Unlocking Nutritional Potential: Multi-OMICS Strategies for Enhancing Millet Nutritional Traits

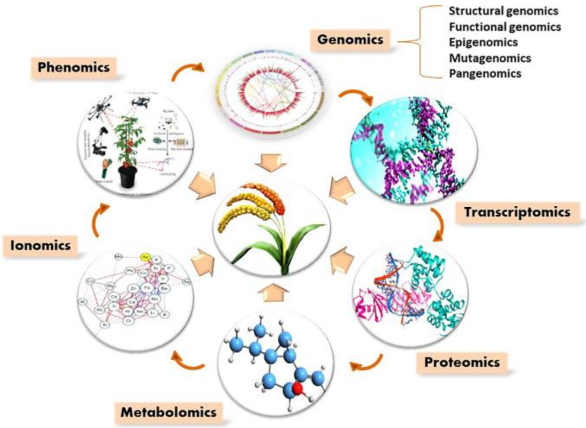
Abstract

Millet grains have long been recognized for their nutritional significance, serving as staple foods for millions around the world. However, unlocking their full nutritional potential remains a crucial challenge. In this study, we explore the application of multi-OMICS (genomics, transcriptomics, proteomics, metabolomics, and phenomics) strategies to enhance the nutritional traits of millet grains. By integrating comprehensive molecular datasets, we aim to elucidate the genetic and biochemical pathways governing key nutritional attributes such as protein content, amino acid composition, micronutrient density, and antioxidant capacity in millets. Through targeted breeding and biotechnological interventions informed by multi-OMICS analyses, we seek to develop improved millet varieties with enhanced nutritional profiles and agronomic performance. This interdisciplinary approach holds great promise for addressing malnutrition and promoting food security, particularly in regions where millets are dietary staples. By harnessing the power of multi-OMICS technologies, we aim to unleash the full nutritional potential of millets, contributing to the development of sustainable and resilient food systems for future generations.

Keywords: multi, millets, future, interventions, amino acids, datasets

Introduction

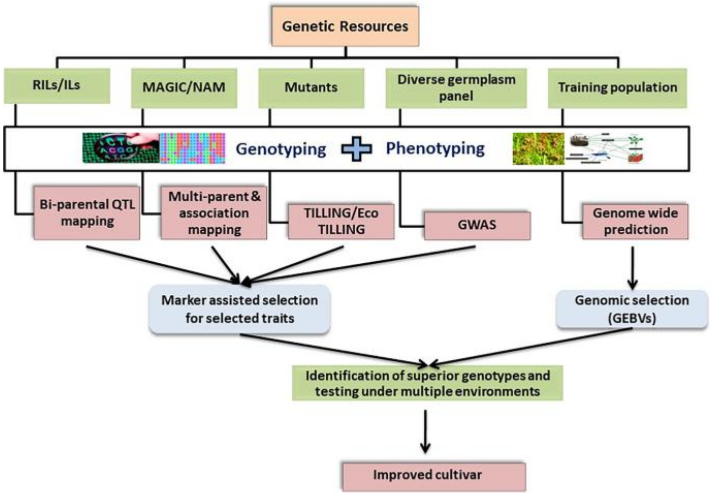
Millets are a kind of annual grass that are separated into main and minor classes. They are characterized by the production of very small seeds. It is important to note that pearl millet (Pennisetum glaucum) and sorghum are considered major millets, whilst other millets are classified as minor millets[1]. Among the minor millets, the following millets are considered to be significant: finger millet (Eleusine coracana), foxtail millet (Setaria italica), small millet (Panicum sumatrense), proso millet (Panicum miliaceum), kodo millet (Paspalum scrobiculatum), and barnyard millet (Echinochloa esculenta). The fact that millets have a C4 physiology and are members of a subfamily of the Poaceae family, namely the Panicoideae and Chloridoideae families, is one of the factors that contributes to their exceptional resistance to severe weather conditions[2]. Because of this, millets are compatible with subsistence farming and have the ability to thrive in an area that is rainfed. Their climatic resilience is shown by the fact that they are able to thrive in harsh growth circumstances, such as severe drought, high temperatures, poor soil fertility, salty soils, and even acidic soils. In addition, millets have exceptional mineral nutrients, fibers, and a high shelf life, which makes them the crop of choice in situations involving global warming[3].



Millets were produced on a global scale in the amount of 89.17 million metric tons, which was grown over an area of 74.00 million hectares. India is the country that produces the most millet, with a total output of 15.53 million tons and an area of 12.45 million hectares dedicated to millet cultivation[4].

After Ukraine, which exports millet at a value of 46.30 million dollars, India is the second-largest exporter of millet. Farmers from all over the world have been cultivating millets, which have a wide range of geographical distributions. Pearl millet is the millet that is grown the most extensively among all millets, accounting for around fifty percent of the millet growing area worldwide. When it comes to cereals, it is the fifth most significant crop in the world, and it is the most important crop in arid and semi-arid areas[5]. Throughout a similar manner, foxtail millet is grown throughout southern Europe and Asia. It is believed to be one of the oldest crops in the world and is ranked second in terms of total millet output worldwide.

Millets have greater agronomic and climate resistance features when grown in severe climatic circumstances, as shown by their worldwide spread and dependence on subsistence farmers. Millets also have a lower water need[6]. On the other hand, in order to achieve the criteria for food and nutritional security in the ever-changing climate circumstances, we need a crop that is resistant to climate change and requires a minimal amount of inputs. As a result, millets have garnered the attention of the scientific community. Millet improvement has a significant obstacle in the form of the efficient characterization of genetic and genomic resources, as well as the use of variants that are particular to traits[7]. The extension of genetic resources may be accomplished via crop improvement initiatives, accurate, efficient, and cost-effective germplasm characterization, and the expansion of genetic resources in order to address the deficiencies in millet research[8]. The improvement of millet is traditionally controlled by breeding programs that are based on phenotypic selection and are used in environments with a high level of environmental noise. In order to develop crop varieties, a prolonged amount of time (five to twelve years) was necessary, and the process was less successful for complicated and low heritable features[9]. The generation of molecular markers that are suitable for the investigation of genetic links among genotypes and the creation of genetic maps for targeted gene and genome-wide association studies may be accomplished via the process of genotyping by sequencing and whole-genome sequencing. The purpose of this study is to provide a summary of the work that has been done to enhance millet with the assistance of contemporary sequencing technologies and to contribute to our understanding of the consequences and uses of these technologies in the future[10].



Global hunger has been on the rise since 2014, and it is estimated that between 720 and 811 million people would be confronted with hunger by the year 2020. Global hunger is caused by a number of key variables, including climate change and the occurrence of regular severe weather events. It is anticipated that climate-affected grain output would reach between 1% and 7% by the year 2060[11]. Developing crops that are able to withstand a high level of abiotic stress is very necessary in order to guarantee a sufficient food supply in the future. When it comes to yield, millets, which are a collective name for coarse grains, are an important crop that ranks sixth in terms of production. For the year

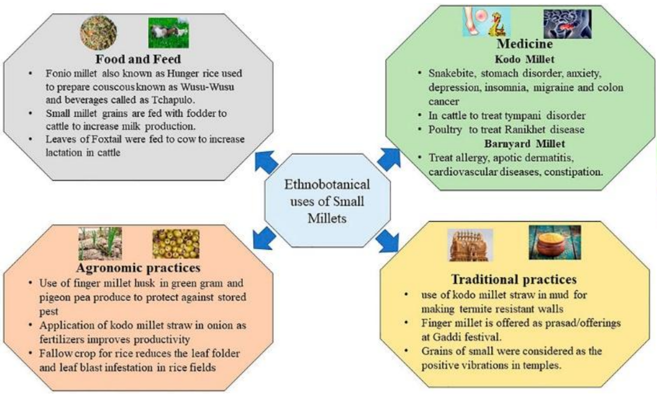
2018, it was predicted that 31 million tons of millet were produced, with more than 96% of that amount being farmed in locations with low soil fertility and inadequate rainfall[12].

