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Review

Biotechnological Improvement of Nutri-Cereal Finger Millet: Current Status and Future Prospects

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Abstract

Climate change and widespread micronutrient deficiencies threaten food security in the semi-arid tropics. Finger millet (*Eleusine coracana* (L.) Gaertn.) is a climate-resilient "nutri-cereal" rich in calcium, zinc, iron and dietary fiber. Finger millet is a promising crop for addressing climate stress and nutrient deficiencies. However, it remains under-explored and relatively neglected in breeding and genetic improvement programs compared to major cereals. This review synthesizes recent biotechnological advances and outlines future directions for finger millet improvement. Foundational resources now include a chromosome-scale reference genome, expanding transcriptome, diverse global germplasm panels, and growing reports of genome-wide association studies (GWAS) and quantitative trait loci (QTL) for key traits including yield, stress tolerance, blast resistance, and mineral contents. Tissue culture studies reported both somatic embryogenesis and direct regeneration. Stable genetic transformation has been achieved in finger millet via *Agrobacterium*-mediated methods, particularly using shoot apical meristem (SAM) and by biolistics (gene gun) methods. Genome editing has not yet been reported, but we propose a practical roadmap leveraging reported tissue culture genetic transformation protocols for applying the CRISPR/Cas system for trait improvements. Using new biotechnological methods, along with pangenome, speed breeding, and helpful microbiomes, will make finger millet a strong and reliable food source for the future.

Keywords: finger millet; biotechnological studies; climate resilience; nutrients; genome editing

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Academic Editors: Edgar Omar Rueda-Puente and Bernardo Murillo-Amador

Received: 7 October 2025
Revised: 17 November 2025
Accepted: 20 November 2025
Published: 25 November 2025

Citation: Ceasar, S.A. Biotechnological Improvement of Nutri-Cereal Finger Millet: Current Status and Future Prospects. *Crops* **2025**, *5*, 87. https://doi.org/10.3390/crops5060087

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1. Introduction

Rising global temperatures and increasingly erratic rainfall patterns have emerged as major constraints to sustainable food production in the semi-arid tropics. Climate projections indicate a global mean temperature increase of 1.5–2.0 °C by mid-century, intensifying droughts, heat waves, and irregular monsoon events [1]. These abiotic stresses reduce yield stability and threaten crop productivity [2,3]. Simultaneously, widespread micronutrient deficiencies, particularly of iron and zinc, affect more than two billion people worldwide, leading to hidden hunger and malnutrition [4,5]. Conventional cereals such as rice and wheat are calorie-rich but poor sources of essential minerals and are highly vulnerable to climate-induced stresses.

Finger millet (*Eleusine coracana* L. Gaertn.) is an allotetraploid millet cultivated across South Asia and sub-Saharan Africa. It offers a unique solution to improving climate-resilient agriculture. Finger millet tolerates high temperatures, intermittent drought, and poor soils [6,7] while providing exceptional levels of mineral nutrients and dietary fiber [8].

Its resilience and nutritional superiority make it a "climate-smart nutri-cereal" for addressing both environmental stress and micronutrient deficiencies in the context of global warming [9]. Nutritionally, finger millet surpasses major cereals such as rice and wheat by providing higher levels of calcium, iron, zinc, and dietary fiber [10]. Nutrient profiling across Asian and African germplasms shows calcium levels ranging from 240 to 450 mg 100 g^{-1}, iron 3–14 mg 100 g^{-1}, zinc 2–4 mg 100 g^{-1}, and dietary fiber 15–20% of seed weight, values that surpass most major cereals [8,11]. Protein content varies from 5 to 9%, with balanced essential amino acids such as leucine, methionine, and phenylalanine [11]. Finger millet is also rich in polyphenols (0.3–3%) and antioxidants, conferring strong nutraceutical properties including anti-diabetic, anti-cancer, and cardioprotective potential [12,13]. Its high calcium and iron bioavailability make it particularly valuable for women, children, and elderly populations in Africa and South Asia, where micronutrient deficiencies are prevalent [14,15]. Its low glycemic index, gluten-free nature, and high levels of essential amino acids, especially methionine and tryptophan, further enhance its value in balanced diets [11]. Beyond nutrition, the crop's deep root system, efficient water-use, and ability to perform in poor soils contribute to sustainable farming under extreme climatic conditions [16,17]. These features position finger millet as a strategic crop for addressing hidden hunger and ensuring climate-smart food security, particularly across sub-Saharan Africa and South Asia, where both nutrient deficiencies and environmental stress are widespread [18].

Despite its nutritional value, finger millet has remained relatively neglected in modern breeding and genetic improvement programs for several decades. It is often cultivated in the semi-arid tropics of less-developed countries in Africa and Asia by subsistence farmers. Grain yields in smallholder farming systems are typically low and unstable, reflecting limited breeding gain [19]. Finger millet has an allotetraploid genome ($2n$ = 4x = 36; AABB) that further complicates genetic and genomic studies such as inheritance analysis, and marker validation [20]. For biotechnological improvement, limited studies on genotype-dependent plant regeneration and genetic transformation studies have been reported. More robust genetic transformation protocols are needed for functional validation and trait introgression in finger millet [21]. Addressing these gaps with genomic resources, genome editing, and genomic selection is essential to understand climate resilience and nutrient-enrichment traits and further improve these traits in finger millet.

Advances in biotechnology now offer unprecedented opportunities to unlock the full genetic potential of finger millet. Emerging resources such as annotated genome [22], transcriptomics [23], proteomics [24] and metabolomics provide molecular insight into stress tolerance and nutrient regulation in millets, including finger millet. Similarly, genome editing, genomic selection, and speed breeding can accelerate varietal improvement beyond the limits of traditional breeding [20,21]. Only a few studies have reported on genome sequencing, genomic selection, and genetic transformation in finger millet. No report is available on genome editing in finger millet till date. This review synthesizes recent progress in genomic resources, functional gene discovery, tissue culture, and genetic transformation in finger millet. It also draws inroads for CRISPR/Cas-based genome editing in finger millet. It further integrates strategies for nutrient biofortification, abiotic stress resilience, and high-throughput phenotyping for crop improvement. By combining molecular innovations, finger millet can be redefined as a climate-resilient, nutrient-dense cereal suited to future food systems.

