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REVIEW

The potentialities of omics resources for millet improvement

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Abstract

Millets are nutrient-rich (nutri-rich) cereals with climate resilience attributes. However, its full productive potential is not realized due to the lack of a focused yield improvement approach, as evidenced by the available literature. Also, the lack of well-characterized genomic resources significantly limits millet improvement. But the recent availability of genomic data and advancement in omics tools has shown its enormous potential to enhance the efficiency and precision faced by conventional breeding in millet improvement. The development of high throughput genotyping platforms based on next-generation sequencing (NGS) has provided a low-cost method for genomic information, specifically for neglected nutri-rich cereals with the availability of a limited number of reference genome sequences. NGS has created new avenues for millet biotechnological interventions such as mutation-based study, GWAS, GS, and other omics technologies. The simultaneous discovery of high-throughput markers and multiplexed genotyping platform has aggressively aided marker-assisted breeding for millet improvement. Therefore, omics technology offers excellent opportunities to explore and combine useful variations for targeted traits that could impart high nutritional value to high-yielding cultivars under changing climatic conditions. In millet improvement, an in-depth account of NGS, integrating genomics data with different biotechnology tools, is reviewed in this context.

Keywords Millet · Next-generation sequencing · Genotyping · Genomic selection · Omics · Genome-wide association study

Introduction

Millets are annual grasses that produce tiny seeds and can be classified into major and minor classes. Major millets include pearl millet (*Pennisetum glaucum*) and sorghum (*Sorghum bicolor*), whereas other millets belong to the minor millet class. Among minor millets, significant millets are finger millet (*Eleusine coracana*), foxtail millet (*Setaria italica*), little millet (*Panicum sumatrense*), proso millet (*Panicum miliaceum*), kodo millet (*Paspalum scrobiculatum*), and barnyard millet (*Echinochloa esculenta*) (Ajeesh Krishna et al. 2022). The principal cultivated germplasm of millets is further subdivided based on panicle morphology and shapes into races and subraces. Millets have C_4 physiology and belong to a subfamily of the Poaceae family, namely Panicoideae and Chloridoideae, contributing to their ability to withstand harsh climatic conditions. Therefore, millets could sustain a rainfed environment and are mainly grown for subsistence farming. For example, sorghum productivity is better than other cereals under rainfed and changing climate conditions. Similarly, pearl millet, proso millet, and kodo millets are well adapted to severe drought, high temperature, low soil fertility, saline, and even acidic soils. It shows millets' climate resilience nature under adverse growing conditions (Das and Rakshit 2016). Millets also

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possess superior mineral nutrients, fibers, and excellent shelf life, making them the crop of choice in global warming scenarios (Kumar et al. 2018).

Globally, millets were produced in 89.17 million metric tonnes from an area of 74.00 million hectares. India is the largest producer of millet, with an area of 12.45 million hectares and a production of 15.53 million tonnes (Anonymous 2020). India ($29.60M) is the second largest exporter of millet after Ukraine ($46.30 M). Millets have been grown by farmers globally with a diverse geographical distribution. Pearl millet is the most widely growing millet among millets, covering around 50 % of the millet growing area globally (Kanfany et al. 2020). At the same time, it is the most important crop in arid and semi-arid regions of the world and ranks fifth among all cereals (Ananda et al. 2020). Similarly, foxtail millet is cultivated in southern Europe and Asia, is considered one of the world's oldest crops, and ranks second in total world millet production (Yang et al. 2012). Unlike many crops consumed by subsistence farmers, finger millet has maintained high socio-economic importance in the Indian and African semi-arid tropics. Interestingly, the Deccan plateau is the only part of the world where the seeds of kodo millets are harvested (Goron and Raizada 2015). Proso millet originated in China and expanded to Eurasia and North America (Bagdi et al. 2011). Most millet varieties are well known for their hardiness, withstand prolonged periods of drought and high temperature, and still produce grain and fodders. This global distribution and dependency of subsistence farmers indicate less water requirement and better agronomic and climate resilience characteristics of millets under adverse climatic conditions.

However, we need a climate-resilient crop with low input requirements to meet food and nutritional security standards in the ever-changing climatic conditions (Satyavathi et al. 2021). Therefore, millets have got the attention of the scientific community. The major challenge in millet improvement is efficiently characterizing genetic and genomic resources and exploiting trait-specific variations (Gimode et al. 2016). We can tackle these shortcomings in millet research by using crop improvement programs and by using precise, efficient, cost-effective germplasm characterization that leads to the expansion of genetic resources.

Millet improvement is conventionally governed by breeding programs based on phenotypic selection encountered with high environmental noise. Developing crop variety required an extended period (5–12 years) and was less effective for complex and low heritable traits. These crop improvement programs are often hampered by a small pool of available genetic variability and inefficient selection methodologies for a suitable variation source (Tuberosa, 2012). Which ultimately results in a gap between the precisely characterized cultivars and varieties for millet improvement. Sequencing technologies could fulfill these gaps in the availability of genomic resources for millets. However, most millets have polyploid genomes that need specific methods for their sequencing. Various millet genomes have been sequenced in recent years, and more are likely to be sequenced in the coming years. Millet species' ploidy levels are different, with many repetitive sequences making their sequencing and genome studies more complex. In this context, different millets, ploidy levels, and genome sizes are compared and presented in Table 1.

Developing genetic and genomic resources could play a crucial role in improving the desired traits of any crop (Tuberosa 2012). The whole-genome sequencing for identifying tightly linked trait-specific markers is feasible due to reduced cost with ever-evolving technologies'. Whole-genome sequencing technologies primarily use first-generation sequencing methods such as the Maxam-Gilbert chemical cleavage and Sanger chain-termination methods. The determination of the reference genomes in *Arabidopsis thaliana* (Kaul et al. 2000), rice (Sasaki, 2005), maize (Schnable et al. 2009), and soybean (Schmutz et al. 2010) through Sanger sequencing were the most symbolic achievement of these methodologies. These crop genome sequencing programs enabled the analysis of genome architecture and accelerated gene characterization in other plant species. However, these methods lack automation and require time and labor. Thus, the high demand for low-cost sequencing has driven the development of high-throughput sequencing technologies that parallelize sequencing, popularly known as next-generation sequencing (NGS) techniques. NGS technologies have made it possible to correlate the phenotypes with genotypes in different crop species at the nucleotide level, increasing precision in crop improvement (Chhapekar et al. 2016). NGS provides an efficient and precise methodology for identifying and tracking genetic variation within large crop populations. It also makes it possible to sequence hundreds or even thousands of related genomes to sample adequate genetic diversity within and between germplasm pools (Kilian and Graner 2012). The increasing availability of high-throughput technology at reduced costs has moved genomics from sequencing a few model species to sequencing any desired crop. The progress in next-generation sequencing (NGS) techniques has revolutionized the detection and gene tagging of specific traits using genetic markers (Ambawat et al. 2020). Similarly, this sequence information help in allele mining, genome editing for designing single guide RNA (SgRNA), promotor identification, functional biology phylogenetic analysis, etc. A schematic diagram of millet development through sequence information using these advanced techniques is represented in Fig. 1.