Pearl millet, finger millet, foxtail millet, proso millet, barnyard millet, tef, fonio, and Job's tears are some of the eleven genera that are included in the classified millet family. It is possible that the genes that confer tolerance to abiotic stress in millets might be transferred to other key Poaceae crops, such as sorghum, maize, and rice, because of the tight phylogenetic link that exists between millets and these other main crops. One example is the transformation of a glutathione peroxidase gene from pearl millet into rice, which resulted in increased resistance to salt and drought[13].

The use of high-throughput sequencing technology has made it possible to read the genomes of millets, which has resulted in the collecting and examination of multi-omics data derived from millets. This data presents new potential for the advancement of genetic enhancement of stress tolerance in important crops and the development of innovative information about the tolerance of plants to abiotic environments. However, the collecting and analysis of this data are time-consuming, particularly for researchers who lack knowledge in bioinformatics and computational resources. This makes the job of data mining of essential stress-tolerance genes a difficult one to do[14].

Historical importance of millets

Millets of a smaller size have a strong cultural and historical value, and they play an important part in the celebrations that take place in tribal communities and temples. These grains were recognized by our predecessors for the nutraceutical and medicinal properties that they were known for. It is common practice in Africa to utilize fonio millet, which is often referred to as "hungry rice," to make a kind of couscous known as wusu-wusu, which is composed of minerals[15]. Similar to how it is employed in the production of beer, finger millet is also used in the production of malted items, which are often utilized in African tribal groups. Within the Eastern Ghats region of India, the Malayali people continue to cultivate and preserve tiny millet landraces. Additionally, the Kolli Hill tribes are responsible for the preservation of landraces of little, foxtail, and proso millets. It seems that these millets include unique alleles, which might be useful for future breeding operations, based on their properties[16]. In the Indian state of Chhattisgarh, little millets have been used for such purposes as the construction of termite-resistant walls, the fertilization of onion fields, and the prevention of insect infestations during storage. For the purpose of baking pots, pot manufacturers in Northern India also make use of straw made from finger millet. The leaves of Kodo millet contain lecithin, which is a compound that is used in the treatment of snakebites, gastrointestinal issues, and joint swelling[17]. The use of Kodo millet straw in the treatment of tympani problem in cattle and Ranikhet illness in poultry has been shown to have remarkable effects. Historically, little millets were combined with fodder in order to boost the amount of milk produced by cows. In recent years, agro-startups in India have begun to use this method in order to encourage increased milk production in rural dairy farms. As part of the Gaddi festival, finger millet is often used as a prasad or offering, and thick pastes made from finger millet flour are used for the treatment of fire burns[18]. In addition to treating blood-related problems, allergies, and atopic dermatitis, barnyard millet is also used to treat cardiovascular diseases, constipation, and other conditions. For the treatment of anxiety, depression, insomnia, migraines, and colon cancer, kodo millet is the nutritional supplement of choice. Chicken pox, heart attack, fever, cholera, and stomach disorders are some of the conditions that may be treated with foxtail millet. These activities highlight the nutritional and therapeutic benefits of tiny millets in our ancestry, and the genetics that underlie these characteristics may be investigated and used to build diets that are more sustainable[19].



Ethnobotanical uses of Small Millets

Food and Feed

- Fonio millet also known as Hunger rice used to prepare couscous known as Wusu-Wusu and beverages called as Tchapulo.
- Small millet grains are fed with fodder to cattle to increase milk production.
- Leaves of Foxtail were fed to cow to increase lactation in cattle

Medicine

Kodo Millet

- Snakebite, stomach disorder, anxiety, depression, insomnia, migraine and colon cancer
- In cattle to treat tympani disorder
- Poultry to treat Ranikhet disease

Barnyard Millet

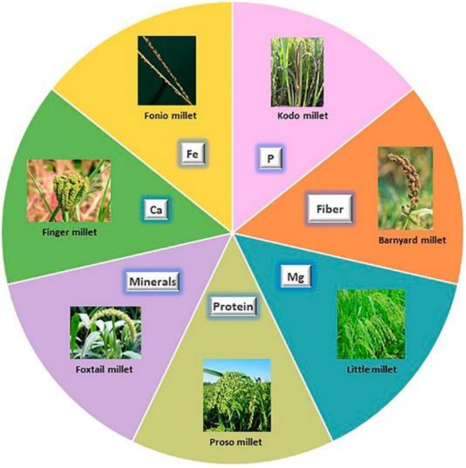
- Treat allergy, apotic dermatitis, cardiovascular diseases, constipation.

Agronomic practices

- Use of finger millet husk in green gram and pigeon pea produce to protect against stored pest
- Application of kodo millet straw in onion as fertilizers improves productivity
- Fallow crop for rice reduces the leaf folder and leaf blast infestation in rice fields

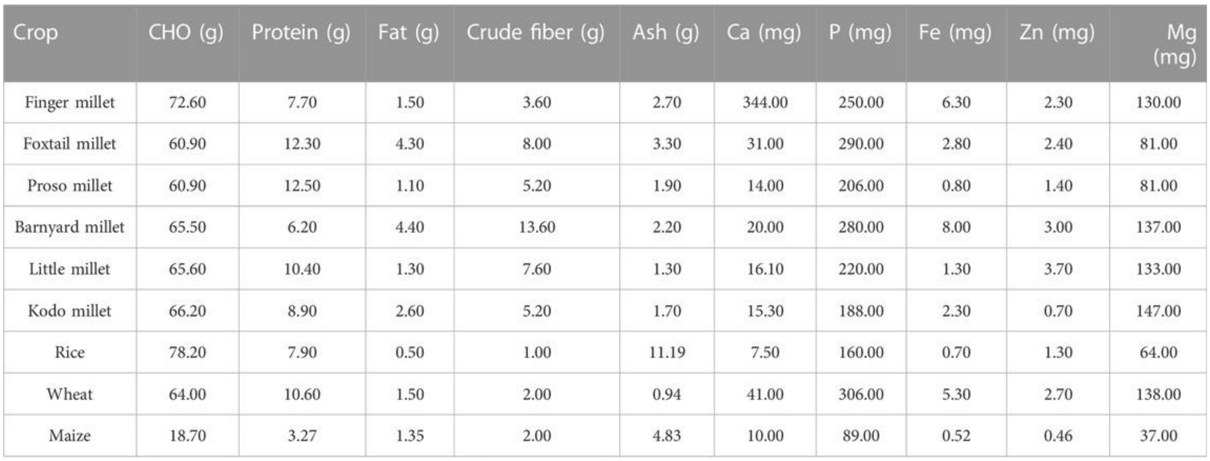
Traditional practices

- use of kodo millet straw in mud for making termite resistant walls
- Finger millet is offered as prasad/offerings at Gaddi festival.
- Grains of small were considered as the positive vibrations in temples.