2. Genetic and Genomic Resources

Finger millet has progressed from scaffold-level genome assemblies to a modern chromosome-scale reference genome. Early de novo genomes for two genotypes, ML-365

and PR-202, provided data on genome size (~1.1–1.5 Gb), gene catalogs, and abundant SSR/SNP resources useful for mapping and marker development [20,25]. This is the first report on the draft genome of finger millet. In 2023, the Devos group developed chromosome-level genome assembly for genotype KNE 796 with complete annotations [26]. It opened avenues for many studies, such as robust synteny analyses, genome-wide association studies (GWAS), QTL mapping, and candidate-gene discovery for stress tolerance and nutrition enrichment traits [26]. The reference genome with annotations is publicly available in Phytozome [27]. The transition from scaffold-level to chromosome-scale reference genomes marks a pivotal advancement for finger millet research. Public availability of these resources, such as through Phytozome, accelerates functional genomics, comparative studies, genome editing, and molecular breeding. Looking ahead, this genomic resource will serve as a foundation for trait pyramiding and the integration of allelic diversity from global collections, driving rapid improvement of finger millet for climate-smart and nutritionally enriched agriculture.

Gene banks at ICRISAT, Hyderabad, India and ICAR-NBPGR, India maintain reasonable accessions from global and local collections. Global finger millet accessions are maintained at ICRISAT and researchers from around the world can freely access these for their studies. As of May 2024, the ICRISAT Genebank hosts 7513 conserved accessions (global collection). From these, 622 were developed as core collections and 80 accessions as mini-core to capture maximal allelic richness with minimal redundancy [28,29]. India's ICAR-NBPGR holds the largest single collection (~10,507 accessions) from India. These are widely used as a source for pre-breeding and evaluation panels across Indian agroecological conditions [30]. With the chromosome-scale reference genome now available for finger millet, allele discovered in core and mini-core panels can be mapped to physical coordinates, accelerating GWAS, and marker deployment [26]. The divided panels, along with regional diversity sets from Africa and South Asia, offer effective, statistically robust subsets for multi-location phenotyping for drought and heat tolerance, blast resistance, and the fortification of grain with Ca, Fe, and Zn. It will provide a connection from conserved diversity to marker-assisted selection and genomic selection pipelines [28,31]. With extensive, globally accessible finger millet germplasm and a chromosome-scale reference genome, future research will harness core and mini-core panels for high-resolution trait mapping and accelerated marker deployment. These resources will drive genomic selection, enabling rapid breeding for stress resilience, disease resistance, and enhanced nutritional quality in diverse environments. A few transcriptome resources were also developed for finger millet under biotic and abiotic stresses (Figure 1; Table 1). Transcriptome analyses in finger millet under salinity, drought, and aluminum toxicity revealed key regulators, including DREB, NAC, WRKY, and HSF transcription factors, ion-homeostasis, ABA-signaling, and transporter genes controlling stress adaptation [32–34].

Table 1. Information about QTL, genes, transcriptomes, and molecular markers linked to abiotic stress resilience in finger millet, along with references.

Trait	Locus/Marker	Type	Evidence Summary	Reference
Drought	*EcGBF3* (G-box binding factor 3)	Gene (TF)	Overexpression of *EcGBF3* improves drought/osmotic tolerance in Arabidopsis	[35]
Drought	*EcCaM* (calmodulin)	Gene	Finger-millet *EcCaM* enhances drought and salinity tolerance when expressed in Arabidopsis	[36]
Drought/ salinity/ oxidative	*EcNAC1*	NAC TF gene	Stress-inducible *EcNAC1* from finger millet is strongly up-regulated by stresses. Overexpression in tobacco enhances tolerance to salinity, drought and oxidative stress	[37]

Table 1. *Cont.*

Trait	Locus/Marker	Type	Evidence Summary	Reference
Drought	*EcGBF3* (G-box binding factor 3)	Gene (TF)	Overexpression of *EcGBF3* improves drought/osmotic tolerance in Arabidopsis	[38]
Heat	UGEP95-candidate HSP70	SSR -candidate gene	Marker sequence associates with *Setaria* HSP70 and Ca-binding EGF-domain genes	
Cross-trait/ diversity resource	DArTseq SNP panel (8778 SNPs)	GBS-SNP markers for GWAS	Genome-enabled DArTseq genotyping of 423 landraces yields 8778 SNPs. Combined with 13 agronomic traits. GWAS and genomic prediction for adaptation and stress-related traits in finger millet.	[39]
Drought	*EcDehydrin7*	Gene	*EcDehydrin7* overexpression in tobacco confers improved drought tolerance	[40]
Drought/ multiple abiotic stresses	*EcCIPK31*-like	Gene	*EcCIPK31-like* in finger millet is induced by dehydration, salinity, heat, cold and oxidative stress.	[41]
Drought/ salinity (heterologous in rice)	*EcNAC67*	NAC TF gene	Salinity-responsive *EcNAC67* overexpression in rice improves tolerance to drought and salinity	[42]
Drought	*EcTAF6*	Gene	*EcTAF6* is induced by NaCl, PEG and methyl-viologen	[43]
Drought	Genome & transcriptome	Genome &+ RNA-seq	RNA-seq under low-moisture stress reveals numerous drought-induced candidate genes and an expanded repertoire of TFs associated with drought tolerance and nutraceutical traits	[20]
Drought-transcriptome & proteome	Drought-responsive DEGs	RNA-seq & label-free proteomics	Time-course drought experiment identifies 80k+ transcripts and 3009 differentially expressed proteins linked to ABA signalling, osmolyte biosynthesis, ROS detoxification and proteostasis modules.	[44]
Heat	*EcDREB2A*	TF gene	*EcDREB2A* overexpression in tobacco improves tolerance to 42 °C stress via enhanced antioxidant activity, better photosynthetic performance and reduced ROS/membrane damage.	[45]
Heat/ER & ROS stress	*EcbZIP17*	Gene	*EcbZIP17* overexpression in tobacco enhances tolerance to heat and other abiotic stresses, increases growth and yield	[46]
Heat	*EcHSP17.8*	Gene	Finger-millet small HSP cloned and strong heat-induced upregulation observed across genotypes	[47]
Salinity	Salinity-responsive DEGs (transporters, TFs, LEA, etc.)	RNA-seq	Whole-transcriptome profiling of contrasting finger-millet genotypes (Trichy 1 vs. Co-12) under 300 mM NaCl reveals differential induction of ion transporters, LEA proteins and TFs linked to salinity adaptation.	[34]
Nutrient stress–phosphorus	*EcPT1–EcPT4* (PHT1 family)	Pi transporter genes	Four high-affinity phosphate transporter genes (*EcPT1–EcPT4*) cloned from finger millet; strongly induced under low-P and AMF colonization in root and shoot tissues.	[48]
Heat	HSP/HSF DEGs	RNA-seq candidates	Heat-responsive HSP/HSF transcripts validated by qRT-PCR in contrasting finger millet genotypes.	[49]
Salinity	*HKT1;5* ortholog(s)	Candidate gene	Na^+ retrieval from xylem reduces shoot Na^+ and comparative analyses support *HKT1;5* as a priority locus in millets	[50]
Salinity	*NHX1/2* ortholog(s)	Candidate gene	Vacuolar Na^+ sequestration via NHX antiporters underpins salt tolerance	[34]
Salinity	Salt-responsive DEGs (transporters, LEA)	RNA-seq candidates	Whole-transcriptome profiling of contrasting finger-millet genotypes under salinity identifies ion transporters and LEA-like genes.	
Cross-trait	GBS-SNP panels	SNP markers	Genotyping-by-sequencing provides thousands of SNPs enabling GWAS for drought-related and adaptive traits	[32,51]
Nutrient stress-low P (association QTL)	SSR markers UGEP19, UGEP68, UGEP13, UGEP90	QTL (P starvation response)	Association mapping in 128 genotypes identifies four QTL for root and shoot biomass and root hair traits under P starvation at seedling stage.	[52]

