NUTRITIONAL SIGNIFICANCE AND BIOTECHNOLOGY BASED IMPROVEMENT OF KEY MILLET CROPS: A REVIEW

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ABSTRACT

In English, “millet (s)” refers to a group of cereal grains. Millet is a staple grain in several impoverished countries, as well as a part of many more prosperous countries’ traditional diets, and it is growing increasingly popular across the world. It is a valuable source of dietary energy. Millet seeds are shown to have various health-promoting properties as well as of their high calorie level. Millets have been utilized for both human food and fodder for around 10,000 years. It thrives in dry, hot regions and produces small, seeded grasses. While millets are a valuable source of protein, calories and minerals, there hasn’t been much research on the potential of biotechnology to enhance millets. Researchers and scientists have conducted studies with millets; here, we assess their nutritional value and write about how biotechnology can improve millet crops.

Key words: Food security, Biotechnology, Pearl millet, Nutritional composition, Finger millet, Foxtail millet.

Introduction

Millets, noted for their tenacity and plasticity, provide both potential and problems during processing, necessitating inventive solutions. Millets are small-seeded grasses from the Poaceae family (Hassan et al., 2021). Nonetheless, underappreciated millets—which have been predicted to become future cereal grains—may flourish in these marginal regions because they can endure the hard soil and environmental circumstances (Rodríguez, Rahman, Thushar and Singh, 2020). According to estimates, over two billion people would be needed globally by 2050 to provide food security, with the majority of output coming from millet grains that are climate-resilient (FAO, 2021). Millets are referred to be “the nutri-cereals of today, and the coarse grains of yesterday.” Their nutritional composition, which includes high fiber, key minerals, and antioxidants, helps greatly to a healthy diet. The nutraceutical benefit of millets emphasizes their medicinal characteristics, providing safeguards for lifestyle-related health problems. On the other hand, the existence of antinutritional variables demands cautious thought. In India, biofortified millet genotypes have shown promise in boosting micronutrient content and mitigating malnutrition issues (Bhosle et al., 2024). Millets are associated with several health advantages, such as reducing the incidence of stroke and cardiovascular disease, controlling blood sugar, promoting losing weight, boosting digestion, boosting immunity, preventing cancer, and reducing inflammation. Millets are a multipurpose grain which may be eaten in many ways. They can be popped like popcorn, pulverized into flour, or cooked similarly to rice. Millets are a great way to supplement any diet and improve your overall health. Sorghum (4.15 MT), pearl millet (9.78 MT), finger millet (1.70 MT), and tiny millets (0.36 MT) are among the millet varieties produced in India in 2021–2022. We show the challenges of Popularizing Millets in Fig. 1. Now, policymakers,
marketers and producers must understand these motivations in order to develop policies that successfully encourage millet usage, eliminate barriers and boost demand (Bhosle et al., 2024).

Small seeded cereals and forage grasses used as food, feed and forage are referred to as “millet” or minor cereals. The citizens of Africa, India, China, and Japan all eat millets (Kothari et al., 2005). Millets are C4 crops that are members of the Poaceae family and its subfamilies, Panicoideae and Chloridoideae. Small millets are renowned for their superior agronomic, physiological, climate-resilient and nutritional qualities while being cultivated in marginal locations (Vetriventhan et al., 2020). The term “millets” refers to a broad category of small-seeded annual C4 Panicoid grasses, including foxtail millet (Setaria italica L.), pearl millet (Pennisetum glaucum L.), finger millet (Eleusine coracana), proso millet (Paspalum miliiuecum L.), kodo millet (Paspalum scorbiculatum L.), bahiagras (Paspalum notatum L.), little millet (Panicum miliare L.), guinea grass (Panicum maximum L.), elephant grass (Pennisetum purpurium L.) and barnyard millet (Echinochola crusgalli L.). These grasses are all members of the monocotolydon group (Arya et al., 2014). They are mostly planted on poor and marginal soils in arid parts of temperate, subtropical, and tropical regions of the world as food and fodder crops (Dwivedi et al., 2012; Lata et al., 2013).