These projects open the avenues for the enormous genetic potential for millet improvement (Table 2 and Table 3). This review will explore NGS technology in crop improvement, focusing on millet improvement. Further application of NGS

Table 1 Major millet species with their scientific name, chromosome number, ploidy level, and estimated genome size

S. N.	Crop	Scientific name	Chromosome number	Ploidy	Estimated genome size	Reference
1	Setaria (foxtail millet)	*Setaria italica* (L.) P. Beauv.	18	2x	490 Mb	Diao and Jia (2017)
2	Finger millet	*Eleusine coracana* (L.) Gaertn.	36	4x	1593 Mb	Ceasar (2021); De Wet et al. (1984)
3	Proso millet/ Broomcorn millet	*Panicum miliaceum* L.	36	4x	923 Mb	Zou et al. (2019); Hunt et al. (2011)
4	Little millet	*Panicum sumatrense* Roth.ex.Roem. & Schult.	36	4x	Not reported	De Wet et al. (1983)
5	Sonoran millet	*Panicum hirticaule*	18	2x	Not reported	Gould and Soderstrom (1970)
6	Pearl millet	*Pennisetum glaucum (L.) R. Br., syn. Cenchrus americanus* Morrone	14	2x	1.79 Gb	Varshney et al. (2017)
7	fonio millet (White)	Digitaria exilis Stap	36	4x	904-956 Mb	Kanlindogbe et al. (2020)
8	Black fonio	Digitaria iburua Stapf.	36	4x	904-956 Mb	Kanlindogbe et al. (2020)
9	Raishan	Digitaria cruciata	72	4x	Not reported	Gould and Soderstrom (1974)
10	Polish millet	Digitaria sanguinalis	36	4x	Not reported	Ahsan et al. (1994)
11	Japanese barnyard millet	Echinochloa crus-galli (L.) P. Beauv	54	6x	1.27 Gb	Guo et al. (2017)
12	Indian barnyard millet	Echinochloa colona (L.) Link)	54	6x	Not reported	Wallace et al. (2015)
13	Burgu millet	Echinochloa stagnina	36	4x	Not reported	Yamaguchi et al. (2005)
14	*Early barnyard grass*	*Echinochloa oryzoides*	36	4x	0.95 Gb	Ye et al. (2020)
15	*Teff*	*Eragrostis tef (Zucc.) Trotter*	40	4x	672 Mb	Rahman et al. (2014)
16	Kodo millet	Paspalum scrobiculatum L.	40	4x	1900 Mb	Jarret et al. (1995)
17	Guinea millet	Urochloa deflexa (Schumach.) H. Scholz (=Brachiaria deflexa)	18, 36	2x, 4x	Not reported	Ganapathy et al. (2021)
18	Browntop millet	Urochloa ramosa (L.) T.Q. Nguyen (=Brachiaria ramosa (L.) Stapf.)	18, 36, 72	2x, 4x	Not reported	Clayton et al. (2016)
19	Taiwan oil millet	Eccoilopus formosanus	40	4x	2.6 Gb	Roscoe et al. (2018)
20	Sorghum	Sorghum bicolor (L.)	20	2x	730 Mb	Paterson et al. (2009)
21	Adlay millet	Coix lacryma-jobi L	20	2x	1684 Mb	Cai et al. (2014)

in genomic selection, genome-wide association studies, different omics platforms, and pangenome in millet improvement have been discussed. Hence, genotyping by sequencing and whole-genome sequencing can lead to the development of molecular markers suited to studying genetic relationships among genotypes and creating genetic maps for targeted gene and genome-wide association studies. Apart from this most frequently used to shorten the selection cycle for crop improvement and reduce the time to release varieties.

Overall, this review summarizes the work done in millet improvement through the help of modern sequencing technologies and adds to our knowledge about their future implications and applications.

NGS as a tool for developing millet genetic resources

Whole-genome sequence data in millets lead to characterizing functionally essential genes such as stress tolerance (Hittalmani et al. 2017). NGS technologies are also helpful in millet improvement by rapidly developing high throughput markers and maps (Serba and Yadav 2016). Single-nucleotide polymorphism (SNP) discovery by whole-genome and targeted genome sequencing via resequencing well-characterized genomes generated enormous genetic resources that can be utilized for millet improvement (Table 4). NGS technologies also help

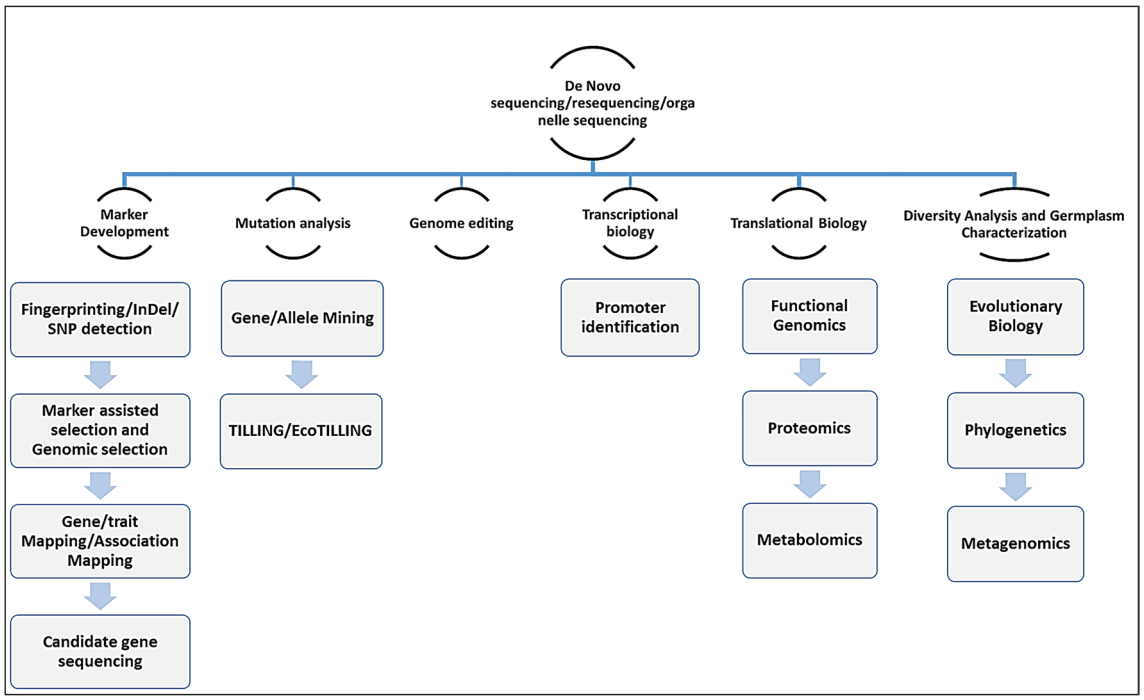

Fig. 1 Next-generation technologies and their implication for millets genetics and breeding

exploit linkage and association mapping to create high and ultrahigh-density crop maps (Poland et al. 2012). Similarly, NGS has exploited allelic diversity in the fine mapping of quantitative trait loci (QTL) for desirable traits using multi-parent populations such as nested association mapping population (NAM) (Yu et al. 2008; Sood et al. 2021) and multi-parent advanced generation intercross (MAGIC) population (Kover et al., 2009). For example, a scheme for NAM and MAGIC populations was proposed to generate a fine map in Pearl millet for drought tolerance (Sood et al. 2021). Li et al. (2021a) used a genome scan approach and sequenced 312 accessions with high depth (>19 fold) to determine genomic signature and the adaptive responses of foxtail millets from different geographical regions. In another report, a landscape genomic approach investigated the impact of climate change on pearl millet cultivation in West Africa. Authors have analyzed the phenotypic and genomic diversity of 173 landraces and identified the early and late flowering varieties as the most vulnerable to climate change in West Africa (Rhoné et al., 2020). These studies suggest the potential for more millet sequencing projects harnessing the climate resilience genetic determinants and developing genetic and genomic resources for further research in the future.