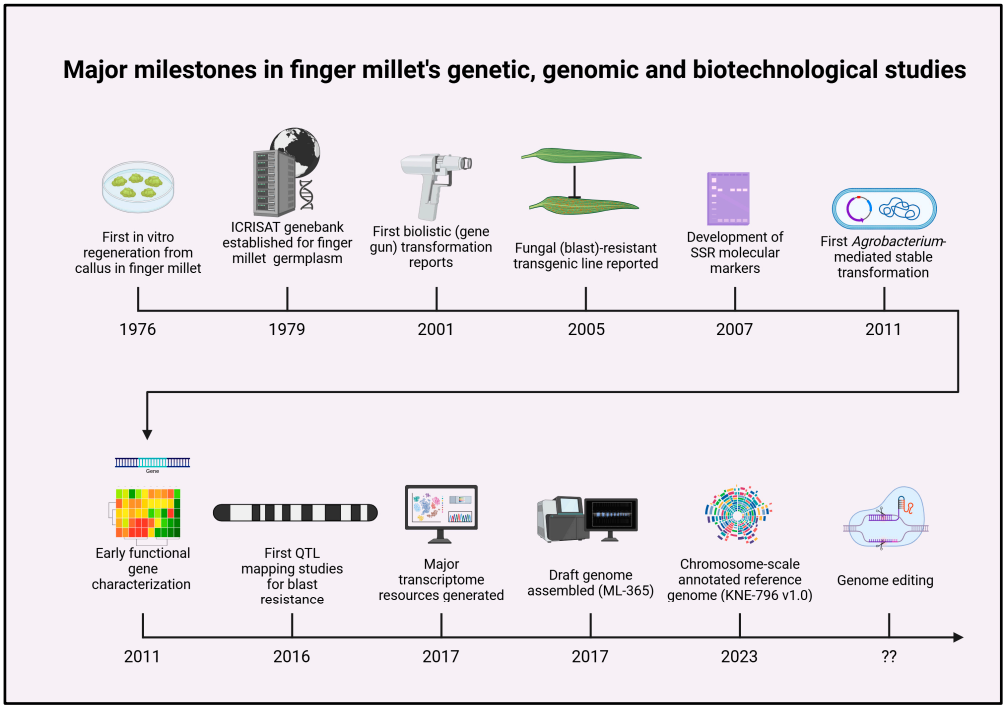


Figure 1. Major milestones in finger millet's genetic, genomic, and biotechnological studies. The journey began in 1976 with the first successful in vitro regeneration from callus cultures, laying the foundation for genetic manipulation. In 1979, the establishment of the ICRISAT genebank enabled global conservation and accessibility of finger millet germplasm. The first reports of biolistic (gene gun) transformation emerged in 2001, followed by the creation of blast-resistant transgenic lines in 2005, marking a breakthrough in trait improvement. By 2007, the development of SSR molecular markers facilitated genetic diversity assessment and linkage mapping. A major advance came in 2011, when *Agrobacterium tumefaciens*–mediated stable transformation was achieved. Subsequent years (2011–2016) saw early functional gene characterization and QTL mapping for blast resistance. In 2017, the generation of major transcriptome datasets and the draft genome assembly (ML-365) provided essential genomic resources. The release of the chromosome-scale annotated reference genome (KNE-796 v1.0) in 2023 marked a new era in functional and comparative genomics. Looking ahead, CRISPR/Cas-based genome editing represents a transformative, prospective milestone for precise trait improvement and accelerated breeding in finger millet.

Finger millet proteomics is not yet fully established and not many reports are available. An integrated RNA-seq and iTRAQ study mapped more than 3000 differentially expressed proteins under drought, implicating ABA signaling, osmolyte metabolism, chaperones, and redox enzymes as core response nodes [44]. Protein profiling of seed samples via LC–MS/MS analysis discovered over 450 proteins, emphasizing nutritionally significant and stress-responsiveness and defense categories in relationship with rice [53]. At the peptide scale, researchers reported two antioxidant peptides purified from finger-millet protein hydrolysates and validated their activities [54]. A recent review summarizes current methods and gaps in millet proteomics, highlighting key priorities such as improved quantification, advances in phospho-proteomics, and the establishment of standardized data protocols [24]. Together, these resources create a comprehensive foundation for linking genetic variation to protein expression. This integrated approach enables robust allele-to-protein validation, which is essential for accelerating the development of climate-resilient finger millet varieties through targeted breeding strategies. By facilitating a better understanding of the molecular mechanisms underlying stress tolerance, these advances support the breeding of crops better suited to withstand changing environmental conditions.