Millets are preferable to main cereal crops like rice and wheat because they include substantial levels of calcium, magnesium, dietary fiber, iron, and protein (Ceasar and Ignacimuthu, 2009; Saha et al., 2006; Ragaee et al., 2006). Globally, micronutrient deficiencies are acknowledged as a major threat to human health (Kanatti et al., 2014). Micro-nutrients like iron and zinc may be found in finger millet (Eleusine coracana L. Gaerth.), a highly self-pollinating crop (Ramakrishnan et al., 2016). Compared to rice and wheat, millet has greater levels of several macronutrients, minerals (such as iron, zinc, phosphorus, calcium, and potassium) and vitamins (Saini et al., 2021). By chelating cations, antinutrients such as phytates, polyphenols and tannins decrease the bioavailability of minerals (Ertop et al., 2017). According to Sharma et al. (2017), millet has several key natural qualities, including low glycemic index, hypolipidemia, and antioxidant properties and other benefits in the Fig. 2.

**Nutritional Profile of Millets**

“A bird that eats rice is frictionless; a tiger that eats Jowar is strong; a nirogi that eats Ragi remains nirogi,” goes an ancient proverb. Since gaining its independence, India has had remarkable growth in its economy, education system, and pursuit of universal health care, which has been a major objective since the writing of the Indian Constitution. However, there is a growing recognition of the need to switch to healthier, more affordable, and easily accessible diets that include millets in light of India’s growing malnutrition problem, which includes both under-nutrition (deficits in vitamins, minerals, and proteins) and over-nutrition (obesity, metabolic syndrome and lifestyle diseases) (Abbasi et al., 2018). When it comes to nutrition, millets are on par with traditional cereals, often even better in terms of calorie value, amount of protein, and macronutrient compositions. They are important components of diets for both humans and animals because they include high quantities of calories, calcium, iron, zinc, fats and protein of excellent quality. Additionally, millets are a great source of vitamins and dietary fiber (Hassan et al., 2021). Please refer to the following review papers for further information on the nutritional value and composition of the main millet’s crops (Sharma et al., 2021; Bhatt et al., 2022; Tripathi et al., 2023; Goswami et al., 2024; Teklu et al., 2024). The features of the major crops of Millets are given in the Table 1.

Pearl millet, is a significant millet that is cultivated in tropical and semi-arid areas of the globe. The nutritional value of its protein is significantly influenced by the
Table 1: Three Major crops of Millets.

<table>
<thead>
<tr>
<th>Millets Name</th>
<th>Features of the Key Crops of Millets</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Finger millet</td>
<td>Soft tissue injury is reduced and the healing process is facilitated. Reduces the risk of cardiovascular disease by lowering plasma triglycerides.</td>
<td>Sarita et al. (2016)</td>
</tr>
<tr>
<td>Pearl millet</td>
<td>The gluten-free nature of pearl millet helps prevent celiac disease. Shigella-induced pathogenicity is inhibited, enhancing the immune system.</td>
<td>Akinola et al. (2017), Ganguly et al. (2019)</td>
</tr>
<tr>
<td>Foxtail millet</td>
<td>Foxtail millet lowers the chance of colon cancer, lower cholesterol and have anti-diabetic properties. Reduces the harm that ethanol causes to the liver.</td>
<td>Yang et al. (2020), Ren et al. (2016)</td>
</tr>
</tbody>
</table>

It is essential to control key crop ideotypes in order to increase agricultural productivity and prevent global food insecurity. Additionally, millets can benefit from biotechnology by being more resistant to biotic and abiotic stressors and having higher nutritional value.

Table 2: The different Amino acid profiles of major three millets.

