over half of the world's population, has been the focus of extensive breeding efforts to enhance yield, disease resistance, and nutritional content. Traditional breeding methods, such as selective breeding and hybridization, have played a crucial role in developing high-yielding rice varieties. Additionally, molecular markers and genomic selection are increasingly being employed to expedite the breeding process by identifying and selecting plants with desired traits, including resistance to pests and diseases. Furthermore, genome editing technologies are being explored to precisely modify genes associated with traits like drought tolerance and grain quality, marking a shift towards more precise and efficient improvements in rice crops.

Millets: The Miracle Grains of 21st Century

• Wheat:

Wheat, another major global cereal crop, has undergone various breeding strategies to meet the increasing demand for food. Conventional breeding approaches have been pivotal in developing wheat varieties with improved yield, disease resistance, and adaptability to diverse environmental conditions.

Recently, genomic tools like molecular markers and genomic selection have been integrated into wheat breeding programs to enhance the accuracy and efficiency of trait selection. Genome editing technologies, such as CRISPR-Cas9, hold promise for introducing specific genetic modifications related to traits like gluten content and resistance to fungal diseases, offering a targeted approach to wheat improvement.

• Maize:

Maize, a versatile cereal crop, has been a subject of continuous improvement efforts to address challenges such as pest resistance, drought tolerance, and increased nutritional value. Traditional breeding methods, including hybridization, have contributed to the development of high-yielding maize varieties.

Molecular markers and genomic selection are employed to accelerate the identification and selection of maize plants with favorable traits. Genome editing technologies are being explored to precisely edit genes associated with traits like resistance to pests and diseases, as well as improving the nutritional profile of maize.

• Sorghum:

Sorghum, known for its resilience in arid regions, has been improved through both traditional and modern breeding approaches. Traditional breeding methods have focused on enhancing traits such as drought tolerance and resistance to pests. Genomic tools like molecular markers have facilitated marker-assisted selection for specific traits, while genomic selection has improved the efficiency of breeding programs by predicting the performance of different sorghum genotypes.

Additionally, genome editing is being explored to introduce precise genetic modifications related to stress tolerance, nutrient content, and other agronomically important traits in sorghum.

• Finger Millet:

Finger millet, a hardy and nutritious cereal crop, is traditionally grown in many parts of Africa and Asia. Breeding efforts for finger millet have historically involved selecting plants with desirable traits such as high nutritional content and resistance to diseases. While molecular markers have been utilized to understand the genetic diversity of finger millet, there is increasing interest in applying genomic tools like genomic selection and genome editing to expedite the development of improved varieties with enhanced nutritional value, yield, and adaptability to changing climatic conditions.

In summary, each cereal crop, including rice, wheat, maize, sorghum, and finger millet, undergoes a unique set of breeding approaches and genomic interventions to address specific challenges and meet the evolving needs of agriculture and food security. The integration of molecular markers, genomic selection, and genome editing technologies represents a paradigm shift towards more precise and efficient crop improvement strategies.

Сгор	Traits Improved	Technique Used	Reference
Rice	Submergence tolerance	MAB	Septiningsih <i>et al.</i> (2009)
Rice	Grain number, dense erect panicles and larger grain size	CRISPR Cas9	Li et al. (2016)
Rice	Maintenance of heterosis	CRISPR Cas9	Khanday <i>et al.</i> (2019) Wang <i>et al.</i> (2019)
Wheat	Leaf rust, fusarium head blight and stripe rust resistance	Speed breeding	Alahmad <i>et al.</i> (2018) Dinglasan <i>et al.</i> (2016)
Wheat	Powdery mildew-resistant	CRISPR Cas9	Wang et al. (2014)
Wheat	Heat tolerance	GWAS	Paliwal <i>et al.</i> (2012)
Maize	Kernel row number	RNA sequencing	Jiang <i>et al.</i> (2018)
Maize	High amylopectin content	CRISPR Cas9	Waltz <i>et al.</i> (2016a)
Sorghum	Low and high nitrogen conditions	RNA sequencing	Gelli <i>et al.</i> (2016)
Finger millet	Salt tolerance	RNA sequencing	Rahman <i>et al.</i> (2014)

Table 12.2: Different Crops with Different Approaches of Improvements

12.5 Conclusion:

The diverse array of cereal crops, including rice, wheat, maize, sorghum, and finger millet, reflects the multifaceted challenges faced by agriculture globally. The efforts to improve these crops involve a combination of traditional breeding methods and cutting-edge genomic tools. Traditional approaches, such as selective breeding and hybridization, have played pivotal roles in developing high-yielding varieties with improved disease resistance and adaptability. The integration of genomic tools, including molecular markers, genomic selection, and genome editing, represents a transformative shift in crop improvement strategies. These tools provide a deeper understanding of the genetic basis of important traits, allowing for more precise and targeted improvements. Molecular markers expedite the selection of plants with desired traits, genomic selection enhances breeding efficiency,

and genome editing offers unprecedented precision in introducing genetic modifications. Each cereal crop faces unique challenges, and the specific genomic approaches employed reflect the intricacies of their genetic makeup and environmental interactions. From enhancing drought tolerance in rice and sorghum to improving gluten content in wheat and addressing nutrient deficiencies in finger millet, genomic tools offer tailored solutions to specific issues.