Finger millet QTL mappings have advanced significantly, evolving from initial SSR and DArT marker scans to high-resolution, SNP-dense GWAS anchored on a chromosome-scale reference genome. This progression has greatly improved the precision and efficiency of identifying genomic loci associated with important agronomic traits. Early mapping efforts using diversity panels identified loci associated with leaf blast resistance and key agronomic traits. For example, markers such as UGEP101 and UGEP95 served as proof-of-concept for the application of marker-assisted selection (MAS) in finger millet [55]. A subsequent high-density linkage map further refined QTL associated with yield components and stress-related indices, including canopy traits linked to senescence and stay-green characteristics [39]. This increased mapping resolution has enhanced the precision of breeding efforts aimed at improving these important traits. For biofortification, multi-environment GWAS pinpointed genomic regions controlling grain iron and zinc content, resulting in the identification of diagnostic SNPs for marker–trait association (MTA) validation. This progress supports more targeted breeding strategies to enhance micronutrient levels in finger millet [56]. Calcium enrichment in finger millet has been investigated through GWAS and association mapping, leading to the identification of candidate transporter and regulatory genes. These studies have also produced breeder-friendly markers, facilitating the selection of calcium-rich lines in breeding programs [57,58]. Collectively, these studies illustrate a coherent pipeline for trait improvement in finger millet. The process starts with the discovery of traits in core, minicore, or regional panels, moves forward to identify SNPs and QTLs under drought and disease stresses, and concludes with multi-location validation. This process will lead to the deployment of these markers through MAS and genomic selection. This systematic approach enables the development of stay-green, stress-resilient, and micronutrient-rich ideotypes, advancing both productivity and nutritional quality in finger millet breeding programs.

3. Physiological and Molecular Basis of Stress Resilience

Finger millet demonstrates strong resilience to abiotic stresses through mechanisms such as osmotic adjustment, enhanced antioxidant defenses, and hormonal cross-talk [6,50]. Physiologically, drought-tolerant genotypes maintain higher relative water content, chlorophyll content, and membrane stability, together with better photosynthetic performance under water deficit conditions [59,60]. Enhanced root system architecture and associated traits, together with improved water-use efficiency, enable sustained carbon assimilation even during prolonged dry spells in finger millet [61,62]. Osmotic adjustment via proline and soluble sugars, together with Na^+ exclusion and maintenance of K^+/Na^+ homeostasis, preserves cellular hydration in the finger millet. This also maintains stomatal conductance and gas-exchange efficiency in finger millet during salinity stress [34,50,63,64]. Heat stress tolerance in tolerant genotypes is associated with higher membrane stability and chlorophyll content, as well as molecular responses such as (dehydration-responsive element-binding protein 2A) EcDREB2A-mediated reactive oxygen species (ROS) scavenging [45,65]. These together support photoprotection and reduced cellular damage during heat stress in finger millet.

During drought conditions, the accumulation of compatible solutes like proline and sugars helps maintain cellular turgor and stabilizes metabolic processes, contributing to the finger millet's ability to tolerate water deficit stress [66]. Integrated transcriptome–proteome analyses have revealed the upregulation of genes involved in osmolyte biosynthesis, ROS detoxification, and the production of defense proteins in finger millet [44]. These molecular responses underpin finger millet's ability to adapt and respond to abiotic stresses such as drought. Overexpression of finger millet's *calmodulin* (*EcCaM)* in Arabidopsis plants resulted in enhanced drought tolerance, characterized by increased proline accumulation

and increased sensitivity to abscisic acid (ABA) [36]. These traits contribute to improved stress adaptation under water-limited conditions in finger millet. Late embryogenesis abundant (LEA) proteins also play a crucial role in dehydration tolerance by helping to maintain membrane integrity during water deficit conditions [50]. This protective function supports cell survival under stress in finger millet. Heat stress leads to protein denaturation and the accumulation of ROS. Finger millet and related millets mitigate these effects through the action of heat-shock transcription factors (HSTFs), which activate molecular chaperones such as heat-shock protein 70 (HSP70) and HSP90, as well as antioxidant systems [67]. Together, these responses help sustain chloroplast integrity and support stay-green phenotypes under high-temperature conditions in finger millet. Salinity stress causes both osmotic and ionic imbalances, primarily due to increased Na^+ influx. Finger millet mitigates these effects through Na^+ exclusion mediated by HKT1;5 transporters and by vacuolar sequestration via NHX antiporters [50]. Coordinated signaling by abscisic acid (ABA), jasmonic acid (JA), and salicylic acid (SA) integrates these stress responses with ROS signaling, allowing for precise regulation and fine-tuning of adaptation to abiotic stress conditions in finger millet.

4. Biotechnological Tools for Finger Millet

4.1. In Vitro Regeneration

Tissue culture techniques have provided the foundation for genetic improvement, genome editing, and functional genomics studies in finger millet. The delivery of constructs and the regeneration of transgenic plants are achieved via tissue culture-based methods. Developing an efficient regeneration protocol is essential for the genetic transformation, genome editing, and regeneration of transgenic plants in finger millet. Early studies demonstrated that both direct organogenesis and somatic embryogenesis (SE) can be achieved from various explants of finger millet, including shoot apices, immature inflorescences, and embryos. Eapen and George (1989) first reported high-frequency somatic embryogenesis from immature inflorescences using 2,4-dichlorophenoxyacetic acid (2,4-D) and kinetin [68], later refined by George and Eapen (1990) through optimized auxin–cytokinin balance [69]. Ceasar and Ignacimuthu (2008) achieved consistent SE and plantlet recovery across Indian genotypes [70]. Poddar et al. (1997) and Kothari et al. (2004) showed that modifying ammonium nitrate or inorganic salts enhanced embryogenic callus formation and shoot induction [71,72]. Satish et al. (2015) established direct regeneration from shoot apical meristems using 6-Benzylaminopurine (BAP), low 2,4-D and spermidine [73,74]. Although these studies demonstrate efficient regeneration from diverse explants, the principal limitation remains the regeneration of stable transgenic plantlets from transformed calli. As a result, the stable transformation for finger millet still exhibits low frequency.

4.2. Genetic Transformation of Finger Millet

Genetic transformation in finger millet has been accomplished using both *Agrobacterium tumefaciens*-mediated and biolistic (gene gun) methods (Table 2). Early *Agrobacterium transformation* efforts with shoot-apex explants achieved stable transformation rates of approximately 3.5–3.9%. Transient GUS expression was observed in about 16–19% of explants [75] (Figure 1). These efficiencies were obtained by optimizing factors such as acetosyringone concentration, co-culture duration, and the inclusion of antioxidants like 100 µM L-cysteine. Building on earlier methods, a shoot apical meristem (SAM) protocol was developed that combined *Agrobacterium* infection with direct regeneration, eliminating the need for a callus phase [76]. This approach shortened the regeneration cycle to approximately 45 days from SAM to greenhouse-ready plant and increased stable transformation efficiency to around 11–12% in responsive cultivars. The protocol also yielded several

single-copy transgenic events, with T1 progeny exhibiting the expected 3:1 segregation ratio [76]. These SAM-based transformation systems could be used for trait stacking and for developing genome editing works in finger millet. However, transformation efficiency still varies among genotypes, highlighting ongoing challenges related to genotype dependence.