High-throughput sequencing technologies have also provided access to the study of the transcriptional regulation of genes. A widely used method for whole transcriptome and RNA sequencing (Wang et al. 2009) works through direct sequencing of complementary DNAs (Severin et al. 2010). NGS technologies and RNA-seq characterize cellular RNA transcripts, identify genes/QTLs, and help understand molecular mechanisms related to defense against biotic and abiotic stresses (Table 5). For example, transcriptome sequencing and gene expression study in contrasting moisture regimes in finger millet (*Eleusine coracana* (L.) Gaertn.) provide insights into drought tolerance, which could be used in future breeding programs (Hittalmani et al. 2017). Similarly, RNA sequencing was used to shed light on the molecular basis of drought tolerance in two pearl millet inbred lines by Illumina Hiseq. This study unraveled the differentially expressed genes and pathways involved in drought tolerance in pearl millet (*Pennisetum glaucum* (L.) R. Br) (Dudhate et al. 2018). NGS strategies for transcriptome analysis have evolved over the years to add a new dimension to crop improvement. These can facilitate genome editing and identify epigenetic marks and patterns to unravel epi-regulation in millet to understand their nature of climate resilience.

NGS-based informatics tools viz. trait-associated markers, cost-effective genotyping platforms, and expertise, increase pace, precision, and gene/QTLs/trait mapping efficiency in millet genome research. This section

Table 2 Millet GBS (genotyping-by-sequencing) projects with their key finding

Crop	Germplasm collection	Sequencing platform	Key findings	Reference
Finger millet	Senegalese group	Illumina	Total 83,875 SNPs were Identified	Hu et al. (2015)
	ICRISAT, VHC lines and AICRP-small millets	Illumina HiSEQ 2000	Total 23K SNPs segregating across the entire collection and several thousand SNPs segregating within every accession	Kumar et al. (2016)
Sorghum	ICRISAT and GRIN	Illumina (San Diego, CA)	Identified the candidate genes for mesocarp thickness (Z) and plant height (qPHT7.1)	Hu et al. (2019)
Foxtail millet	Diverse panel from 10 different countries	Illumina Hiseq4000	10 K SNPs were identified and MTAs for three important agronomic traits, FLW (flag leaf width), GY (grain yield), and TGW (thousand-grain weight) identified	Gupta et al. (2014); Jaiswal et al. (2019)
Pearl millet	ICRISAT-Niger	Ion Torrent Proton Sequencer	Total of 54,770 SNPs were identified and used in population structure analysis	Kumar et al. (2018)
Proso millet	USDA germplasm bank	Illumina HiSeq 2000	Total of 2,412 SNPs were identified and GWAS study for seed morphology traits identified 10 MTAs	Boukail et al. (2021)
Little millet	ICRISAT	Illumina HiSeq 2500	Total of 2245 SNPs were identified and tested heritability with different agronomic traits.	Johnson et al. (2019)
Barnyard Millet	ICRISAT	Illumina HiSeq 2500	Total of 8217 discriminatory SNPs were identified and used in study of population structure and phylogeny	Wallace et al. (2015)
Kodo millet	ICRISAT	Illumina HiSeq 2500	3461 SNPs identified and test heritability with different agronomic traits	Johnson et al. (2019)

will discuss crucial genome sequencing efforts and omics interventions through NGS of specific millets.

Sequencing efforts for generating genomic resources

Foxtail millet

Several millet crops have been sequenced in the last decade and have been used to generate genomic information for different stress-related traits (Zhang et al. 2012). A draft genome (~423 Mb) of foxtail millet was produced using Illumina Genome Analyzer II and HiSeq 2000 and annotated 38,801 genes. In the same year, a high-quality reference genome sequence was developed for foxtail millet (*Setaria italica*) in different cultivars and identified SNP markers for genetic mapping (Bennetzen et al. 2012). This study helped to understand the molecular basis of adaptation for drought tolerance. Authors identified six drought-associated gene clusters with significantly more gene members in drought-tolerant species (Setaria and Sorghum) than in drought-susceptible species (Maize and rice). The latest Setaria genome sequenced from *xiaomi*, a rapid-cycling mini foxtail millet mutant due to mutation in *Phytochrome* C (PHY C) gene, together with efficient transformation system, establish an ideal C4 model system (Yang et al. 2020). Sequencing information also served as a source for developing recombinant inbred lines between landrances from Japan and China Taiwan in foxtail millet (Fukunaga et al. 2022). Authors have used NGS technology to map the candidate genes responsible for morphological and agronomical traits. Similarly, comparative genomics studies through sequencing provided evolutionary clues about C3 and C4 photosynthesis. For example, a comparative analysis of essential photosynthetic genes involved in the C4 metabolic cycle in grasses like Brachypodium, sorghum, maize, and foxtail millets revealed that all the genes involved in C4 carbon fixation pathways were also present in C3 plants like rice. Interestingly, functional changes in these genes in the C3 cycle lead to the C4 pathway's evolution (Shi et al. 2020). These studies signify the importance of sequencing projects

Table 3 Millet genome sequencing projects with their strategies and key findings

Crop	Genotype used	Sequencing strategy/platform	Key findings	Reference
Pearl millet Cenchrus americanus (L.) Morrone	Tift 23D2B1-P1-P5	Whole genome shotgun (WGS) and bacterial artificial chromosome (BAC) sequencing /Illumina HiSeq 2000	~1.79 Gb draft whole genome sequence with 38,579 genes and substantial enrichment for wax biosynthesis genes, which may contribute to heat and drought tolerance	Varshney et al. (2017)
Sorghum *Sorghum bicolor* (L.) Moench	BTx623	Whole-genome shotgun sequence	~730-megabase ~27,640 genes. About 24% of genes were grass-specific and 7% are sorghum-specific. Recent gene and microRNA duplications may contribute to sorghum drought tolerance.	Paterson et al. (2009)
Foxtail millet	'Zhang gu' and 'A10'	Illumina second-generation Sequencer	~86% genome coverage, 423 Mb genome size with 38801 genes	Zhang et al. (2012)
	'Yugu1' and 'A10'	ABI3730xl capillary sequencer; 454 FLX platform; Illumina Genome Analyzer II platform	~80% genome coverage, 510 Mb genome size with24000-29000 genes	Bennetzen et al. (2012)
Finger millet (*Eleusine coracana (L.)* Gaertn)	cultivar PR202 (IC: 479099)	Novel multiple hybrid assembly workflow that combines next-generation with single-molecule sequencing, followed by whole-genome optical mapping using the Bionano Irys® system	~78.2 % genome coverage, 1.5 GB genome size, and 62,348 genes	Hatakeyama et al. (2018)
	ML-365	Illumina HiSeq4000 and NextSeq500, SOLiD sequencing technologies	Approximately 82% of total estimated genome size, 1196 Mb genome size, 85,243 genes have higher collinearity with foxtail millet and rice as compared to other Poaceae species	Hittalmani et al. (2017)
Proso millet (Broomcorn millet) (Panicum miliaceum L.)	Landrace (accession number 00000390) originating from Northern China	Combination of short-read sequencing, single-molecule real-time sequencing, Hi-C, and a high-density genetic map, PacBio sequencing	~923 Mb genome size, 55,930 protein-coding genes and 339 microRNA genes	Zou et al. (2019)
	Longmi4	Combination of PacBio sequencing, BioNano, and Hi-C (in vivo) mapping	~887.8 Mb genome size with63,671 genes	Shi et al. (2019)
Teff (*Eragrostis tef*)	Tsedey (DZ-Cr-37)	Illumina and 454	Representing 87% of the genome size, 672 Mb genome size, 28113 genes, and first allotetraploid assembled de novo	Cannarozzi et al. (2014)
Japanese Barnyard millet (*Echinochloa crus-galli)*	STB08	Illumina HiSeq 2000 system	Representing 90.7% of the predicted genome size, 1.27 Gb genome size with 108,771 genes	Guo et al. (2017)