12.6 References:

- 1. Alahmad, S., Dinglasan, E., Leung, K. M., Riaz, A., Derbal, N., Voss-Fels, K. P., ... & Hickey, L. T. (2018). Speed breeding for multiple quantitative traits in durum wheat. *Plant methods*, *14*(1), 1-15.
- 2. Dinglasan, E., Godwin, I. D., Mortlock, M. Y., & Hickey, L. T. (2016). Resistance to yellow spot in wheat grown under accelerated growth conditions. *Euphytica*, 209, 693-707.
- 3. Gelli, M., Mitchell, S. E., Liu, K., Clemente, T. E., Weeks, D. P., Zhang, C., ... & Dweikat, I. M. (2016). Mapping QTLs and association of differentially expressed gene transcripts for multiple agronomic traits under different nitrogen levels in sorghum. *BMC plant biology*, *16*, 1-18.
- Hossain, A., Islam, M. T., Maitra, S., Majumder, D., Garai, S., Mondal, M., ... & Islam, T. (2021). Neglected and Underutilized Crop Species: Are They Future Smart Crops in Fighting Poverty, Hunger and Malnutrition Under Changing Climate? *Neglected and underutilized crops-towards nutritional security and sustainability*, 1-50.
- 5. Jiang, Q., Tang, D., Hu, C., Qu, J., & Liu, J. (2016). Combining meta-QTL with RNAseq data to identify candidate genes of kernel row number trait in maize. *Maydica*, 61(4), 1-9.
- 6. Khanday, I., Skinner, D., Yang, B., Mercier, R., & Sundaresan, V. (2019). A maleexpressed rice embryogenic trigger redirected for asexual propagation through seeds. *Nature*, 565(7737), 91-95.
- 7. Li, M., Li, X., Zhou, Z., Wu, P., Fang, M., Pan, X., ... & Li, H. (2016). Reassessment of the four yield-related genes Gn1a, DEP1, GS3, and IPA1 in rice using a CRISPR/Cas9 system. *Frontiers in plant science*, *7*, 377.
- 8. Paliwal, R., Röder, M. S., Kumar, U., Srivastava, J. P., & Joshi, A. K. (2012). QTL mapping of terminal heat tolerance in hexaploid wheat (*T. aestivum* L.). *Theoretical and Applied Genetics*, *125*, 561-575.
- Rahman, H., Jagadeeshselvam, N., Valarmathi, R., Sachin, B., Sasikala, R., Senthil, N., ... & Muthurajan, R. (2014). Transcriptome analysis of salinity responsiveness in contrasting genotypes of finger millet (*Eleusine coracana* L.) through RNAsequencing. *Plant molecular biology*, 85, 485-503.
- Septiningsih, E. M., Pamplona, A. M., Sanchez, D. L., Neeraja, C. N., Vergara, G. V., Heuer, S., ... & Mackill, D. J. (2009). Development of submergence-tolerant rice cultivars: The Sub1 locus and beyond. *Annals of Botany*, 103(2), 151-160.
- 11. Singh, R. K., Muthamilarasan, M., & Prasad, M. (2021). Biotechnological approaches to dissect climate-resilient traits in millets and their application in crop improvement. *Journal of Biotechnology*, *327*, 64-73.
- Singh, S., Yadav, R. N., Tripathi, A. K., Kumar, M., Kumar, M., Yadav, S., ... & Yadav, R. (2023). Current Status and Promotional Strategies of Millets: A Review. *International Journal of Environment and Climate Change*, 13(9), 3088-3095.

- 13. Waltz, E. (2016). CRISPR-edited crops free to enter market, skip regulation. *Nature Biotechnology*, *34*(6), 582-583.
- 14. Wang, C., Liu, Q., Shen, Y., Hua, Y., Wang, J., Lin, J., ... & Wang, K. (2019). Clonal seeds from hybrid rice by simultaneous genome engineering of meiosis and fertilization genes. *Nature biotechnology*, *37*(3), 283-286.
- 15. Wang, Y., Cheng, X., Shan, Q., Zhang, Y., Liu, J., Gao, C., & Qiu, J. L. (2014). Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. *Nature biotechnology*, *32*(9), 947-951.
- 16. Xu, Y., Liu, X., Fu, J., Wang, H., Wang, J., Huang, C., ... & Zhang, A. (2020). Enhancing genetic gain through genomic selection: from livestock to plants. *Plant Communications*, 1(1).
- 17. Zenda, T., Liu, S., Dong, A., & Duan, H. (2021). Advances in cereal crop genomics for resilience under climate change. *Life*, *11*(6), 502.