A second *Agrobacterium*-mediated transformation strategy, developed by the Kothari group, utilized seed-derived regenerative callus as the target tissue [77]. By optimizing parameters such as infection density, acetosyringone concentration, co-culture duration, and incorporating $CuSO_4$ into the regeneration medium, they enhanced T-DNA delivery and increased the recovery rate of stable transformants in finger millet [77]. Under optimized conditions, including lower incubation temperatures around 22 °C, the group achieved transformation efficiencies of up to approximately 44% at the callus stage [77]. However, the stable recovery of whole plants from these calli was lower, and the overall process was more time-consuming compared to the faster SAM-based protocols.

The SAM-based *Agrobacterium* approach offers faster transformation cycles (~45 days) and favors single-copy, Mendelian insertions, though it remains genotype-dependent. However, the *Agrobacterium*-mediated transformation in finger millet remains relatively inefficient compared with rice. Using shoot-apex explants, only 3.8% stable transformation was achieved [74], later improved to 11.8% through protocol refinement [76]. The callus-based systems reached ~44% transformation frequency but with a variable stable plant recovery stage [77,78]. These comparisons highlight that efficient regeneration of stable transgenic plants remains a major bottleneck in finger millet, despite incremental methodological advances.

For biolistic transformation in finger millet, parameters were optimized using seed-derived callus as the target tissue, such as 1.0 µm gold particles, two shots, ~1100 psi rupture disk pressure, a 3 cm macrocarrier gap, 12 cm flight distance, and 0.4 M sorbitol treatment for osmotic conditioning before and after bombardment [79]. Under these conditions, a transformation efficiency of approximately 45.3% (based on selection) was achieved, with transgene integration in T_0 plants confirmed by Southern blot and stable transmission observed in the T_1 generation [79]. Biolistic transformation is less sensitive to genotype differences and accommodates larger DNA constructs but typically results in higher copy number and more variable transgene insertions compared to *Agrobacterium*-mediated methods.

A few traits have also been engineered in finger millet via genetic transformation. For example, blast resistance was achieved by introducing a crustacean PIN antifungal protein using biolistics [78]. Additionally, the rice *chitinase 11* (*chi11*) gene was introduced via *Agrobacterium*-mediated transformation, resulting in 3.5–3.9% stable transformation rates [80]. For abiotic stress tolerance, overexpression of the sorghum *sorghum bicolor Vacuolar H^+-Pyrophosphatase* (*SbVPPase*) gene in finger millet led to improved salt tolerance, enhanced ion homeostasis, and increased antioxidant enzyme activities [81]. In parallel, several finger millet transcription factors have been functionally validated by expression in other plants, demonstrating their regulatory roles in abiotic stress adaptation. For instance, NAC Domain-Containing Protein 67 (EcNAC67) conferred enhanced drought and salinity tolerance by improving osmotic adjustment and maintaining photosynthetic efficiency in transgenic rice [42]. Similarly, *Basic Leucine Zipper Domain-Containing Protein 17* (*EcbZIP17*) expression in tobacco plants promoted improved growth, antioxidant activity, and multi-stress resilience under adverse environmental conditions [46].

Seed-callus transformation methods expand the range of amenable genotypes, while biolistics accommodates larger genetic constructs and recalcitrant varieties, although with a greater risk of high copy-number insertions. Current priorities include developing genotype-flexible regeneration systems, expanding gene editing-ready germplasm resources, and achieving low-copy transgene delivery for complex trait stacking. Advance-

ments such as standardized, high-throughput SAM pipelines, use of morphogenic regulators, and precise developmental-stage control hold promise for improving transformation frequencies. Looking ahead, CRISPR/Cas systems with DNA-free delivery are expected to facilitate more efficient and precise trait stacking in finger millet breeding programs.

Table 2. Details on genetic transformation studies reported in finger millet. The method of transformation, explants used, strain, plasmid, genes, and transformation efficiency are listed with references.

Year	Method of Transformation	Explant	Strain	Plasmid	Selection Gene	Reporter Gene	Candidate Gene	Reference
2001	Biolistic method	Seed callus	-	-	-	*ActI/UqI/ RbcS/Ft/uidA*	-	[82]
2005	Biolistic method	Embryogenic calli	-	*pPur*	*bar*	*uidA*	*PIN*	[78]
2006	Biolistic method	Shoot tip callus	-	*TG0063* of *pCAMBIA* series	-	-	*PcSRP*	[83]
2011	*Agrobacterium*-mediated	Shoot apex	LBA4404	*pCAMBIA1301*	*HptII*	*uidA*	-	[75]
2011	*Agrobacterium*-mediated	Seed callus	LBA4404	*pBI-121*	*nptII*	*uidA*	-	[77]
2011	*Agrobacterium*-mediated	Seed callus	EHA105	*pCNL-56*	*nptII*	*uidA*	-	[77]
2012	*Agrobacterium*-mediated	Shoot apex	LBA4404	*pHyg-Chi.11*	*HptII*	-	*Chi11*	[80]
2014	*Agrobacterium*-mediated	Seed callus	LBA4404	*pCAMBIA1301*	*HptII*	*uidA*	*SbVPPase*	[81]
2014	*Agrobacterium*-mediated	Seed callus	EHA105	*pCAMBIA1380*	*HptII*	*uidA*	*mtlD*	[84]
2017	*Agrobacterium*-mediated	Shoot apex	EHA105	*pCAMBIA1301*	*HptII*	*uidA*	-	[76]

5. Prospectus for CRISPR-Based Genome Editing in Finger Millet

Finger millet offers an excellent test material for precise gene editing because it combines nutritional promise with climate-resilience. Finger millet is a nutritionally rich grass that serves millions in semi-arid regions, yet genetic improvement has progressed slowly. No report is available to date on genome editing in finger millet. Recent advances in CRISPR/Cas technologies across foxtail millet, sorghum, maize, rice, and wheat now provide clear blueprints for precise gene editing in these crops. The following section synthesizes these lessons and maps a practical gene editing pathway for finger millet from target selection to edited line release.

The prospects for deploying CRISPR/Cas system in finger millet are highly promising. The crop's established compatibility with *Agrobacterium*-mediated transformation of shoot apices, coupled with efficient direct regeneration protocols, provides a reliable entry point for the delivery of genome-editing tools such as CRISPR/Cas9, base editor, and prime editor (Figure 2) [75,76]. This foundation enables precise gene editing and accelerates the development of improved finger millet varieties. In sorghum, CRISPR/Cas-mediated gene edits have been successfully introduced using *Agrobacterium*-mediated delivery in embryo-derived tissues, resulting in reliable inheritance of the targeted modifications [85,86]. These studies provide valuable practical guidelines for guide RNA (gRNA) design, selectable marker strategies, and optimized tissue culture schedules, all of which can be adapted to accelerate genome editing efforts in finger millet (Figure 2).