Table 4 Millet GBS (genotyping-by-sequencing) projects with their key finding

Crop	Germplasm collection	Sequencing platform	Key findings	Reference
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Sorghum	ICRISAT and GRIN	Illumina (San Diego, CA)	Identified the candidate genes for mesocarp thickness (Z), plant height (qPHT7.1)	Hu et al. (2019)
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Pearl millet	ICRISAT-Niger	Ion Torrent Proton Sequencer	54,770 SNPs were identified and used in population structure analysis	Kumar et al. (2018)
Proso millet	USDA germplasm bank	Illumina HiSeq 2000	2,412 SNPs identified and GWAS study for seed morphology traits identified 10 MTAs	Boukail et al. (2021)
Little millet	ICRISAT	Illumina HiSeq 2500	2245 SNPs identified and test heritability with different agronomic traits.	Johnson et al. (2019)
Barnyard Millet	ICRISAT	Illumina HiSeq 2500	8217 discriminatory SNPs identified and used in study of population structure and phylogeny	Wallace et al. (2015)
Kodo millet	ICRISAT	Illumina HiSeq 2500	3461 SNPs identified and test heritability with different agronomic traits	Johnson et al. (2019)

Table 5 Transcriptomics studies in the improvement of millet

Crop	Genotype used	Experiment type	Sequencing strategy	Trait	Reference
Pearl millet	ICMB 843 and ICMB 863	Green house	Illumina Hiseq	Drought tolerance	Dudhate et al. (2018)
	inbred lines viz. PPMI 953, PPMI 1108, PPMI 627, 5540 B	Field	ION S5 Next Generation Sequencer	Iron and zinc content	Satyavathi et al. (2021)
	Tifleaf 3	Growth chamber	PacBio Sequel and Illumina	Heat stress and drought stress	Sun et al. (2020)
	ICMB 01222 and ICMB 081	Green house	Illumina HiSeq 2500	salinity stress	Shinde et al. (2018)
Sorghum	RTx430 and BTx642	Field	Illumina HiSeq.	Drought induced biotic and metabolic response	Varoquaux et al. (2019)
Black Sorghum	BTx378 and RTx3362	Growth chamber	NovaSeq 6000 (Illumina Inc.)	Light quality	Fedenia et al. (2020)
Foxtail millet	Yugu 2 and An 04	Incubator	Illumina (NEB, USA)	Salt stress	Pan et al. (2020)
	Damaomao and Hongnian	Climate controlled Incubator	Illumina HiSeq-TM 4000	Water deficiet	Xu et al. (2019)
Broomcorn millet	Yumi No. 2 and Yumi No. 3	Glasshouse condition	IlluminaHiSeq2500	Salt stress	Yue et al. (2016)
Finger millet	GPU-28	Gravimetric approach	Illumina NextSeq 50	Drought stress	Parvathi et al. (2019)

for generating genomic resources for crop improvement in foxtail millet.

Finger millet

NGS has also solved the problems encountered in millets' genome assembly of allopolyploid species. The allopolyploid finger millet genome was sequenced utilizing multiple hybrid assembly workflows and generated ~78.2 % genome coverage, 1.5 GB sequence data, and 62,348 protein-encoding genes. This system demonstrates the super-scaffolding and long-range homoeologous complexity in finger millet (Hatakeyama et al. 2018). Whole-genome sequencing of finger millet genotype ML-365 (drought tolerant and blast disease-resistant) was performed using Illumina and SOLiD (Sequencing by Oligonucleotide Ligation and Detection) sequencing technologies. This led to identifying about 2866 drought-responsive genes, 1766 genes as R-genes, and 330 genes involved in calcium transport and accumulation (Hittalmani et al. 2017). Similarly, in cultivated finger millet (*Eleusine coracana* (L.) Gaertn) genotypes KNE755 and KNE796 identified 10,327 (Simple Sequence Repeat) SSRs, and 23,285 non-homeologous SNPs by using both Roche 454 and Illumina NGS technologies. These sequencing projects on allopolyploid crops open the avenues for sequencing many more millet genomes to understand their genomics (Gimode et al. 2016). Also, the climate resilience nature of finger millet could be deployed in conventional crops through candidate gene identification for future climate-smart crops.

Sorghum

Advances in next-generation sequencing (NGS) technologies and improved genome assembly algorithms have facilitated the de novo assemblies of the *Sorghum bicolor* (Jowar) genome. Whole-genome shotgun sequencing resulted in ~730 Mb sequence data encoding ~27,640 genes. About 24% of genes are grass-specific, and 7% are sorghum-specific (Paterson et al. 2009). The authors also suggested recent gene and microRNA duplications may contribute to drought tolerance in sorghum. Further refinement in genome sequencing data suggested size of the genome sequence is 700 Mb (Mace et al. 2013). The sequence information help in mining sorghum genomic variation to facilitate the genetic research of complex traits. In sorghum, large-scale SNP datasets using genotyping-by-sequencing were obtained. Further, by reusing sequencing data of 10,323 sorghum genotypes total of 459,304-SNP were identified. These SNPs data were additionally used for the GWAS study, and many candidates' SNP were determined for different important traits (Hu et al. 2019). For example, Girma et al. (2019) reported a strong candidate gene for the ABORTED MICROSPORES bHLH transcription factor, which controls male fertility. Efforts in sorghum sequencing led to the identification of evolutionary variations responsible for better performance in harsh climatic conditions, which can pave the way for developing varieties sustaining climatic fluctuations.

Pearl millet

NGS technologies have also helped identify and tag genes controlling the trait of interest in millets (Serba and Yadav, 2016). Whole-genome sequencing of pearl millet generated ~1.79 Gb draft sequence with 38,579 genes by using whole-genome shotgun (WGS) and bacterial artificial chromosome (BAC) sequencing in Illumina HiSeq 2000 platform (Varshney et al. 2017). The authors reported substantial enrichment for wax biosynthesis genes, which may contribute to heat and drought tolerance in this crop. This study further revealed enrichment for wax biosynthesis, tolerance to heat, and drought stress genes in the arid zone (Varshney et al. 2017). NGS platforms and high throughput phenotyping technologies have been utilized in foxtail and pearl millet to identify marker-trait associations (Varshney et al. 2017; Jia et al. 2013). In pearl millet, resequencing data was obtained using the RAD-Seq approach to establish marker-trait associations for genomic selection, define heterotic pools, and predict hybrid performance. The information obtained using NGS technologies has been expected to facilitate genomic research at the molecular level, ultimately improving millets productivity and enhancing the bioavailability of vitamin A, iron, and zinc. These technologies also enable the population structure study by identification of SNPs. Kanfany et al. (2020) identified 54,770 high-quality SNPs markers for a population structure study in 309 inbred lines by arranging in four Ion Proton Next-Generation Sequencers (Kanfany et al. 2020). Overall, we need more sequencing efforts to identify the hidden variations in resource use efficiency and nutritional values in pearl millet. Sequencing data would undoubtedly help develop new crop varieties in less time for pearl millet in the future.