Nutritional value in millets

A substantial crop that offers great nutritional advantages, especially in terms of micronutrient and protein profiles, little millets are a key agricultural option. Patients suffering from diabetes and celiac disease might benefit greatly from these species since they do not contain gluten and have a greater fiber content than other vegetables. This cultural legacy is still being preserved by traditional farmers via the use of festivals and customs[20]. The pandemics that are on the rise and the weather that has never been seen before have caused a reevaluation of the nutritional advantages that these crops provide. Finger millet and foxtail millet are the two types of tiny millets that have bigger cultivation areas. Finger millet is well-known for the calcium, phosphorus, iron, and zinc that it contains, but foxtail millet is chosen due to the amount of protein and minerals that it has[21]. A low glycemic index, high phosphorous and magnesium content, and high phosphorous content are all characteristics of barnyard millet. On the other hand, Kodo millet has the greatest phosphorous content and the strongest radical scavenging activity. In contrast to Proso millet, which has a high protein profile and necessary amino acids, little millet is abundant in polyunsaturated fatty acids (PUFA) and flavonoids[22]. The high levels of iron, dietary fiber, crude protein, flavonoid, GABA, and riboflavin that are found in fonio millet make it a popular grain in African countries. Another miracle millet that can be found in Africa is teff, which has the smallest grain size and the greatest calcium and iron content respectively. Because of the value-added goods that are being produced, the demand for tiny millets is growing in emerging countries. As a result, the commercial manufacture of their products have to take into consideration their quality characteristics[23]. Because tiny millets are cereals with small grains and a lesser milling recovery than other grains, it is important to ensure that the seeds are of a consistent size and are not fractured. The grains of tiny millets may vary in colour from light yellow to brown, with the majority of customers preferring grains with a light yellow to brown tint for use in flour and cooking. Furthermore, in comparison to black grains, yellow grains have a higher level of aromatic properties. Kodo millet, tiny millet, barnyard millet, and foxtail millet are the types of small millets that have been shown to have the greatest resistant starch values[24]. Small millets are also an excellent source of resistant starch. These grains have the potential to be used in a variety of sectors for the production and processing of resistant starch, as well as for their nutraceutical value. In order to make rice analogues from tiny millet flours, heat extrusion and gelatinization are used. These methods result in rice analogues that are more desired than regular rice kinds. Small millets are particularly well-liked for use as animal feeds because, in addition to their nutritional advantages, they also have value as fodder and forage. On the other hand, rancidity is a problem when it comes to storing these flours in homes, which is why more improved breeding procedures are required in order to extend the shelf life of tiny millet flour alternatives[25].



Crop	CHO (g)	Protein (g)	Fat (g)	Crude fiber (g)	Ash (g)	Ca (mg)	P (mg)	Fe (mg)	Zn (mg)	Mg (mg)
Finger millet	72.60	7.70	1.50	3.60	2.70	344.00	250.00	6.30	2.30	130.00
Foxtail millet	60.90	12.30	4.30	8.00	3.30	31.00	290.00	2.80	2.40	81.00
Proso millet	60.90	12.50	1.10	5.20	1.90	14.00	206.00	0.80	1.40	81.00
Barnyard millet	65.50	6.20	4.40	13.60	2.20	20.00	280.00	8.00	3.00	137.00
Little millet	65.60	10.40	1.30	7.60	1.30	16.10	220.00	1.30	3.70	133.00
Kodo millet	66.20	8.90	2.60	5.20	1.70	15.30	188.00	2.30	0.70	147.00
Rice	78.20	7.90	0.50	1.00	11.19	7.50	160.00	0.70	1.30	64.00
Wheat	64.00	10.60	1.50	2.00	0.94	41.00	306.00	5.30	2.70	138.00
Maize	18.70	3.27	1.35	2.00	4.83	10.00	89.00	0.52	0.46	37.00

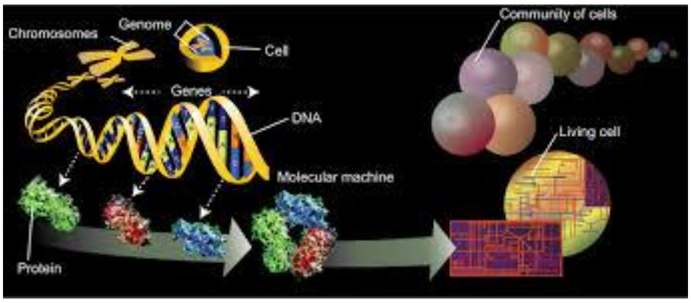
Sources: Dey et al., 2022; Gopalan et al., 2009; Geervani and Eggum, 1989; Longvah et al., 2017.

Concept of omics

Biotechnological approaches to overcome abiotic constraints require a comprehensive understanding of the target crop species and stress tolerance mechanisms, which includes measurements from the whole plant to the molecular level. Plant stress response is influenced by the type, duration, and severity of stress, and recent efforts have focused on decoding the molecular response of plants to abiotic stresses[26]. Model plants like Arabidopsis and rice were the focal point of research on plant stress responses, while orphan crops like millets lagged behind. Recent progress in this area has been significantly enhanced by the development of foxtail millet as a model system to investigate evolution, physiology, and the genetics of stress tolerance in millets. Foxtail millet's small genome size, diploidy, self-polination, short generation time, and efficient transformation platform have made it an ideal choice for molecular genetic studies in millet crops[27].

However, sequencing information alone is not enough to resolve queries pertaining to stress-responsive gene function, regulatory networks, and biochemical pathways involved. More comprehensive approaches such as quantitative and qualitative analyses of gene expression products at the transcriptionomic, proteomic, and metabolomic levels, along with bioinformatics and systems biology approaches, are necessary to address these issues.

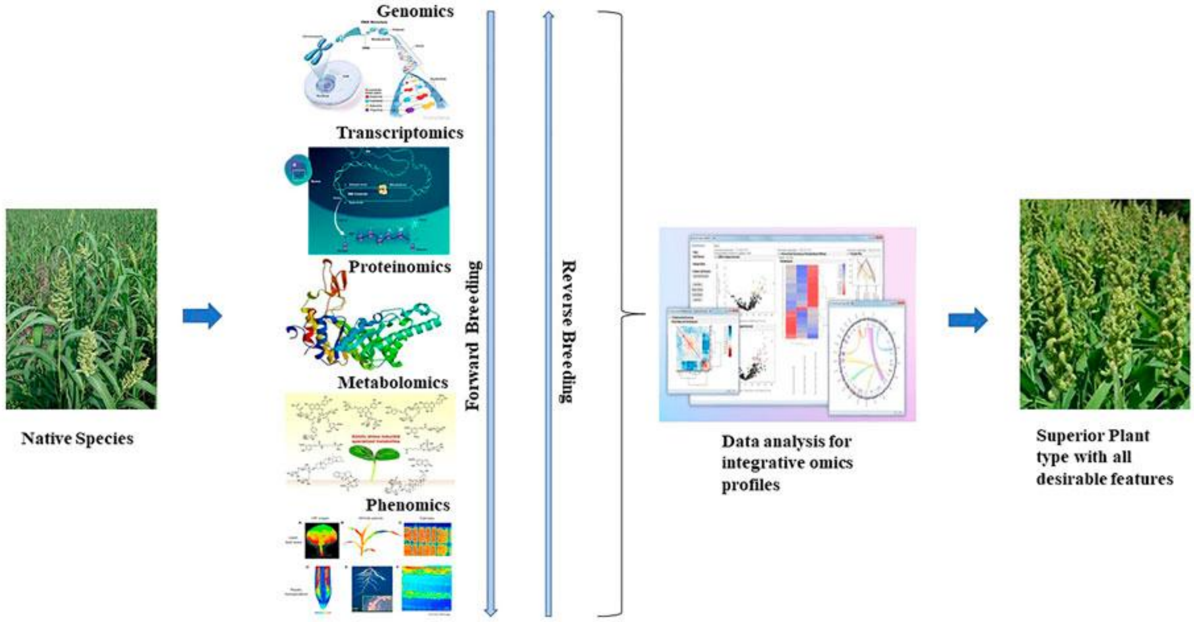
Omics refers to all biotechnological applications that require knowledge of stress response at molecular level, including genomics, functional genomics, genetic engineering, gene expression, protein or metabolite profiles, and their overall phenotypic effects in response to environmental perturbations. Recently, 'omic' approaches have been efficiently utilized to understand the molecular and genetic basis of abiotic stress perception, response, and tolerance in millet crops[28].