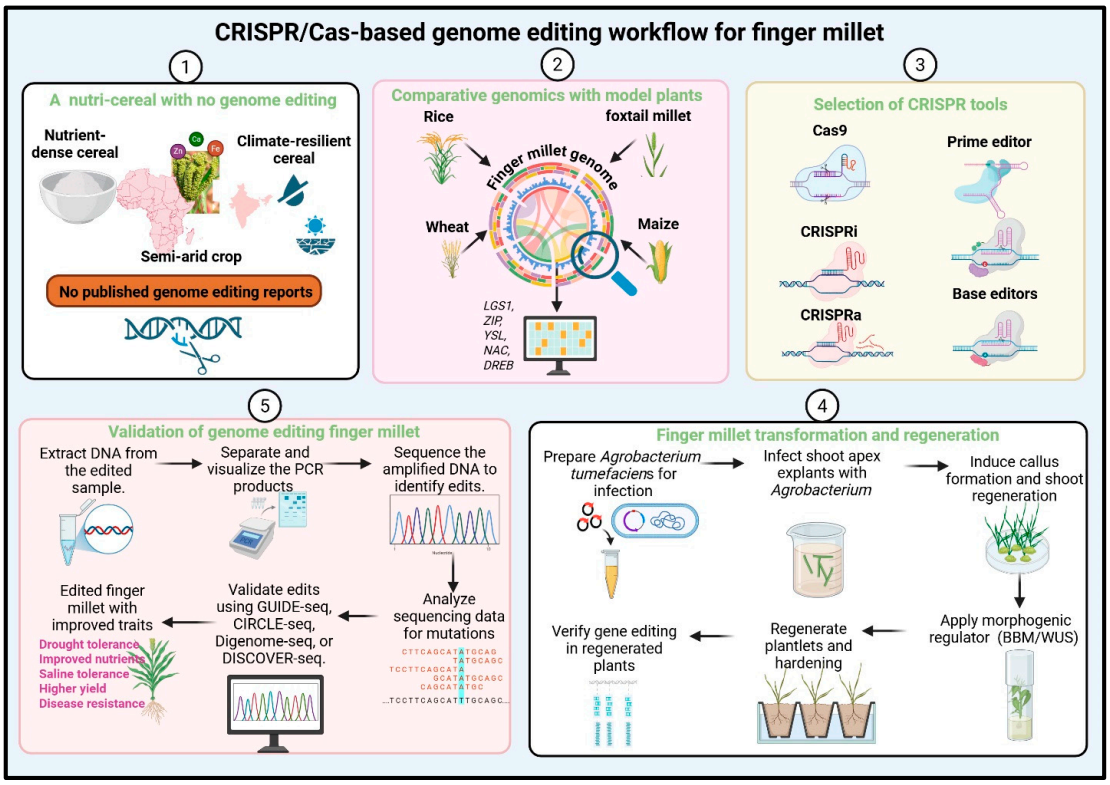


Figure 2. Proposed CRISPR/Cas-based genome editing workflow for finger millet. This schematic outlines the stepwise strategy for establishing genome editing in finger millet, a nutrient-dense, climate-resilient cereal with no published genome editing reports. (1) Context and significance: Finger millet is a semi-arid crop rich in micronutrients (Fe, Zn, and Ca) and resilient to stress but lacks reported genome editing studies. (2) Target discovery through comparative genomics: Candidate genes (e.g., LGS1, ZIP, YSL, NAC, DREB) are identified by comparison with model cereals including foxtail millet, rice, maize, and wheat. (3) Selection of CRISPR tools: suitable editors such as Cas9, CRISPR interference (CRISPRi), CRISPR activation (CRISPRa), base editors, and prime editors are chosen depending on the desired modification. (4) Transformation and regeneration: Shoot apex explants are infected with *Agrobacterium tumefaciens*, followed by callus induction, shoot regeneration, and optional application of morphogenic regulators (BBM, WUS) to enhance transformation efficiency. Edited plantlets are regenerated and verified. (5) Validation and trait assessment: Edits are confirmed using PCR and sequencing-based assays (GUIDE-seq, CIRCLE-seq, Digenome-seq, or DISCOVER-seq). Edited lines are evaluated for improved traits such as drought tolerance, nutrient enrichment, salinity tolerance, higher yield, and disease resistance.

Sorghum serves as a model for trait-to-gene targeting by genome editing, particularly through the strigolactone pathway. Key genes such as *LOW GERMINATION STIMULANT 1* (*LGS1*) have been shown to confer field-relevant resistance to Striga (a major parasitic weed), as proved by targeted gene editing [87,88]. These genetic pathways are preserved across grass species, including finger millet, establishing a definitive framework for adapting trait-based gene editing methodologies to enhance resistance in finger millet. In foxtail millet, researchers have achieved efficient genome editing, including base editing of herbicide target alleles and targeted modifications in the *MATRILINEAL* gene [89–91]. Edits in *MATRILINEAL* have enabled the development of doubled haploid pipelines, allowing for rapid fixation of edited alleles in foxtail millet [89]. These advances demonstrate the potential for accelerated breeding and trait improvement using CRISPR/Cas tools in finger millet. Advances in maize and wheat have demonstrated the power of base and prime editing for highly precise genome modifications, such as promoter modifications and targeted point mutations. Notable achievements include the landmark engineering of the

ARGOS8 promoter to enhance drought tolerance and robust knockout of the *MLO* gene for broad-spectrum disease resistance in maize, wheat and rice [92–95]. These approaches provide valuable templates for applying precise genome editing in finger millet to improve key agronomic traits. For finger millet, immediate priorities include establishing routine construct delivery systems in meristematic tissues, utilizing CRISPR/Cas tools to enhance nutritional traits, particularly in carotenoid and folate pathways, as well as to initiate pilot projects with prime editing to introduce precise promoter and coding sequence modifications. The creation and sharing of specialized toolkits by millet or cereal researchers, such as reliable CRISPR/Cas constructs tested in cereals, efficient pegRNA and base editor systems from foxtail millet, and flexible methods for transforming sorghum, could help develop efficient genome editing systems for finger millet. These collaborative efforts could make finger millet genome editing more standardized, efficient, and reproducible and offer useful models for improving finger millet. These will be instrumental in efficiently transferring targeted edits into farmer-preferred genotypes, reducing the number of breeding cycles required for trait integration. CRISPR/Cas base and prime editing technologies offer the ability to precisely refine finger millet alleles in key transporter and transcription factor genes without introducing foreign DNA. For example, cytosine or adenine base editors can induce specific amino acid changes in ZIP and YSL transporter proteins, potentially enhancing iron and zinc loading in the grain. This targeted approach enables the improvement of nutritional traits while maintaining the crop's genetic integrity. Promoter engineering can modulate *SWEET* and *NRT* expression to balance carbon and nitrogen flux. Prime editing can install or repair functional motifs in NAC, DREB, and bZIP regulators that govern drought and grain filling in finger millet.