<table>
<thead>
<tr>
<th>Amino acid (g/100g)</th>
<th>Pearl Millet</th>
<th>Finger Millet</th>
<th>Foxtail Millet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucine</td>
<td>14.1</td>
<td>10.8</td>
<td>13.60</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>5.1</td>
<td>4.3</td>
<td>4.59</td>
</tr>
<tr>
<td>Methionine</td>
<td>1.0</td>
<td>2.9</td>
<td>3.06</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>7.6</td>
<td>6.0</td>
<td>6.27</td>
</tr>
<tr>
<td>Valine</td>
<td>4.2</td>
<td>6.3</td>
<td>5.81</td>
</tr>
<tr>
<td>Threonine</td>
<td>3.3</td>
<td>4.3</td>
<td>3.68</td>
</tr>
</tbody>
</table>

Source: doi: 10.20944/preprints202401.0253.v1
A C4 grass with high cross-pollination (2n = 2x = 14), pearl millet (P. glaucum L.) R. Br., syn. (Cenchrus americanus L.) (Morrone) has the capacity to produce biomass and has a great photosynthetic efficiency (Varshney et al., 2017). In the dry and semi-arid ecologies of Sub-Saharan Africa and South Asia, where temperatures are high and irregular rainfall is a problem, P. glaucum is a significant cereal crop. Due to its short development phases, rapid growth and high photosynthetic efficiency, it is a crop that thrives in shorter growing seasons and is able to tolerate harsh circumstances. It also has an inherent adaptability to low fertility soils (Yadav and Rai, 2013; Serba et al., 2020). In Canada, North America, the United States, Mexico, Central Asia and Brazil, pearl millet is emerging as a significant crop for feed, relay crop, fodder and food. Pearl millet is significant due to its nutrient-rich grains for human consumption, as well as dry and green forage for cattle (Rai et al., 2008). Pearl millet is an agronomically strong crop with a great nutritional profile and high abiotic stress tolerance (Satyavathi et al., 2021). Pearl millet can blossom at 42°C, grow in 250 mM NaCl, and yield grain with as little as 250 mm of precipitation (Gupta et al., 2015).

Notwithstanding these qualities, pearl millet is sometimes regarded as an orphan crop since its research and development progress is less advanced than that of other staple crops (Bani et al., 2022; Srivastava et al., 2022; Shrestha et al., 2023). Considerable study has been done on pearl millet since its genome was sequenced in 2017 (Varshney et al., 2017). Understanding pearl millet’s physiological and molecular responses to abiotic stressors has been the focus of several research initiatives (Fritsche-Neto et al., 2012). By clustering changes in physiological traits, such as CC and RWC associated with gene expression, Shinde et al. (2023) examined for genes regulating physiological changes, like as chlorophyll content (CC) and relative water content (RWC), in response to abiotic stress using “weighted gene co-expression network analysis” (WGCNA). The relationships between genes and characteristics were characterized as modules, with each module being identified by a separate color name. Genes exhibiting comparable expression patterns and a propensity for co-regulation and functional relatedness are grouped together as modules. The studied genotype Tift 23D2B1-P1-P5 has a draft complete genome sequence of around 1.79 Gb, which includes an estimated 38,579 genes, according to Varshney et al. (2017). Varshney et al. (2017) re-sequenced and examined 994 pearl millet lines, enabling knowledge of population structure, genetic diversity and domestication. Use of these resequencing data allows for marker trait relationships for genomic selection, to identify heterotic pools, and to predict hybrid performance. Senthilvel et al. (2008) showed the possibility of EST-derived SSR primer pairs in pearl millet. As stated for other crops, EST-derived SSRs provide a cost-effective marker development option in pearl millet. Highlighted the significant enhancement for wax biosynthesis genes, that might contribute to heat and drought tolerance in this crop. Pearl millet researchers now have access to a substantial number of valuable SSRs in addition to the approximately 100 genomic SSRs that had previously been accessible because to the resources produced in this work. Animasaun et al. (2015) used Inter-Simple Sequence Repeat markers to perform Polymerase Chain Reaction after extracting genomic DNA from each accession. MEGA 4.0 software was used to examine the data for genetic diversity. 48 loci with 410 bands overall and 56.25% polymorphism were produced. Three primary axes contributed considerably (70.20%) to the reported variances, according to a primary Coordinates analysis.