Multi-omics techniques accelerate the genetic dissection of complex traits

Genomics regarding millets

The process of genetically deconstructing agronomic variables and determining the phenotypic variations that correlate to those features is essential for making correct breeding designs. DNA sequencing technology has led to considerable improvements in sequencing throughput and quality over the last four decades. These advancements have resulted in cost reductions and have made it easier to progress genomic research and functional analysis. Plant genomes have been decoded by the use of next-generation sequencing (NGS), also known as massively parallel sequencing (MPS)[29]. There have been less than 300 complete genome assemblies at the chromosomal level, which represent about 900 species belonging to the green plant group. Consequently, this has made available a wealth of genetic diversity resources for the purpose of genomic breeding of crops. Recent developments in high-throughput sequencing technology have made it possible to access the reference genome in over 800 different plant species. The majority of these plants have been built from scratch using third-generation sequencing (TGS)[30]. Pacific Biosciences (PacBio) and Oxford Nanopore Sequencing (ONT) platforms have been instrumental in the fast development of the pan-genome over the last twenty years. The pan-genome was first suggested in 2005. Both presence/absence variations (PAVs) and structural variations (SVs) are examples of complicated features that may be dissected using structural differences that are discovered by pan-genome research. These differences play an important part in the process. Given these structural disparities, it is necessary to revaluate the foundations upon which phenotypes are built[31].

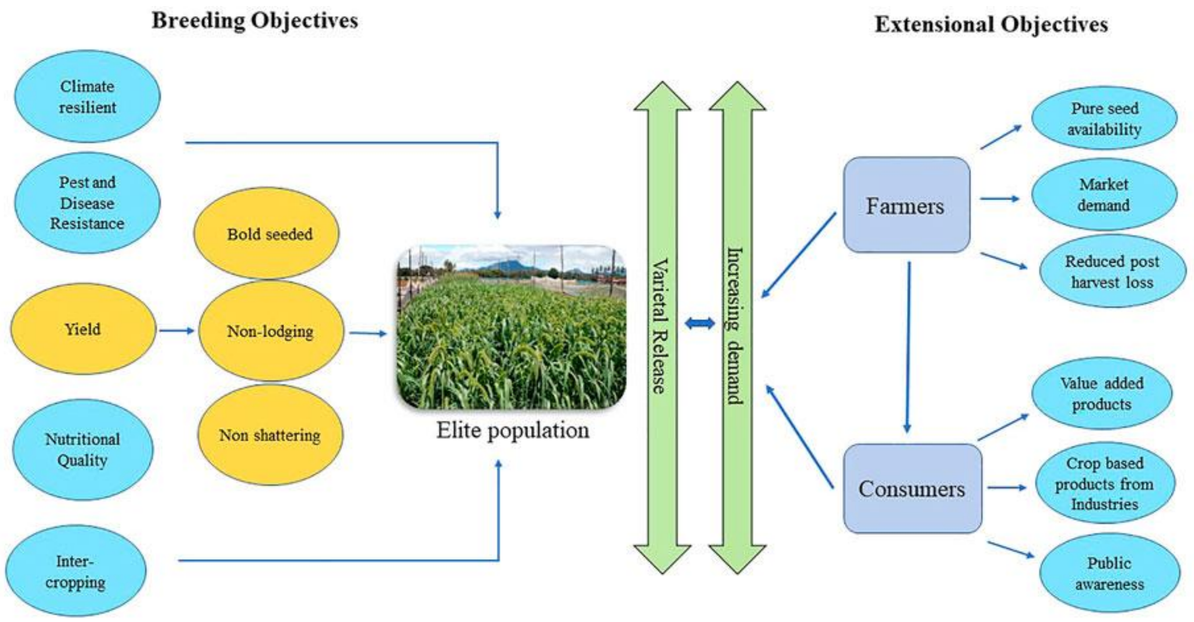


It has been shown that SVs are connected with a broad spectrum of biotic and abiotic stress tolerance in a variety of crop species. A complex SV that included numerous transposable elements (TEs) was responsible for inducing resistance to the sugarcane mosaic virus in maize. This resistance was produced at the Scmv1 locus. Resistance to late blight in potato was gained by two genes, R1 and ELR, which were introgressed from wild potato but were absent in cultivated potato[32]. These genes were responsible for the acquisition of resistance. Through the use of graph-based pan-genomes, re-sequenced data have been reinvigorated in the process of intercepting new genetic variations, particularly for big specific variants. Research on the rice pangenome has shown that SVs and gene copy number variations (gCNVs) facilitated environmental adaptation and artificial selection throughout the process of evolution and domestication. This was discovered in rice. SVs may be valuable for identifying heterotic groups and choosing parental lines for the creation of hybrid crops, as shown by a previous research that was conducted on 25 inbred lines of maize[33]. The study found parallels between PAVs and participation in heterotic groups. The comprehensive genome composition heterogeneity that is outlined by genomes and pan-genomes makes it easier for plant scientists to investigate functional alleles for phenotypic variations and for breeders to increase genetic resources to enhance crop germ plasm in changing climates. In general, this makes it possible for novel methodologies to be established[34].

Epigenomics

Understanding the changes in gene regulation that occur as a result of epigenetic alterations of DNA sequences has become more important thanks to the development of epigenomics as a tool. Through the use of these modifications, such as DNA methylation, histone modifications, and chromatin accessibility, we are able to get a better understanding of how plants adapt to changes in their environment without causing any changes to their DNA sequences[35]. In order to explore the methylation status that is involved in the regulation of gene expression in plants that are subjected to environmental challenges, it is usual practice to make use of techniques such as whole genome bisulfite sequencing (WGBS), methyl-sensitive amplification polymorphism (MSAP), and methylated DNA immunoprecipitation (MEDIP). Mulberry, mungbean, and rice are examples of drought-resistant plants that have a more stable methylome. These plants include differentially methylated regions (DMRs) that connect genes that are predominantly engaged in programmed cell death and stress response. Rice genotypes that were subjected to high temperatures were analyzed using differentially methylated epiloci (DME), which revealed the presence of twelve stress-related genes. When alfalfa plants were subjected to salinity stress, the methylome level of the plants rose, and the treatment with 5-AzaC decreased their salt tolerance[36].