The gRNA expression in finger millet could begin with endogenous RNA polymerase III promoters (*U6* and *U3*), as they yield precise small RNA transcripts with reliable initiation and termination. Evidence from rice, sorghum, and other grasses shows that *U6* and *U3* promoters drive strong editing and multiplexing and often work across cereals [86,96,97]. When native U6 and U3 promoter sequences are not yet available for finger millet, orthologous promoters from rice or maize can be effectively used to drive gRNA expression. It is important to avoid long thymidine (T) tracts in the gRNA scaffold and to ensure that U6-driven gRNA begin with a guanine (G) and U3-driven gRNA start with an adenine (A) for optimal transcription efficiency [98–100]. If RNA polymerase III-driven gRNA expression proves suboptimal, alternative strategies can be employed by expressing gRNAs from RNA polymerase II promoters in finger millet. This can be achieved by flanking the gRNAs with hammerhead and hepatitis delta ribozymes or by using tRNA spacers, both of which ensure accurate processing of the gRNAs [101,102]. These approaches also facilitate high-order multiplexing, allowing multiple gRNAs to be expressed simultaneously under strong promoters such as maize ubiquitin or rice actin, thereby increasing genome editing efficiency in finger millet.

Rigorous validation of genome edits in finger millet should establish a clear connection between edit specificity and trait-level performance. The process should begin with in silico gRNA selection to maximize on-target efficiency and minimize off-target effects. This should be followed by confirmation of edits at both on-target and potential off-target sites using amplicon deep sequencing [103,104]. Quantitative analysis tools such as CRISPResso2 can then be used to measure indel spectra and allele frequencies, ensuring accurate assessment of editing outcomes and their correlation with phenotypic traits. In addition to targeted validation, it is essential to incorporate unbiased discovery of genome editing outcomes in finger millet using genome-wide assays. Techniques such as GUIDE-seq in living cells, CIRCLE-seq as an in vitro screen, Digenome-seq for biochemical footprinting, and DISCOVER-seq to monitor chromatin-bound repair factor activity provide

comprehensive assessment of both on-target and off-target events [105–108]. Integrating these approaches ensures a robust evaluation of edit specificity and genome integrity, supporting safe and effective deployment of genome editing technologies in finger millet improvement. Together these provide complementary coverage across sequence and chromatin contexts. Phenotyping should be conducted across both controlled and managed stress environments, utilizing standardized assays of abiotic stresses. High-throughput measurements such as canopy temperature and fluorescence imaging, combined with multi-time point assessments, will provide detailed insights into the physiological and agronomic impacts of genome edits [109]. This comprehensive phenotyping approach ensures robust evaluation of trait performance under relevant environmental conditions.

6. Challenges and Future Prospects for Biotechnological Improvement of Finger Millet

Finger millet research faces bottlenecks that begin with low transformation and regeneration efficiencies. These limit genotype coverage and slow the editing process, despite progress with shoot apex systems and direct regeneration. The crop is an allotetraploid with subgenomes that contain extensive duplication and high repeat content, which complicates gRNA design because paralogs and homeologs create multiple, closely related targets and increase the need for multiplexed edits to achieve complete editing. Experience from wheat shows that coordinated editing of homeoalleles is feasible but requires careful gRNA placement and delivery that supports concurrent cleavage, using tRNA or ribozyme processing to express several gRNAs from compact cassettes [94,102]. Base editors enable precise allele refinement without introducing double-strand breaks, but their effective use requires subgenome-level targeting and rigorous off-target assessment, especially in repetitive genomic regions [110]. Developing a high-quality pangenome and subgenome-level annotations for finger millet will significantly reduce false positives during in silico gRNA prediction and enhance the accuracy of linking specific edits to phenotypic outcomes, ultimately supporting more efficient and reliable genome editing efforts.

Future research in finger millet should harness artificial intelligence (AI) for guided target discovery, leveraging pangenome-based selection to identify and prioritize gene targets for editing. Despite advances in tissue culture, transformation, and genome editing, finger millet improvement still faces low regeneration efficiency, genotype dependence, and limited genomic resources. Future efforts should integrate biotechnology with AI for predictive modeling of gene–trait associations and stress responses (Figure 3). Machine-learning approaches using multi-omics and environmental data can accelerate genotype selection, optimize transformation conditions, and enhance stress-tolerance prediction [111,112]. Such AI-driven biotechnological frameworks will enable precision breeding and rapid trait deployment for climate-resilient finger millet cultivars. AI can accelerate CRISPR/Cas construct design pipelines for finger millet. Deep-learning models trained on large editing datasets can help predict gRNA on-target efficiency, off-target risk, and PAM-proximal base preferences, enabling in silico screening of thousands of gRNAs before wet-lab work [113]. This will save a lot of time, labor and cost for finger millet genome editing. For transporters and metabolic enzymes controlling nutrient use or stress tolerance, AI models can prioritize functional codons of genes or regulatory motifs in promoters for base or prime editing to enhance activity or regulation [114]. In finger millet, combining these AI-CRISPR tools with existing genome and transcriptome resources would rapidly pinpoint edit sites linked to drought, salinity and micronutrient traits [115].