Finger millet (Elusine coracana L.)

The finger millet (E. coracana L.) (Gaertn.), also called Mandua or Ragi in India, is a significant food crop that is extensively grown throughout the world’s arid and semi-arid regions, particularly in East Africa, India and other Asian nations (Subastri et al., 2015). The Poaceae family includes the finger millet subspecies coracana, which is thought to belong to a native crop of Central Africa (Upadhyaya et al., 2011). Tetraploid (2n=4x=36), the cultivated finger millet has morphological characteristics with E. indica (2n=18) and E. africana (2n=36). Globally, micronutrient deficiencies are acknowledged as a major threat to human health (Kanatti et al., 2014). According to Dida et al. (2008), finger millet (E. coracana L.) was domesticated in India around 3,000 years ago and is divided into two subspecies: africana and coracana (Dida and Devos, 2006). One crop that farmers grow in the semi-arid tropical regions of India and Africa is finger millet, which is highly significant from a socioeconomic standpoint on a global scale (Krishna et al., 2020). Eight percent of the world’s millets are produced, and eleven percent of those are finger millet (Bennetzen et al., 2003). According to Ramakrishnan et al. (2016), finger millet is a largely self-pollinating crop that is a good supplier of micronutrients including iron and zinc. Calcium (0.38%), dietary fiber (18%) and phenolic compounds (0.3–3%) are present in finger millet. Finger millets are rich in antioxidants, antimicrobials, anti-tumorogenic, anti-diabetic and atherosclerogenic characteristics (Sarita, 2016).
One of the most important responsibilities of the current plant breeding initiatives is sustainable agricultural development. While traditional plant breeding techniques have increased food yields, they are not without restrictions. Tools and procedures based on genetic engineering and molecular marker-assisted breeding, which are more focused and need less time are recommended in such a situation. Some of the transgenics created in finger millet for agricultural enhancement were covered by Mundada et al. (2021). Through the use of sequence variations for nutritional attributes from other cereals and resistance gene homologues of rice for blast, comparative genomics has opened the road for marker-assisted selection. A basic knowledge of dietary variation, drought tolerance and salinity tolerance has been provided by transcriptomics research. However, little is known about the genetics of many significant finger millet features, necessitating rigorous research by biologists from several fields. The finger millet genome decoded by Sood et al. (2019) will allow candidate genes for nutritional and agronomically significant features to be identified. In the near future, advancements in genome assembly, genomic selection, and genome editing will yield a wealth of data and opportunities for comprehending the genetics of intricate features.

In order to decipher the molecular mechanisms of entire finger millet systems under various conditions, new opportunities were presented by the analysis of transcriptome/RNA-seq data generated by various high-throughput sequencing platforms and their integration through systems biology. These opportunities included the identification of key genes involved in drought stress, growth and development, disease resistance, synthesis of bioactive compounds, proteins, carbohydrate and Ca accumulation, and so forth. The fundamentals of finger millet transcriptome analysis, from data creation to data analysis, are emphasized by Pathak et al. (2022). High throughput platforms and multi-layered “Omics” techniques have recently been employed to identify the genes and proteins involved in various metabolic and signaling pathways and their regulatory mechanisms in order to gain insight into the molecular genetics of biosynthesis and biomolecule homeostasis in numerous model organisms, including rice and Arabidopsis. On the other hand, virtually little knowledge on the molecular biology of seed development in FM exists in the scientific community.