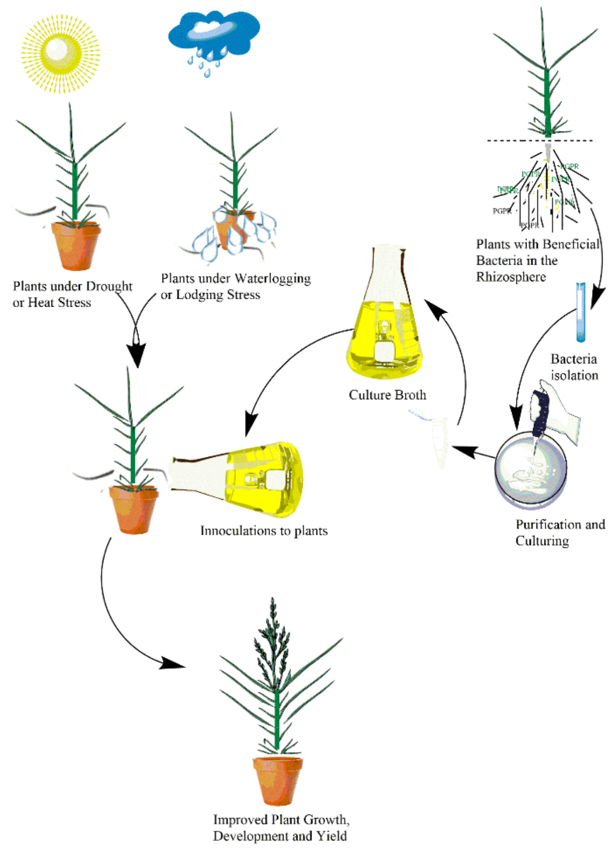
Histone modifications and chromatin remodeling are two essential epigenetic mechanisms that regulate gene expression by modifying the chromatin state and activating transcription regulators. Both of these processes are seen in bacteria. The use of immunoprecipitation is essential for the detection of epigenome-level histone changes. Chromatin immunoprecipitation sequencing (ChIP-seq) and its modified variant, ChIP-exo, are two examples of immunoprecipitation techniques[37]. ChIPmentation, CUT&Tag, and DamID are a few more approaches that may be used for histone profiling. For the purpose of determining the association between DNA-methylation sites and histone proteins, ChIP-seq is a technique that is often used. Chromatin accessibility profiling has been implemented in a wide variety of plant species by the use of DNase-seq, Mnase-seq, FAIRE-seq, and ATAC-seq procedures. Studies on chromatin accessibility have made it feasible to construct transcriptional networks that react to changes in the environment in rice, A. thaliana, maize cold-tolerant lines under stress, and grapevine. These networks are able to adapt to environmental changes[38]. The development of organs and the differentiating of cells are both affected by epigenetic alterations, which are also present in all animals. The findings of these research add to our knowledge of how plants react to environmental signals by altering the expression of their genes, as well as how chromatin-based control of gene expression is likely to be a crucial component of these responses[39].



Transcriptomes

The study of the whole collection of RNA transcripts that are produced by the genome of an organism to be found in a cell or tissue is referred to as transcriptomics. Not so long ago, it has been used widely in crop breeding for the purpose of investigating gene expression under a variety of situations. Traditional methods of profiling, such as differential display-PCR (DD-PCR), cDNAs-AFLP, and SSH, have been shown to have a low level of resolution. Because of the development of enhanced throughput sequencing, plant scientists are now able to perform substantial study on transcriptomics. RNA-seq, which makes use of NGS technologies, makes it feasible to define the transcriptome with more precision than is achievable with microarrays[40]. Studies of the transcriptome also include a number of other types of non-coding RNAs, including micro RNAs (miRNAs), circular RNAs (circRNAs), long non-coding RNAs (lncRNAs), ribosomal RNAs (rRNAs), and short interfering RNAs (siRNA). These non-coding RNAs are responsible for a variety of functions, including the following: microRNAs are involved in the slicing and post-transcriptional modification of target mRNAs; long non-coding RNAs serve as important regulators in several vital biological processes; and circRNAs act as miRNA sponges, transcriptional and splicing regulators, and moderators of primary gene expression in plants. For the purpose of constructing the global landscape of these mRNAs in plants, such as maize, Chinese cabbage, and citrus, whole-transcriptome sequencing in plants was used[41].

Studying the reactions of plants' transcriptomes at the cellular level has been of the utmost importance in recent years. This is due to the fact that various cell types perform distinct biological tasks throughout the process of plant growth and development. For the purpose of researching plant functional genomes and transcriptional activity at the single-cell level, a high-resolution technique known as single-cell RNA-sequencing (scRNA-seq) is gaining more and more popularity[42]. Researchers have been able to achieve advancements in plant hybridization, polyploid genetics, and reproductive biology by using this technology, which has allowed them to investigate heterogeneity in plants across various cell types. On the other hand, up to this point, much fewer studies have been conducted on the high-throughput single-cell transcriptome in plants than there have been in mammals. It is possible that this information may be put to greater use in breeding programs that have unique and focused aims to aid in agricultural output and quality, plant adaptations to climate change, and plant tolerance to biotic and abiotic stresses[43].



Resource developments in the field of "multi-omics" for the purpose of mainstreaming orphan millet crops

Millets, which are examples of orphan crops, possess certain qualities that may assist in increasing their output and lowering the rates of food insecurity and malnutrition they cause. On the other hand, as compared to other cereal crops, the genetic resources needed for orphan crops are behind the times, which results in gradual advancements. As a main input for breeding, genetics is an essential component, and genomics is an effective instrument for characterization[44]. In spite of the fact that genomic resources knowledge on molecular markers and physical/genetic maps for orphan crops, such as millets, is growing, the efforts that are being made to develop these materials are insufficient, which hinders their progress.

Recent developments in next-generation sequencing (NGS) and the availability of whole-genome sequencing (WGS) have contributed to a better understanding of the genetics of many characteristics as well as the mapping of quantitative trait loci (QTLs associated with those traits)[45]. Foxtail millet, finger millet, proso millet, teff, and Japanese barnyard millet are the five tiny millets that have had their genomes made accessible to the public. Millets have been shown to have a number of alleles, QTLs, and new genes, which together have shed light on the functions that these genes play in imparting stress tolerance, nutritional, and agronomic characteristics[46].

Researchers are motivated to conduct high-throughput investigations because of the genetic advancements that are based on WGS on millets. With the information that was obtained from these investigations, it is possible that the functions of genes that are involved in nutrient signalling and abiotic stress responses might be investigated. Additionally, plant breeders could use this information to generate better millet varieties[47].

The field of genomics has made tremendous advancements in the study of millets, with studies using relatively novel methodologies including as transcriptomics, proteomics, metabolomics, fluxomics, and ionomics. The information that we have about the connection between genotype and phenotype has been considerably enhanced as a result of rapid technological advancement and high-throughput sequencing methods that are user-friendly[48].

In the field of plant genetics, the functional genomics technique known as transcriptomics has been extensively used, resulting in the discovery of genes that are expressed differently in a variety of biological processes when subjected to certain environmental conditions. It was the first millet whose transcriptome was made public by the sequencing of the total RNA of the root, stem, leaf, and spike. Foxtail millet was the recipient of this honour[49].