Other millets

NGS has also expedited structural and functional genomics studies in minor millet, assisted their improvement, and provided the foundation for studying exceptional stress tolerance and C4 biology in millets. To understand its genome assembly, sequencing of broomcorn millet generated ~103.4 Gb Illumina paired-end reads (150 bp) in cultivar Longmi 4. Interestingly, authors have identified 15 ABA or WDS (water-deficiency stress) responsive genes. Four genes were highly expressed across tissues/ stages in stress/stress-free conditions. This indicates stress resilience expression of stress tolerance genes in broomcorn millet that could be

utilized for integration in conventional crops through transgenic or marker-assisted selection (Shi et al. 2019). Zou et al. (2019) reported about 55930 protein-coding genes and 339 microRNA genes present in the genome of broomcorn millet using Illumina short-read coupled with Pac-Bio long-read genome sequencing technology. The identified candidate genes coexisted for all three C4 subtypes of the carbon fixation mechanism. This study will help us better understand the C4 mechanism in plants and provide insight into the genetic modification of C3 plants into C4 to improve their stress tolerance capability (Zou et al. 2019).

Other efforts of the whole millet genome include sequencing teff (*Eragrostis tef*) cultivar Tsedey (DZ-Cr-37) by Illumina and 454 representing 87% of the genome size, 672 Mb sequence reads, and 28113 genes. This is the first allotetraploid assembled de novo and paved the way for other allotetraploid crops (Cannarozzi et al. 2014). Similarly, sequencing of Japanese Barnyard millet (*Echinochloa crus-galli*) cultivar STB08 by Illumina HiSeq 2000 system represented 90.7% of the predicted genome size, 1.27 Gb sequence reads, with 108,771 protein-encoding genes (Guo et al. 2017).

At present, the genomic resources available for *P. miliaceum* are several types of molecular markers, such as simple sequence repeat (SSR) and single-nucleotide polymorphism (SNP) markers and expressed sequence tags (ESTs). The first genetic linkage map of proso millet using SNP markers was discovered through genotype-by-sequencing (GBS) using NGS protocols (He et al. 2014). Initially, 69,981 SNPs were identified for major and minor alleles. Further, QTL was determined for different traits using this data (Rajput et al. 2016). In other millets, such as barnyard millet and kodo millet minimal attempts have been made to discover the genomic structure and associated downstream processes due to genome complexity (Renganathan et al. 2020).

More efforts in sequencing and resequencing millet crops are required to completely utilize the potential at the genetic level to translate in field conditions for millet and conventional crops.

Omics-based millet genomic resources

Omics technologies are integrated approaches involving high throughput technologies, analytical methods, bioinformatics, and computational analysis. These technologies comprise the detailed study of genes (genomics), mRNA (transcriptomics), proteins (proteomics), metabolites (metabolomics), and epigenetics (epigenomics) that have provided insights into the molecular mechanisms for understanding the complex interactions between genes, proteins, and metabolites within the resulting phenotype of millets (Tables 5 and 6). Overview of the use of omic resources in millets improvement is represented in Fig. 2.

Transcriptomics

Transcriptome profiling provides a detailed account of genes differential expression patterns and valuable information on millet climate resilience. For example, transcriptome analysis showed differentially expressed genes for salinity in finger millet and low moisture stress tolerance in contrasting genotypes (Hittalmani et al., 2017; Rahman et al. 2014). Comparative transcriptome analysis in barnyard millet identifies candidate genes for drought tolerance, Fe and Zn accumulation (Jayakodi et al. 2019), and submergence tolerance (Nah et al. 2015). Recently, proteomics and metabolomics studies have been conducted to characterize stress-specific proteins related to post-transcriptional and post-translational modifications (Vetriventhan et al. 2020). Several investigations were conducted on finger millet in grain filling stages, identifying calcium-binding proteins (Singh et al. 2016). Transcriptomic and proteomic technologies were combined to identify genes and proteins responsible for abiotic stress tolerance. The finger millet samples were treated with drought at different stages to determine the drought response mechanism. A total of 80,602 differentially expressed genes (DEGs) and 3009 differentially expressed proteins (DEPs) were identified at the transcriptomic and proteomic levels, respectively. Further, it suggested that the coordination of the DEGs-DEPs was essential to the enhanced drought tolerance response in the finger millet (Li et al., 2021b). Although genome sequencing remains cumbersome and costly, NGS has significantly reduced the cost of expression studies, which could be utilized to identify the stress-related gene expression in different varieties to obtain stress tolerance.

Proteomics

Apart from this, proteomics techniques identify different proteins expressed in mechanisms such as biotic, abiotic stresses, etc. For example, the comparative proteomic analysis of foxtail millets identified 29 differentially expressed salt-tolerant responsive proteins. It provides new insights into salt stress responses in foxtail millet (Veeranagamallaiah et al. 2008). The proteomic analysis of finger millets identified EcJAZ protein that could act as the signaling hub for the JA and other phytohormone signaling pathways in response to a diverse set of stressors and developmental cues to provide survival fitness to the plant (Sen et al. 2016). The shotgun proteomics approach in pearl millet investigates differential protein expression for drought tolerance. Using this approach in pearl millet, a total of 2281 proteins were quantified, and leaf tissue showed the highest number of significant changes (120), followed by roots (25) and seeds (10). Increased levels

Table 6 Other omics studies in the improvement of millet crops

Crop	Sample used	Experiment	Techniques used	Key findings	Reference
Foxtail millet	Seedling of Yugu1variety	Differential protein expression in drought condition	LC-MS and HPLC	Identified 252 up-regulated and 69 down-regulated proteins and it play role in stress defense responses, photosynthesis, carbon metabolism, ROS scavenging, protein synthesis, etc	Pan et al. (2018)
	Grains	Differential expression of protein in high concentration of selenium	HPLC and MS	123 differentially expressed proteins identified and it may control carbohydrate and amino acid metabolism	Liang et al. (2020)
	Five Leaves stage of 150 accessions	mGWAS	LC-ESI-MS/MS system	Identified Candidate gene for important metabolite synthesis	Wei et al. (2021)
Pearl millet	Leaves, roots and seeds of 843-22B and ICTP8203 genotypes	Differential protein expression with and without water	LC-MS/MS	Tissue- and phenotype-specific marker proteins of drought stress identified	Ghatak et al. (2021)
	Seed sample of 197 genotypes from PMiGAP	mGWAS	FIE-HRMS (flow infusion electrospray ionization high-resolution mass spectrometry)	738 candidate genes were identified for metabolite, vitamin and starch synthesis etc.	Yadav et al. (2021)
Sorghum	Leaves ICSV700, IS2205 and Swarna	Differential protein expression of susceptible and tolerant parents for insect pest C. partellus	LC-MS/MS	Produce two-fold higher number of proteins in tolerant parents.	Tamhane et al. (2021)