To better comprehend the intricacies of seed developmental biology and nutrient partitioning, multi-omics data may be combined with systems biology. This knowledge may be applied to enhance physiologically necessary compounds for the large-scale production of nutrients in seeds and nutraceuticals by route engineering and biotechnology (Kumar et al., 2022). Proteomics, metabolomics, transcriptomics, and genomes are examples of omics methods that have gained importance recently in the study of finger millets’ ability to withstand abiotic stress. With the use of an omics-based method, Panda et al. (2022) provided insight into the advancement and potential of genetic modification in creating finger millet genotypes that are resistant to abiotic stress. In order to address the issues of FM production in the face of climate change, Wambi et al. (2019) offered a thorough review of the FM genetic and genomic resources-aided treatments that might support the three pillars of Climate-smart agriculture (CSA). Moreover, it provided very rich data on other valuable sources of variation found in FM genetic resources that have been selected to increase FM resistance to a range of climate-related stressors. In particular, second-generation genomic biotechnologies (e.g., TILLING and Eco TILLING) that are lacking and have not gotten much attention were compared with genome-wide technologies like genomic selection (GS), gene pyramiding, and gene expression. In order to provide a thorough understanding of promising concepts for finger millet breeding, Mbinda and Masaki (2021) examined the breeding strategies currently being used to improve resistance to disease and discussed potential future directions toward the development of new blast-resistant finger millet varieties. Realistic instances demonstrating the application of sophisticated molecular techniques to the development of durably blast-resistant cultivars are also included in the paper. The methods described here are high-throughput, reasonably priced approaches that drastically shorten the generation cycle and speed up research and breeding initiatives. They offer a substitute for traditional breeding methods in the quick introduction of disease resistance genes into susceptible, advantageous cultivars.

**Foxtail millet (Setaria italica L.)**

A valuable crop for food and fodder in dry areas, *Setaria italica* L. as a member of the Poaceae family has the potential to be used for C4 biofuel. According to Zhang et al. (2012), it serves as a model system for additional biofuel grasses like pearl millet and switchgrass. Most Asian nations, particularly northern China, utilize this as an ancient crop for food, feed and forage. Because of the crop’s well-known innate tolerance to drought, it is typically planted in dry, hilly regions with limited soil. Furthermore, diabetics have been advised to eat foxtail millet (Wang et al., 2011). A significant small-scale crop that has been grown for millennia worldwide is foxtail...
millet. It is a vital staple meal to millions of people and ranks second in the world’s total millet output. About 90% of the land in India is under cultivation of foxtail millet, with the three main growing states being Andhra Pradesh, Karnataka and Tamil Nadu (Makwana et al., 2023). Cultivated from the wild species green foxtail (S. viridis) more than 8000 years ago, it has close ancestry with numerous important feeds, bioenergy and food grasses such as sugarcane, maize, sorghum and switchgrass. Due to its tiny size and the recent sequencing and annotation of its small diploid genome (Bennetzen et al., 2012; Jia et al., 2013), it has become increasingly popular as an instance for C4 plants. Recombinant haplotype stacking and screening in fixed genetic backgrounds are accelerated by doubled haploid (DH) technologies (Jacquier et al., 2020). Cheng et al. (2021) showed that only knocking out SiMTL can induce haploid induction in foxtail millet. The predict that HIR may be enhanced by establishing simtl lines with various genetic histories or in conjunction with altering additional genes via the CRISPR-Cas9 technique in foxtail millet, as various inductor lines have varying HIR learned from maize (Jacquier et al., 2020).