Due to the fact that the number and expression of RNAs do not immediately correspond with the amount of proteins and metabolites, proteomics and metabolome profiling are vital for understanding the functional features and cellular metabolism of organisms. To get an understanding of the flow of biological information that lies underneath complicated characteristics, it is necessary to use a systems biology approach that incorporates the integration of numerous omics data, modelling, and the prediction of physiological activities[50].

The study of proteomics allows for the straightforward investigation of intricate cellular biochemistry by gaining a grasp of the structure and function of proteins under certain circumstances. Proteins operate as linkages between the transcriptome and metabolomic profiles, allowing for the dissection of the real physiological and metabolic status of their respective cells. An in-depth research of these proteins may be able to outline the process of cellular adaptation under stressful circumstances. Long-term cellular adaptation is mediated by the synthesis of "stress-responsive proteins," which are proteins that are produced in response to stress[51].

The word "metabolism" refers to the comprehensive analysis of the metabolome, which is defined as the whole collection of tiny biomolecules or intermediates that are produced by cellular metabolism in a biological sample. It may be impacted by changes in the transcriptome and proteome, but it can also be affected by the chemical, physical, and nutritional environment of a cell. It is determined by the coding capacity of the genome of a cell, and it can be affected by changes in the transcriptome and proteome[52].

In the field of fluxomics, the many approaches that are used to ascertain the rates of metabolic processes that occur in living organisms are referred to as metabolic flux. Metabolomics and fluxomics are two types of omics technologies that supplement other omics technologies at the metabolite level. Both of these technologies are directly tied to the physiology and phenotype of an organism, individual organ, or cell[53].

Millets are a crop that is widely suited to low-input agriculture and exhibit a significant amount of genetic diversity for essential mineral elements such as calcium, zinc, and iron. In order for stress-resilient and nutritionally superior features to work properly, the availability of nutrients (minerals/ions) and their accumulation are the primary factors that determine their effectiveness. In the process of radial transfer of nutrients and water from the soil to the root vasculature, membrane transport proteins play a vital role in the process of nutrient and water intake from the soil[54].

Due to the fact that disadvantaged farmers are unable to pay costly fertilizers and the manufacture of these fertilizers is energy-intensive and harmful to the environment, it is of the utmost importance to improve the efficiency with which crops utilize nutrients. Ionomics, which is the study of the metal, non-metal, and metalloid compositions of cells, has the potential to give novel methods for finding genes and proteins as well as their regulatory pathways that are associated with mineral accumulation, transportation, cross-talk, and stress resistance[55]. The assessment of mineral contents has been made possible by high-throughput electronic analytical methods such as inductively coupled plasma-atomic emission spectroscopy (ICP-AES) and inductively coupled plasma-mass spectrometry (ICP-MS)[56].

Using bioinformatics methods, researchers have found a number of different mineral nutrient transporter proteins in finger millet. There was an up-regulation of metabolic pathways in pearl millet that was subjected to drought stress, according to a comparative transcriptome study conducted under drought stress. In a different research, 63,090 and 26,299 unique transcripts and genes were discovered, which led to a better understanding of the processes that pearl millet uses to tolerate drought[57].

A comparative and quantitative proteomic analysis was conducted on foxtail millets, and the results showed that there were 2,474 proteins that were differently expressed under drought stress conditions. Of these proteins, 321 were substantially expressed (252 were up-regulated, and 69 were down-regulated), and they were classified as stress and defence responsive proteins[58]. In addition, proteins and enzymes that play a role in the processes of photosynthesis and carbon metabolism, the elimination of reactive oxygen species (ROS), the production and control of proteins, the metabolism of fatty acids and amino acids, polyamine biosynthesis, hormone metabolism, and alterations to cell walls were discovered. Through the use of LC-MS-based target metabolome profiling in foxtail millet, Li et al. (2018) conducted an investigation into the comprehensive profile, natural variation, and species-specific accumulation of primary and secondary metabolites[59].

Transgenic breeding, mutant breeding, and crossbreeding are the three primary methods that are now being used in contemporary agriculture for the purpose of crop development. On the other hand, the stochastic character of these techniques places limitations on them, which makes them labor- and time-intensive. Crop development initiatives have been changed as a result of advancements in genome editing, notably clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (CRISPR/Cas9)[60]. However, despite the scientific advancements that have been shown in the CRISPR/Cas9 system for crop improvement, there has not been a significant amount of progress made in the use of these technologies to enhance orphan crops. It has been proved that genome editing is an excellent technique for orphan crops. This was demonstrated by Lemmon et al. (2018), who created genomic resources and efficient transformation in the orphan Solanaceae crop known as "groundcherry" (*Physalis pruinosa*)[61].

Agriculture systems are no exception to the rule that research and innovation are essential components of every aspect of existence. Instead of concentrating on model plants and big crops, research on genetic analysis need to primarily concentrate on the frugality and rusticity of orphan crops. Utilizing the most recent developments in phenotyping and "omics" technology, in addition to the variety of germplasm that is already accessible, is recommended for the improvement of millets[62]. The availability of more genomic materials contributes to the advancement of our knowledge of the genetics of orphan crops. Approaches that use "multi-omics" are urgently required in order to speed crop advances in millets and other orphan crops. This is necessary in order to fully harness the genetic potential that lies under the surface and to comprehend the molecular process that underlies abiotic stress tolerance[63]. By integrating the data obtained from integrated omics, it would be possible to investigate potential "genes/proteins/pathways" that may be altered via the use of transgene-based (overexpression/silencing) or genome-editing (CRISPR-Cas) techniques. In the not-too-distant future, it is intended that genome editing techniques will make a substantial contribution to the cultivation of new plant varieties that are able to withstand a variety of abiotic challenges and will also assist in the acceptability of these goods by the general population[64].