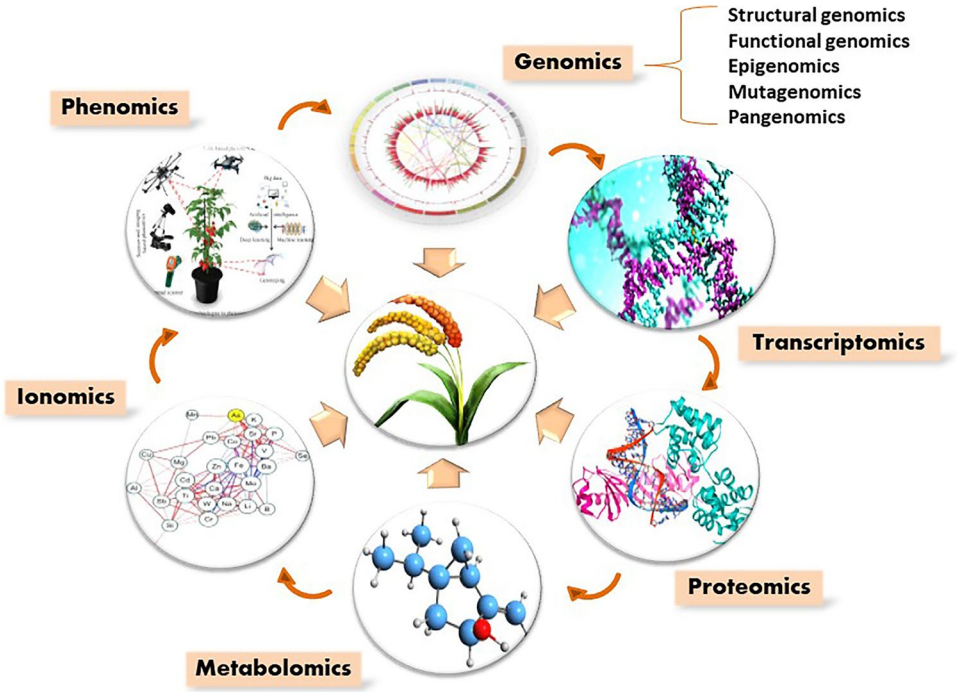

Fig. 2 An overview of the use of omics approaches for millet improvement

of root proteins observed in increased root length point to impaired shoot–root communication under drought stress (Ghatak et al. 2016). Differential protein expression analysis of Zhangzagu10 and its parental lines in foxtail millet identified 4015 proteins . The cluster analysis revealed that the DEP expression profiles of Zhangzagu10 were closer to those of the male parent. The DEPs were significantly enriched in the metabolic pathways, photosynthesis, and stress response. These proteomics analyses of differential expression in hybrid millet and its parents could provide valuable information for the practical application of heterosis in breeding hybrid millet (Wang et al., 2021a). Similarly, proteomic studies in foxtail millet revealed kernel protein concentration and distribution change under drought and salinity stress (Li et al. 2019). More proteomics studies need to identify molecular chaperones governing the climate resilience nature of these nutri-cereals.

Comparative genomics

The genome sequence information of millets is not well explored. Therefore, comparative genomics is essential in identifying genes/QTLs linked to biotic and abiotic stress resistance. For example, Babu et al. 2014 identified the gene responsible for blast resistance in finger millets utilizing comparative genomics information for millets. Based on homology, a total of 47,142 genes were identified in 20,374 gene families of broomcorn millet and 11,773 in foxtail millet by comparative genomics. Further, most of these genes were also identified as candidate genes for abiotic stress tolerance (Zou et al. 2019). More such studies could also provide insight into the evolutionary relationship between grasses in changing climates.

Metabolomics

Metabolomic studies on millet could also provide insights into the metabolic functions of different metabolites for climate resilience. For example, metabolic reconstruction and multiple omics mapping were performed in foxtail millet using transcriptome, proteome, and targeted metabolome data (de Oliveira Dal'Molin et al., 2016). Authors have identified a significant abundance of metabolites that plays a role in C4 metabolism. Similarly, integrated metabolomics analysis in foxtail millet cultivar An04 and Yugu2 under salinity stress implied that lysophospholipid, phenylpropanoid, lignin, and flavonoid biosynthesis pathway play a crucial role in the seed germination stage (Pan et al. 2020). Targeted metabolomics analysis of 150 foxtail millet germplasm identified 330 annotated metabolites (Wei et al. 2021). Further, mGWAS study of the generated data identified genes for complex physiological traits. Large-scale multi omics analysis of 398 foxtail millet accessions elucidated the domestication-related genomic region along with common variants influencing the metabolite traits and having anti-inflammatory properties (Li et al. 2022). Further authors have identified the grain color affecting gene and validated it through genome editing in foxtail millet (Li et al. 2022). At the grain-filling stage total of 2014 metabolites associated with foxtail millet were identified, which suggested stage-specific metabolic properties

(Wang et al. 2023). In pearl millet metabolome analysis and mGWAS of 197 inbred lines identified 738 probable candidates for further exploration (Yadav et al. 2021). Metabolomics analysis in Proso millet (*Panicum miliaceum* L.) identified 2082 differential metabolites related to photosynthesis, energy metabolism, and anthocyanin production essential to drought tolerance (Cao et al. 2022). Different color proso millet varieties were subjected to metabolomics analysis, which identified 672 metabolites that brought changes in phenolic acid and flavonoid accumulation, attributing to their different grain color (Li et al., 2021c). This analysis reveals the crucial role of metabolomics in dissecting stress-related traits and their associated genes and metabolites for generating future-generation climate-smart crops (Pandey et al. 2022).

In conclusion, these omics technologies have enormous potential to generate resources that can be used for millet improvement. Integrating multi-omics technologies will provide a comprehensive framework for understanding millets' molecular mechanism and climate resilience that can be utilized to improve these nutri-cereals.

Epigenomics

The DNA associated with histone proteins forms complex chromatin structures which are prone to undergo certain mechanisms such as DNA methylation, histone modifications, and small RNA-mediated methylations. Alterations of chromatin structures lead to the inaccessibility of genomic DNA to various regulatory proteins such as transcription factors ultimately modulate the gene expression. The advancement of sequencing technology provides an opportunity to study epigenetic mechanisms at the genome-wide level. Epigenomics using high-throughput technologies would widen the understanding of mechanisms as well as functions of regulatory pathways in plant genomes which will further help in manipulating these pathways using genetic and biochemical approaches. A comprehensive study of phenotype can be correlated with genotype to epigenotype and phenotype and also with methylation QTL or epiQTL are possible with high throughput NGS. It will further accelerate the breeding program of millets (Yadav et al. 2018). Genome-wide DNA demethylation induced by salt stress in foxtail millets and modulate gene expression. Hemimethylation in pearl millets has a key role in regulating defense response and also provides tolerance against SA application (Ngom et al. 2018). Different methylation patterns in two foxtail millet varieties provide novel epigenetic points of view (Zhang et al., 2012). DNA methylation and RNA sequence analysis showed that the dynamics change of DNA methylation plays a crucial role in grain development in foxtail millets (Wang et al., 2021a). As our understanding will progress to select, identify, and manipulate epigenetic factors/epialleles to lead to the discovery of novel diversity. This diversity could be used for millet breeding programs.

Application of NGS technologies for millet improvement

Recent plant breeding studies of many species have demonstrated the utility of combining molecular assessments of genetic distance into trait-linked SNP genotyping during the development of parent lines to maximize yield gains due to heterosis (Baggett et al. 2021). SSRs are the molecular marker of choice to determine genetic diversity, but the methods historically used to sequence them have been burdensome. But targeted NGS technology allowed it to validate only the most informative region influencing heterotic groups (Baggett et al. 2021). These studies suggest that the NGS technology is ultimately used to characterize gene/alleles controlling agronomic traits and will serve as a source of markers for molecular breeding in millets (Fig. 3). NGS provides high throughput genotyping of crop plants, and these genotyping data are directly used in the following techniques for improving millets.

Genome-wide association mapping (GWAS) and genotyping by sequencing (GBS)

NGS and GBS have tremendous potential to enhance breeding efficiency in millets to mitigate a changing climate effects. Quantitative traits of plants are regulated by many genes and their genotype interaction with the environment. To unravel the genetic architecture of such characters, natural variation within species can be explored by studying genotype-phenotype relationships. Linking phenotypes with single-nucleotide polymorphism has become standard for such analyses through GWAS (Scheben et al. 2017).