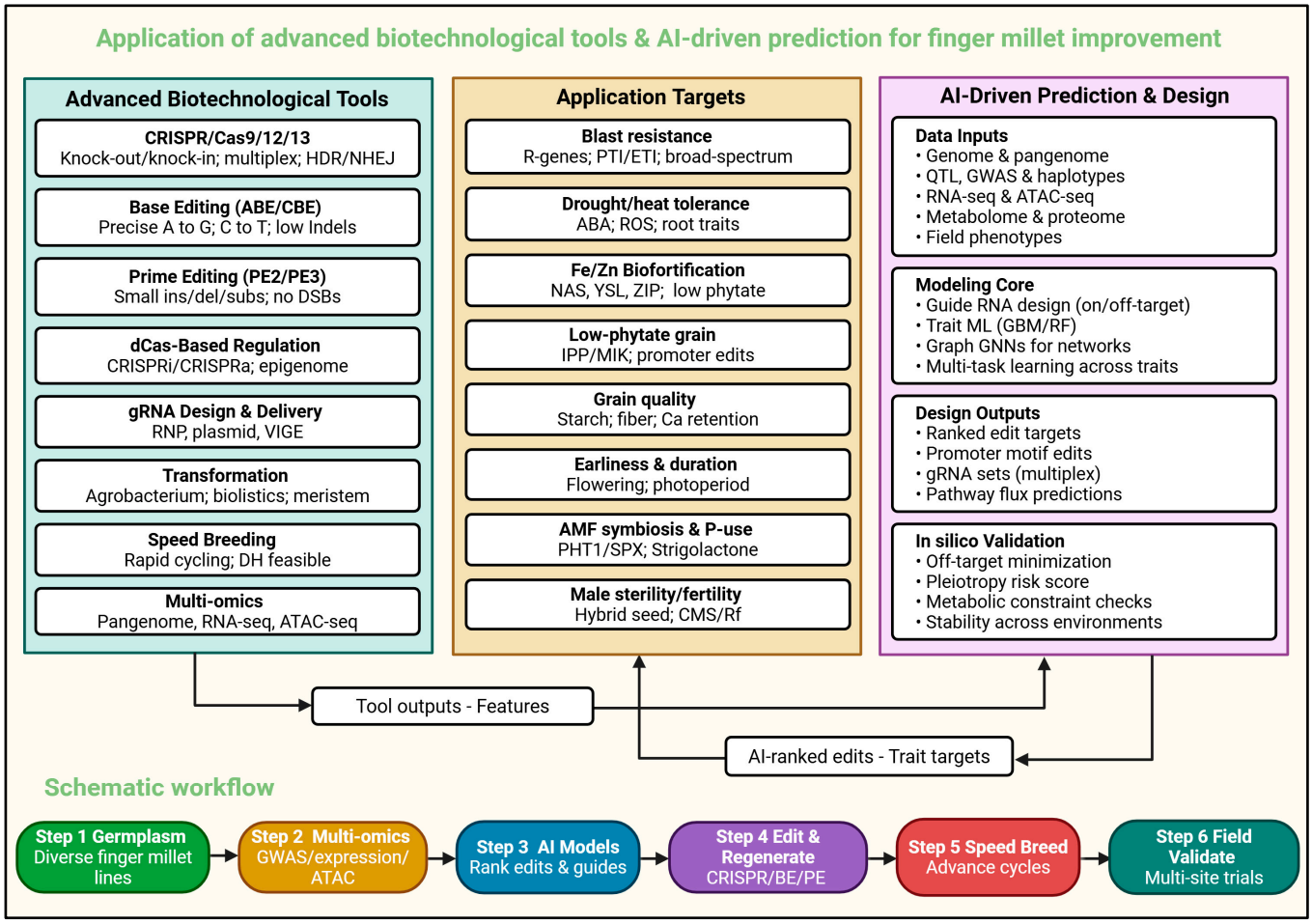


Figure 3. Application of advanced biotechnology tools and AI-driven prediction in finger millet. The left panel compiles advanced biotechnological tools such as CRISPR/Cas nucleases, base and prime editors, CRISPRi/a regulators, RNAi/VIGS/VOX, Agrobacterium and biolistic transformation, robust regeneration pipelines, and speed breeding—which both generate actionable datasets and enable precise genome modifications. The right panel outlines an AI-driven design stack that integrates multi-omics and phenotyping like genome and pangenome resources, QTL/GWAS haplotypes, RNA-seq and ATAC-seq, metabolome/proteome profiles, and field phenotypes. Machine-learning modules such as guide on/off-target predictors, gradient-boosting and random-forest trait models, graph neural networks for regulatory and co-expression networks, and sequence transformers prioritize trait-linked genes, propose promoter-motif edits, assemble multiplex gRNA sets, and draft HDR/prime-editing donors, followed by in silico validation of off-targets, pleiotropy, metabolic constraints, and environmental stability. The central panel lists breeding targets like blast resistance; drought and heat tolerance; Fe and Zn biofortification and low-phytate grain; improved grain quality; earliness; lodging resistance; AMF symbiosis and phosphorus-use; and reproductive control for hybrid seed. The bottom schematic summarizes the schematic workflow.

Efforts should also focus on designing crop-supportive microbiomes that enhance nutrient uptake and stress tolerance and on deploying improved varieties within climate-smart agricultural systems. Importantly, aligning research outcomes with supportive policy frameworks and active farmer participation will be crucial to ensure that scientific advancements translate into tangible benefits in farmers' fields and contribute to sustainable food systems. AI can prioritize causal genes and regulatory variants when it is trained on multi-omics data and paired with genomic prediction. Deep learning and machine learning models already improve genomic prediction across crops and they can be extended to recommend genotype-by-environment matches for diverse agroecologies where finger

millet is grown [116,117] Pangenome resources reveal the presence or absence of variation and structural haplotypes that underlie adaptation, and a finger millet pangenome would allow for association tests that do not miss nonreference alleles [118].

Microbiome engineering can complement genetics by improving phosphorus and nitrogen acquisition and by improving abiotic stress tolerance in finger millet. Core strategies include selection of phosphate-solubilizing bacteria that release mineral phosphorus and deployment of arbuscular mycorrhizal fungi that expand the effective root surface for nutrient capture and modulate water relations during drought [119–121]. Future designs should treat the plant and microbiome as a key strategy and should use synthetic communities that are isolated from successful finger millet farms. Integration into climate-smart low-input systems requires agronomy that stabilizes yield while reducing external inputs. Ecological and sustainable intensification principles point to rotations with legumes, residue retention that feeds soil carbon, precise placement of small nutrient doses, and water-saving practices that fit rainfed conditions where finger millet grows well [122,123]. Robust phenotyping of canopy temperature, spectral indices, and ionomics should accompany variety and microbiome packages to confirm that physiological gains translate to nutrition and resilience outcomes in farmer fields. These collective efforts will ultimately place finger millet as a prominent climate-resilient and nutrient-dense crop suited for future climates.

Funding: This research received no external funding.

Data Availability Statement: No new data were created for this work.

Acknowledgments: The author thank Rajagiri College of Social Sciences for all supports.

Conflicts of Interest: The author declares no conflicts of interest.

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