Foxtail Millet

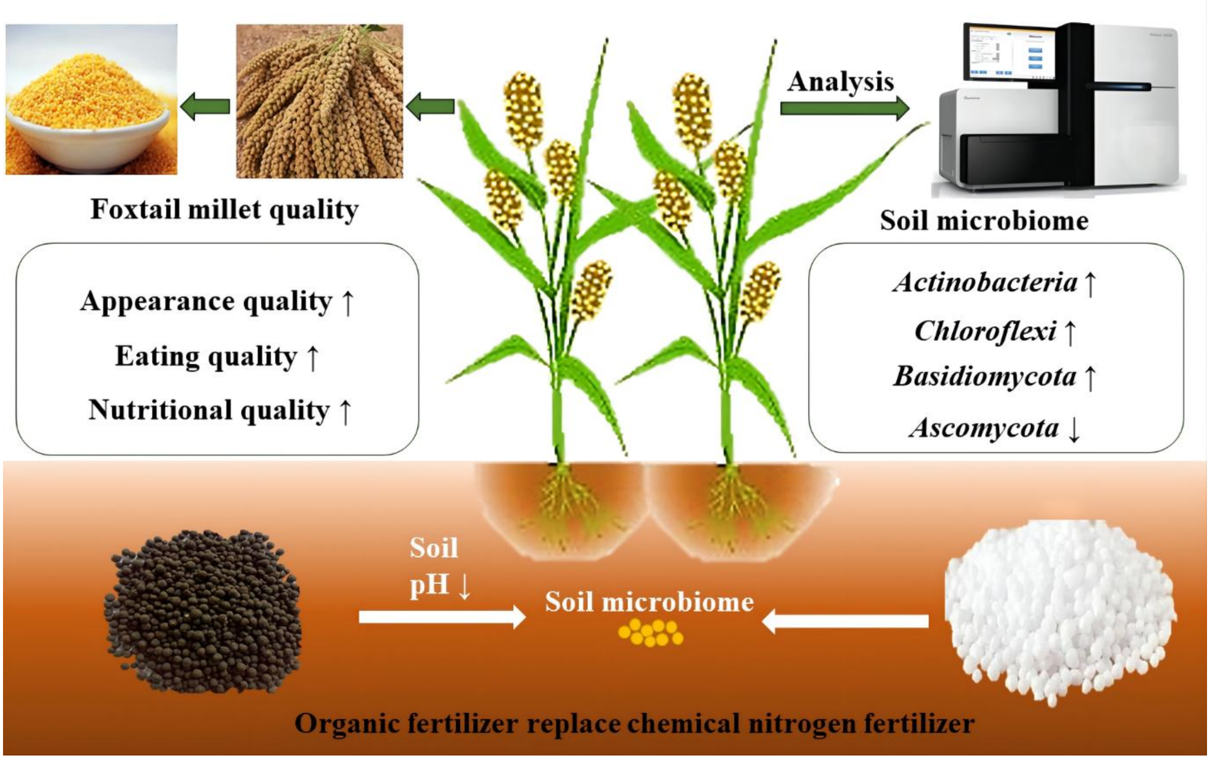
In order to facilitate marker-assisted breeding (MAB) and crop improvement, the foxtail millet genome sequence, as well as genetic and physical maps, provide excellent tools for the discovery and characterization of genes and quantitative trait loci (QTLs) for a variety of agronomic variables associated with the plant. It was via an intervarietal cross that the first RFLP-based map was generated[65]. This map was then used in the process of developing comparative genetic maps of foxtail millet and rice. Since that time, a number of different groups have produced simple sequence repeat (SSR) markers that are based on genomic or EST data. These markers are intended to be used in a variety of genotyping applications, as well as phylogenetic and transferability research on foxtail millet and other grass species.

In foxtail millet, intron length polymorphic (ILP) markers have been produced, and they have shown their relevance in the areas of cross-species transferability, germplasm characterisation, comparative mapping, and genomic connection studies between millets and non-millets[66]. Large-scale production of foxtail millet genomic resources is now feasible as a result of the availability of the genome sequence of Setaria virus. A foxtail millet landrace known as 'Shi-Li-Xiang' was sequenced in order to find single nucleotide polymorphisms (SNPs), indels, and structural variants (SVs) in comparison to two reference genome sequences. Researchers created a total of 9,576 and 7,056 SSR markers using 42,754 transcripts obtained via referenced based assembly using the foxtail millet genome and 60,751 transcripts from de novo assembly, respectively. These transcripts were used to construct the SSR markers[67].

Based on the data obtained from the Setaria genome sequencing, a number of molecular markers based on microRNA (miRNA) have also been produced. Recently, single nucleotide polymorphisms (SNPs) have been evolved into potent molecular markers. SNPs are a kind of single base substitution, insertion, or deletion that is often seen in a population. Within a unique DREB2-like gene, discovered a synonymous single nucleotide polymorphism (SNP) that is connected with dehydration tolerance[68]. Additionally, they established an allele-specific marker (ASM) for the same. From a total of 916 different foxtail millet accessions, a total of 2,584,083 single nucleotide polymorphisms (SNPs) were discovered. Of these SNPs, 845,787 were used in the construction of a foxtail millet haplotype map[69].

These marker resources are utilized effectively in genomics-assisted breeding, the evaluation of genetic relationships, the study of domestication-related geographical structure and phylogenetic relationships between foxtail millet and wild relatives, the evaluation of genetic diversity, population structure, and linkage disequilibrium in a variety of foxtail accessions, and the investigation of significant agronomic characteristics. In order to offer researchers and breeders with unlimited access to the genetic and genomic resources that have been generated, comprehensive online resources like as the marker database and the transcription factor database have been built[70].

Foxtail millet is a grass crop that has the potential to be used for bioenergy, which makes it a target for genetic modification. Some of the most significant ways to transgenomics include particle bombardment and Agrobacterium-mediated genetic transformation; nevertheless, the effectiveness of both procedures is very low. Using plasmids pROKf40s, pROKf40an, and pROKf40i, Liu et al. (2009) reported that the transformation of SiPf40 was achieved by the use of particle bombardment. Only one paper has shown that foxtail millet has enhanced its ability to tolerate abiotic stress, despite the fact that agrobacterium-mediated transformation is an increasingly common method[77].



Pearl millet

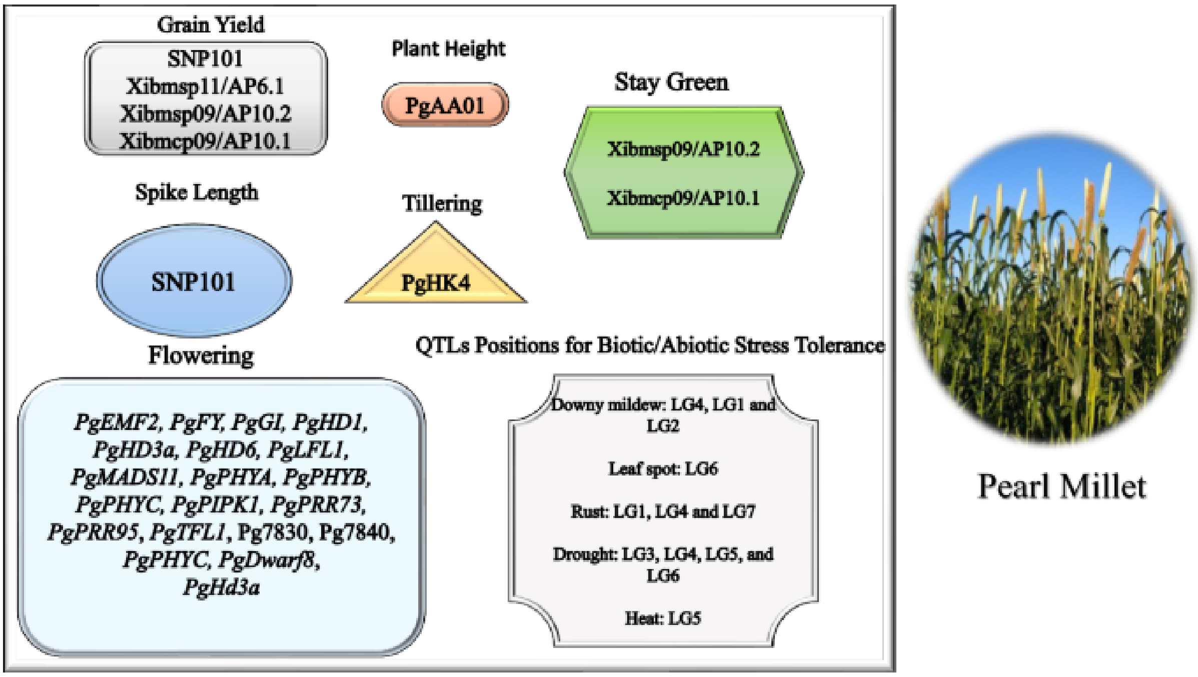
Pearl millet is a tough cereal crop that is mostly cultivated in dry and semi-arid tropical parts of Asia and Africa for the purpose of producing grain, fodder, and stover. It is a C4 Panicoid cereal crop that is annual, has tiny seeds, is extensively cross-pollinated, and has a big genome size. Additionally, its life cycle is rather brief. The genetic and genomic resources of pearl millet are extensive. These resources include DNA-based molecular markers such as RFLP, STS, AFLP, SSRs, diversity arrays technology, SNPs, and conserved intron spanning primer (CISP) markers. Pearl millet is abundant in these resources[71].