GBS has been used efficiently in millets to generate a vast repository of SNPs for studying genetic diversity and establishing marker-trait associations (Sehgal et al. 2012) (Table 3). GBS has enormous potential to generate many high-quality markers in orphan species like finger millet, where marker number is currently limited. Hu et al. (2015) performed a genome-wide screening to identify the role of the WRKY transcription factor in pearl millet (PgWRKY). Genome-wide association mapping and comparative genomics identify genomic regions governing grain nutrient content traits in finger millet. One hundred ninety genotypes were evaluated for minerals and protein content, implying GBS and GWAS integrated strategy to identify marker-trait associations (MTAs). Authors have identified candidate

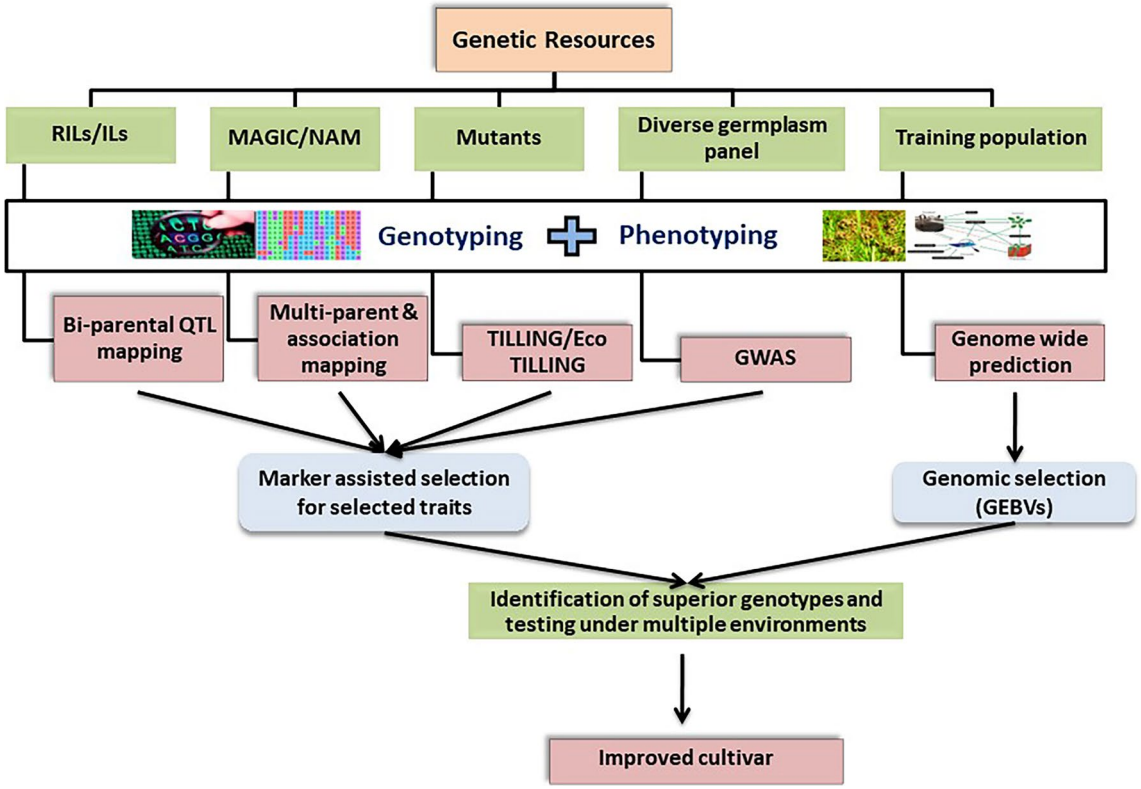

Fig. 3 Role of NGS in the genomic-assisted breeding program of millets improvement

genes governing significant associations through comparative mapping with other cereals. Genotyping by sequencing (GBS) of finger millets identified 169,365 SNPs. Through GWAS identified, 418 markers linked with mineral content. A total of 18 markers showed the homology with candidate genes having putative functions in binding, remobilization, or transporting metal ions (Chanwala et al. 2020). GWAS study in sorghum was used to determine the thirty-seven putative genes associated with epicuticular wax content that might be responsible for its better adaptability to biotic and abiotic stress (Puranik et al. 2020). GWAS study was also done in minor millets, such as in proso millet, and identified 13 marker traits associated with critical agronomic and seed traits (Elango et al. 2020). In conclusion, these studies help to identify climate resilience genetic information that can be utilized for millet and other crop improvements.

Genomic selection (genomic assisted selection)

Genomic selection is a novel breeding approach for the rapid passage of superior genotypes based on genomic estimated breeding values assessed from the training population using high-density DNA markers uniformly distributed across the genome (Meuwissen et al. 2001). Genotypic characterization of a population assists in harnessing useful information for genomic-assisted breeding. Characterization of the genomic resources in light of genetic diversity and population structure is necessary to accelerate millets' genetic improvement for target traits. In this regard, 309 inbred lines were evaluated for population genomics using GBS to understand the extent of pearl millet genetic diversity for hybrid development. For achieving a higher rate of genetic gain, a world association mapping panel called the Pearl Millet inbred Germplasm Association Panel (PMiGAP) was developed at ICRISAT to map traits related to drought tolerance, grain Fe and Zn content, nitrogen use efficiency, components of endosperm starch, and grain yield (Srivastava et al. 2020). To compare three GS models (Jarquin et al. 2020) using grain yield and dense molecular marker information of pearl millet obtained from two different genotyping platforms, namely conventional GBS RAD-seq (Miller et al. 2007; Baird et al. 2008) and tunable GBS (Ott et al. 2017) to improve the selection procedure and hybrid prediction ability. Further, in sorghum, genomic prediction for grain yield and drought tolerance is enhanced by multi-traits analysis (Velazco et al., 2019). Due to the limited genomics studies and millet germplasm availability, genomic selection emerges as an

essential tool for improving the genetic gain of millets and helping in their improvement.

Pan-genome reconstruction

Pan-genome refers to the collection of core and variable genes within a species. The pan-genome study provides better insight into species diversity, domestication, and breeding history and provides a complete characterization of species genes' content diversity. Luo et al. (2016) developed a sorghum SNP database (SorGSD) with abundant SNPs and other resources related to genetics and genomics by using published sorghum genome re-sequencing data from 48 accessions (Luo et al. 2016). Further, the updated Sorghum Genome SNP Database (SorGSD) contains SNPs from 48 sorghum accessions mapped to the reference genome BTx623 (v2.1) and, with the addition of new data and new features, renamed it to Sorghum Genome Science Database (SorGSD), which contains 289 sorghum lines with both SNPs and small insertions/deletions (INDELs), which were aligned to the newly assembled and annotated sorghum genome BTx623 (v3.1) (Liu et al. 2021). Ruperao et al. (2021) produced a pan-genome for genetically diverse race-specific sorghum accessions using a reference genome, then added novel genome sequences from 176 sorghum accessions . Sorghum 13 genomes assembled to represent the cultivated and wild relatives. Authors have integrated them with three other published genomes to generate a pan-genome of 44,079 gene families with 222.60 Mb of the new sequence identified to explore genetic diversity within its primary gene pool (Tao et al. 2021). Wang et al. (2021b) constructed chromosome-level genome assemblies for two important sorghum inbred lines, Tx2783 and RTx436, consisting of 19 and 18 scaffolds, respectively, with contig N50 values of 25.60 and 20.30 Mb. Therefore, pan-genomes of sorghum represent an essential resource for millet improvement and gene discovery for various stress tolerance (Wang et al., 2021b). More studies on pan-genome certainly improve our knowledge of species diversity, domestication, and breeding to generate better crops sustaining the pressure of climate change.