Lata et al. (2014) used a group of 122 foxtail accessions, 45 of which were examined in a previous study, to confirm this allele-specific marker (ASM). The SiDREB2 QTL was responsible for approximately 20% of the overall PV for relative water content (RWC), indicating the significance of this QTL for foxtail millet’s ability to withstand dehydration. The NAC (NAM, ATAF, and CUC) transcription factors unique to plants have a variety of roles in the control of stress and development. Puranik et al. (2013) discovered a transcript known as SiNAC, which encodes the NAC protein, in a salt stress subtractive cDNA library of S. italica seedlings. According to Puranik et al. (2013) there is evidence to suggest that SiNAC encodes a membrane-associated NAC-domain protein, which could act both a transcriptional activator in response to stress and developmental control in plants. An Agrobacterium-mediated transformation method for foxtail millet was created by Wang et al. (2011). In this section researchers describe the system’s optimization through enhanced regeneration system effectiveness and improved gene delivery circumstances. For the purpose of callus induction and regeneration, immature inflorescence explants of foxtail millet cv. Jigu 11 with lengths ranging from 0.5 to 1.0, 1.1 to 1.5, 1.6 to 2.0, and >2.0 cm were cultivated on modified MS medium. By using blast analysis using AtFPGS1/2/3 sequences of proteins, Zhang et al. (2022) found Setaria italica FP-GS2 (SiFPGS2) loci within foxtail millet. SiFPGS2 belonged to the FP-GS subfamily, had the tetra-hydrofolypolyglutamate synthase domain and could bind tetrahydrofolate (THF) as an alternative substrate, according to the findings of phylogenetic tree analysis, function of protein domain analysis, and docking study. SiFPGS2 can be exploited as a potential gene for genetically engineered folate biofortification in Gramineae crops. Zhang et al. (2012) generated a 423 Mb draft genome that was anchored to nine chromosomes and identified 38,801 genes.

Present research status and future directions

In the last ten years, sorghum, pearl millet, foxtail millet, and finger millet have all benefited from the effective use of GWAS to discover the critical genes governing growth, development, stress tolerance, nutrient usage efficiency and nutritional quality attributes. But development in other lesser millets is still in its early stages. Genetic modification of climatic resistance, photosynthesis, and accumulation of nutrients in rice and wheat may be made possible by the genetic deconstruction of these intricate features in millets. The advancement of GWAS analysis in identifying QTLs responsible for complex characteristics in sorghum and other millets is emphasized by Vellaichamy et al. (2023). Maurya et al. (2022) talked about the proteomic developments and genomic techniques that are currently accessible for examining the abiomic stress tolerance of foxtail and pearl millet. A free, web-accessible, user-friendly millets multi-omics database platform (Milletdb, http://milletdb.novogene.com) has been developed. The Milletdb contains six millets and their one related species genome, graph-based pan-genomics of pearl millet and stress-related multi-omics data, which enable Milletdb to be the most complete millets multi-omics database available. Milletdb can simplify the functional genomics analysis of millets by providing users with 20 different tools (e.g., ‘Gene mapping’, ‘Co-expression’, ‘KEGG/GO Enrichment’ analysis, etc.). On the Milletdb platform, a gene PMA1G03779.1 was identified through ‘GWAS’, which has the potential to modulate yield and respond to different environmental stresses. Sun et al. (2023) discovered which the stress-related PLATZs TFs (transcription factors) family grows in 87.5% of millet accessions and contributes to vegetative development and abiotic stress responses with Milletdb tools. Milletdb can help researchers with crucial gene mining, genome editing, and molecular breeding of millets. Raut et al. (2023) investigated millet breeding improvements for improved characteristics, developments in processing methods and the influence of biotech and climate-smart farming practices. In this instance, we provide them with further
recent and significant articles that discuss the application of biotechnology to improve the major millets crops (Ceasar, 2022; Wilson and VanBuren, 2022; Shinde et al., 2023; Ingle et al., 2023; Rouamba et al., 2024; Haq et al., 2024; Jaiswal et al., 2024).

**Conclusion**

These technical and scientific advances provide prospects for overcoming existing hurdles and expanding millets’ position in global food systems. Also included are case studies from several countries, notably India, demonstrating successful millet planting projects, integration into national food policy and community-led activities. These examples provide useful insights into practical strategies for promoting millets. It is anticipated that in the near future, these high-throughput genotyping, sequencing and phenotyping innovations will contribute to better breeding techniques and higher-quality research on minor millet crops in response to changing climatic circumstances.

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**Conflict of interest**

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