Genetic maps have been established in order to identify and breed potential QTLs for a variety of phenotypes, such as terminal drought tolerance, components of drought adaptation, grain and stover production, and more. Through the use of segregating populations produced from two separate crossings between H 77/833-2 and PRLT 2/89-33, as well as ICMB 841 and 863B, a significant quantitative trait locus (QTL) for terminal drought tolerance in pearl millet has been discovered and mapped on linkage group 2 (LG 2). The QTL in question has been regarded as a significant target for MAS, with the objective of enhancing grain production and grain stability in pearl millet under a range of different terminal stress situations[72].

A large grain yield (GRYLD) QTL was also found on LG 2, and it was co-mapped with QTLs for harvest index (HI), grain number, and grain mass when the plant was subjected to severe terminal stress. Additional quantitative trait loci (QTLs) that are connected with drought tolerance of grain production in late stress conditions were discovered on a variety of different linkage groups[73].

This DT-QTL was shown to promote growth and productivity characteristics under saline and alkaline circumstances by restricting Na+ buildup in pearl millet leaves. The performance of LG 2 main QTL for terminal drought resistance was evaluated under salt stress, and the results showed that this DT-QTL was responsible for improved growth and productivity. In another research, it was shown that the drought-resistant parent, together with two QTL-near isogenic lines, recorded a greater yield under salt stress throughout post-flowering development stages. This was in comparison to the drought-sensitive parent[74].

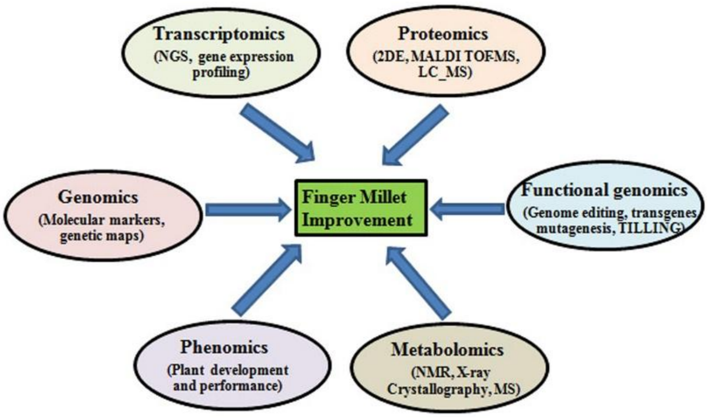
The transformation of pearl millet has mostly been accomplished by the use of biolistic gene delivery technologies, with the first report using particle bombardment in the year 1991. Since then, a number of different organizations have reported on biolistic transformations that were performed against fungal diseases by using a variety of target tissues and transgenics. On the other hand, there has been no report of a genetically altered pearl millet that is more resistant to abiotic stress. Furthermore, the technique of Agrobacterium-mediated transformation employing shoot apices has only been described relatively recently[78].



Finger millet

Finger millet is a nutri-millet that is resistant to the effects of climate change and has a vast collection of superior genes and alleles. It has a genome that is identified as AABB and is classified as an allotetraploid crop. Due to the fact that finger millet blooms are quite tiny and possess a self-pollinating character, breeding attempts have been restricted. There have been very few publications on the morphological, nutritional, and quantitative characteristics of finger millet, hence the germplasm pool of this grain has not been defined[75]. Through the use of RAPD and microsatellite techniques, Dida et al. (2008) discovered that domesticated finger millet cultivars show a greater degree of genetic variability than previously thought. The creation of a marker-based genetic linkage map of the A and B genomes of finger millet came about as the first significant accomplishment in the field of finger millet genomics. When finger millet and rice were compared, it was found that there were substantial levels of preserved collinearity between the gene ordering of both of these grains. On the other hand, there is a dearth of research that is specifically focused on abiotic stress in finger millet, despite the fact that genomics studies for biotic stress tolerance in finger millet have been limited[76].

Despite the nutritional value of finger millet and the priority it has for better grain production and stress tolerance, there has been minimal progress made in the genetic enhancement of this crop via the use of transgenic technology. Previous research has used biological techniques and agrobacterium-mediated transformation, both of which have been applied to shoot explants. However, there is just one publication that has reported on the generation of transgenic finger millet that has enhanced resistance to numerous stresses, such as drought, salt, and oxidative stress. The persistent expression of the mannitol-1-phosphate dehydrogenase (mtlD) gene extracted from bacteria was the means by which this tolerance was attained in this work[79].



Conclusion

In conclusion, the utilization of multi-OMICS strategies presents a transformative approach to unlocking the nutritional potential of millet grains. Through the integration of genomics, transcriptomics, proteomics, metabolomics, and phenomics data, we have gained unprecedented insights into the genetic and biochemical mechanisms governing key nutritional traits in millets. This multidisciplinary approach has enabled us to identify candidate genes, metabolic pathways, and biomarkers associated with protein content, amino acid composition, micronutrient density, and antioxidant capacity in millet grains. By leveraging these molecular insights, targeted breeding efforts have been initiated to develop improved millet varieties with enhanced nutritional profiles and agronomic performance. Through marker-assisted selection (MAS), genomic selection, and gene editing techniques, breeders can now expedite the development of high-yielding millet cultivars with superior nutritional qualities, tailored to meet the diverse dietary needs of consumers worldwide. Moreover, biotechnological interventions informed by multi-OMICS analyses offer promising avenues for further enhancing millet nutritional traits. Through the manipulation of gene expression, metabolic pathways, and biochemical processes, researchers can engineer millet crops with enhanced protein quality, increased micronutrient content, and improved bioavailability. Biotechnological approaches such as biofortification, metabolic engineering, and synthetic biology hold great potential for addressing specific nutritional deficiencies and enhancing the overall nutritional value of millet grains. Furthermore, the adoption of multi-OMICS strategies has broader implications for global food security and nutrition. Millets, with their exceptional nutritional resilience and climate resilience, are poised to play a pivotal role in sustainable agriculture and resilient food systems. By harnessing the nutritional potential of millets, we can address malnutrition, combat micronutrient deficiencies, and promote dietary diversity, particularly in regions where millets are dietary staples.

However, realizing the full potential of multi-OMICS approaches for enhancing millet nutritional traits requires concerted efforts from researchers, policymakers, and stakeholders across the value chain. Collaboration between academia, government agencies, non-governmental organizations, and the private sector is essential to facilitate knowledge sharing, technology transfer, and capacity building in millet research and development. Moreover, efforts to promote millet consumption and value addition must be accompanied by supportive policies, investment in infrastructure, and consumer awareness campaigns. By fostering a conducive enabling environment, we can create new market opportunities for millet-based products, enhance livelihoods for smallholder farmers, and contribute to sustainable rural development. In conclusion, the application of multi-OMICS strategies offers a powerful paradigm for enhancing the nutritional value, agronomic performance, and socio-economic impact of millet crops. By embracing innovation, collaboration, and inclusive development approaches, we can unlock the full potential of millets as a sustainable solution to global nutrition challenges, ensuring food security, health, and well-being for generations to come.

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