Potential use of NGS for millet improvement

Various conventional breeding methods release several notable cultivars in millets, viz. mass, pure line, and pedigree selection from local landraces/cultivars. In India, 248 varieties of six millets (finger millet—121, foxtail millet—32, proso millet—24, kodo millet—33, barnyard millet—18, and little millet—20) released by conventional breeding (Ghatak et al. 2016). Hybridization followed by selection using the contact method, controlled hybridization following hand emasculation, or hot water emasculation has been a vital breeding method in minor millets. However, these emasculation techniques often constrain millet improvement through hybridization due to the small size of the flowers. Hence, advanced biotechnological tools are required to develop millet cultivars with novel traits presumed to provide a new pace in millet improvement.

Assisted selection

The advancements in DNA markers and mapping populations for traits of interest with the refinements in phenotyping and statistical analysis have led to identifying and mapping QTL associated with the critical agronomic and stress tolerance along with the tightly linked DNA markers. These resources can be employed in marker-assisted selection. MAS used in generation advanced in sorghum. Similarly, the high-density map identified the QTL and improved several agronomic traits of foxtail millets. The marker-assisted backcrossing improves biotic, abiotic, and other nutritional traits. Recently, MAS efficiently transferred drought-tolerant QTL in the most widely cultivated cultivar HHB 226 of pearl millet (Bidinger et al. 2005). Besides this, many reports show that MAS improves millet's biotic and abiotic stress tolerance and nutrient content. These reports indicated that the genome database swift the genetic and molecular studies of the natural stress tolerance ability of millets and very quickly transfers to the desired genotype of millets.

Mutation breeding

In crop improvement programs, continuous reduction in genetic diversity results from increased focus of breeders on so-called elite cultivars. This reduction in genetic diversity can be managed by mutation breeding (Smartt and Simmonds 1995). The mutation-based methods evolved with the advancement of sequencing technology. The draft sequence of crop plants helps the breeder to screen the genomic region responsible for the variation. Traditional gene mapping is complicated, time-consuming, and requires large distribution of molecular markers. But with the emergence of resequencing technology, we can quickly map the genes with the help of bioinformatics tools (Guo et al. 2014). Application of mutation breeding earlier reported in pearl millet for delayed seedling emergence, reduced plant height, delayed maturity, and reduced seed set (Burton et al. 1974; Burton and Powell, 1966) through ethyl methanesulfonate (EMS) treatment of seeds in water solution at various dosages. Similarly, finger millet mutants have been developed for delay seedling emergence (Ambavane et al. 2014) in proso millet for early maturity and high-yielding capacity (Bhave et al. 2016) and in kodo millet for high seed viability (Jency et al. 2016). Mutational breeding resulted in 13 successful small millet

cultivars (finger millet 8, kodo millet 3, and little millet 2) in India (Ghatak et al. 2016). Besides this technology, NGS opens the gate to integrating many advanced biotechnological tools with mutation breeding that could accelerate the millet improvement program. These technologies include mutation-based methods (viz. MutMap, MutMap+, SHOREmap, Mutmap-Gap, QTL-Seq), GWAS (Genome-wide association study), genomic selection, genome editing, and multi-omics approaches. Such variation would be instrumental in generating genetic resources to understand the climate resilience characteristics of millets.

Transgenic approaches

Along with selection and hybridization, the genetic transformation of millets is necessary for millet improvement. The millet improvement program utilized a combination of plant tissue culture and transgenic approaches. For example, in finger millet, a transgenic variety resistant to leaf blast disease was developed using the *antifungal protein* (*PIN*) gene from prawn (Latha et al. 2005) and *Chitinase11* gene (*Chi11*) from rice through *Agrobacterium*-mediated transformation (Antony Ceasar and Ignacimuthu, 2011). Salinity stress-tolerant finger millet was developed using the serine-rich protein (PcSrp) gene from *Porteresia coarctata* (Mahalakshmi et al. 2006). The Na^+/H^+ *antiporter* of *Pennisetum glaucum (PgNHX1)* and *Arabidopsis thaliana vacuolar H+-pyrophosphatase (AVP1)* was utilized to develop the salinity tolerant finger millet (Jayasudha et al. 2014). Many reports suggested using a transgenic approach in finger millet for drought, salinity stress tolerance, and high chlorophyll retention using the *mannitol-1-phosphate dehydrogenase* (*mtlD*) gene from bacteria (Hema et al. 2014). Similarly, transgenics in finger millet were developed for herbicide resistance (Bayer et al. 2014) and improved zinc concentration (Ramegowda et al. 2013). In foxtail millet, transgenics were designed for a high tolerance against drought and salinity stress. The aquaporin PgAQPs differential expressed under drought stress of pearl millet. Transgenic tobacco developed by heterogenous expression of PgPIP2;6 genes exhibited better adaptation against significant abiotic stresses such as drought, high VPD, heat, and cold stresses (Reddy et al. 2022). With the advent of advanced transformation protocols and new-generation genome editing tools, the characterization of millets will provide a better understanding of their functions at different climate adversaries.

Genome editing

Genome editing technologies are becoming user-friendly for developing crop plants exempted from GMOs (Genetically Modified Organisms) regulations to cope with changing climate and ensure future food security in millets (Ceasar, 2021). Sequence-specific targeted genome editing technologies viz. ZFNs, TALENs, and CRISPR technologies are worthy of a boon in crop improvement and can be used to achieve desired traits in the target genotype. The CRISPR/Cas9 system has been used for genome editing of cinnamyl alcohol dehydrogenase (CAD) and phytoene desaturase (PDS) genes in sorghum (Liu et al. 2009). It is also reported that CRISPR/Cas9 improves the grain quality by editing Alpha-Kafirin Gene in sorghum (Li et al. 2014). There are only two successful reports of genome editing in millets, both in foxtail millet. In the first, the *Phytoene desaturase* gene was targeted through CRISPR-Cas 9 system, whereas in the second example, *S. italica Matrilineal* (*SiMTL*) gene was targeted for double haploid induction (Lin et al. 2018; Cheng et al. 2021). The scarcity of the application of genome editing in millets could be due to the complex genome in some millets, the non-availability of regeneration protocols for tissue culture, and the very recent sequencing of their genomes. However, these technologies' precise editing ability and modern transformation protocols could be game-changers for millet genetic improvement programs.

Future perspectives

Millets are climate-resilient nutri-cereals necessary for food and nutritional security in developing countries. NGS technologies are breakthroughs in the millet improvement program. Two critical applications of NGS, viz. de novo sequencing and resequencing, are attractive options for studying large and complex millet genomes, pre-breeding activities, and identifying useful genomic variations in millets. As discussed, it adds a new dimension to differential gene expression analysis, identifying candidate genes for important economic traits, quality parameters, and resistance against biotic and abiotic stresses. The multi-omics application of NGS provided new information for trait-specific improvement in millet whose genomes are yet to be sequenced. The progress of sequencing technology leads to the available resequencing data of the genomes of different millets. These resequencing data could rapidly identify genes governing other traits through mutation-based techniques and advancement in data analysis tools, and many plants' draft genomes are available. Further, NGS and other mutation methods and sequencing technologies can rapidly identify the gene responsible for any traits. Last but not least, sequence information of a genome upfront to facilitate the applications of the CRISPR/Cas toolkit in new plant or crop species. There is every reason to believe that these enormous efforts in millet sequencing will pay a considerable dividend to be essential and applied plant research in the near future.

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Data availability Not applicable.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication All authors are aware and agree to publish this study.

Competing interests The authors declare no competing interests.

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