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Review Article

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Mutation Breeding and Metabolomics Integration for Finger Millet Improvement: Toward Mechanism-Based Crop Enhancement

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Abstract

Finger millet is a climate-resilient cereal widely cultivated in Africa and Asia, valued for its high calcium, dietary fibre and essential amino acid content. However, its genetic improvement has lagged behind major cereals due to limited genetic variability and insufficient research investment. Mutation breeding has been widely used to generate novel genetic variation for crop improvement, while metabolomics enables comprehensive profiling of biochemical variation linked to phenotypic traits. These approaches have independently improved agronomic performance and nutritional quality while enabling identification of key metabolites and metabolic pathways linked to plant performance. However, their integration within a single experimental framework in finger millet remains unexplored, limiting linkage between induced genetic variation and functional biochemical traits and constraining precision breeding. This mini review summarizes current knowledge on mutation breeding and metabolomics in finger millet and proposes a conceptual framework for their integration in crop improvement. The complementary roles of induced mutagenesis in generating genetic diversity and metabolomics in capturing downstream biochemical responses are highlighted. Integration of these approaches offers opportunities for early selection of superior genotypes using metabolite-based markers and for improved understanding of trait expression. Key limitations include high analytical costs, limited metabolomic databases and lack of integrated datasets. Future research should prioritize multi-omics integration, advanced data analytics and metabolite-guided selection to accelerate development of improved finger millet varieties with enhanced agronomic and nutritional traits.

Keywords: Finger Millet, Mutation Breeding, Metabolomics, Crop Improvement, Genetic Variability, Multi-Omics Integration

1. Introduction

Finger millet has long supported populations across sub-Saharan Africa and South Asia due to its exceptional adaptability to marginal environments [1]. The crop thrives under conditions characterized by low soil fertility, erratic rainfall, and high temperatures, where many major cereals perform poorly [2]. Finger millet grains are rich in calcium, dietary fibre, essential amino acids, and micronutrients, making the crop highly valuable for improving food and nutritional security among vulnerable populations [3]. Despite these advantages, finger millet remains under-researched and underutilized compared to major cereals such as wheat, rice and maize [4]. Limited research investment has slowed genetic improvement and contributed to yield stagnation, highlighting the need for innovative strategies to accelerate genetic gain in finger millet.

Improving finger millet productivity remains a major challenge due to limitations in conventional breeding approaches. Conventional breeding primarily relies on selection from existing genetic variability and hybridization strategies. However, the self-pollinating nature of finger millet restricts recombination, resulting in a narrow genetic base within cultivated germplasm [5]. This constraint limits opportunities for improving complex traits such as yield and nutritional quality. Phenotypic selection is

often influenced by environmental variability, which can obscure true genetic differences among genotypes [6]. Long breeding cycles, coupled with limited access to advanced analytical tools, further constrain selection efficiency and genetic progress. These constraints call for approaches that expand genetic variability and improve selection precision through mutation-based and omics-assisted tools.

Mutation breeding has been used to generate novel genetic variation in crop improvement programs [7,8]. It involves the use of physical mutagens, such as gamma rays and X-rays, and chemical mutagens, such as ethyl methanesulfonate, to induce changes in DNA [9]. These changes include point mutations, insertions, deletions, and chromosomal rearrangements, which can result in new beneficial traits [10]. This approach has contributed to the development of improved crop varieties with desirable traits, including enhanced yield, early maturity, and improved grain quality, without the introduction of foreign DNA [7-11]. However, the process is inherently random, requiring the screening of large populations and multiple generations to identify and stabilize beneficial mutations. Despite its success in several crops, application in finger millet remains limited, and the efficient identification of useful variants continues to constrain its effectiveness. The efficient identification of superior variants requires integration of high-throughput phenotyping and metabolomics within mutation breeding pipelines.

Metabolomics has emerged as a powerful tool for the comprehensive profiling of small-molecule metabolites that reflect the physiological and biochemical state of plant tissues [12,13]. Metabolites, including sugars, amino acids, organic acids and secondary compounds, represent the final outputs of gene expression and provide direct insight into functional biological processes [14]. Advanced analytical platforms such as GC–MS, LC–MS and NMR spectroscopy (Table 1) enable high-resolution detection of these compounds [12-15]. Metabolomics has been used in crop breeding programs to identify biochemical markers associated with important traits and to uncover variation that is not detectable through phenotypic evaluation alone [16]. Metabolomic research in finger millet remains limited, and its application in breeding pipelines is still at an early stage.

Integrating mutation breeding with metabolomics offers a promising strategy for improving selection efficiency and interpreting trait expression. This integration can enable early and more precise identification of superior genotypes through metabolite-based markers and provide insights into the metabolic pathways underlying key traits. Importantly, no study has systematically integrated mutation breeding and metabolomics in finger millet, leaving a critical gap in linking genetic variation with biochemical traits and limiting precision breeding. This mini review summarizes the current knowledge on mutation breeding and metabolomics in finger millet and to propose a conceptual framework for their integration to enhance selection efficiency and accelerate crop improvement.

Analytical Technique	Metabolite Type	Strengths	Limitations	Reference
GC-MS	Volatile compounds, organic acids, sugars	High sensitivity and reproducibility	Requires derivatization, limited for non-volatiles	[17]
LC-MS	Non-volatile metabolites, secondary metabolites	Broad coverage, high sensitivity	Complex data analysis	[18,19]
NMR	Multiple metabolite classes	Non-destructive, reproducible	Lower sensitivity, expensive	[20]

Table 1: Metabolomics Platforms for Finger Millet Analysis

2. Mutation Breeding in Finger Millet

Mutation breeding has emerged as a powerful strategy for generating genetic variability beyond the limits of natural diversity [9-21]. This approach involves the deliberate exposure of plant material to physical or chemical mutagens to induce heritable genetic changes, thereby generating novel allelic variation with potential phenotypic benefits [22]. Physical mutagens such as gamma rays are widely applied due to their strong penetration capacity and ability to induce diverse mutation types [23]. Chemical mutagens, including ethyl methanesulfonate and sodium azide, predominantly generate point mutations that alter gene function at the nucleotide level [24,25]. These mutations influence gene expression, protein activity, and metabolic pathways, ultimately expanding the functional genetic base of crops with limited variability. However, optimization of dose and exposure is essential to balance mutation efficiency and plant survival [26-28].

The effectiveness of mutation breeding depends on efficient mutation induction followed by systematic population advancement and selection. The M1 generation represents the initial mutagenized population, where mutations are present but often not phenotypically expressed due to chimerism [29]. Expression and segregation of mutations become evident in the M2 generation, enabling selection of desirable phenotypes. Subsequent generations are required to confirm trait stability and heritability. Beneficial mutations occur at low frequencies, making large population sizes essential for capturing rare advantageous variants. Multi-environment evaluation is also necessary to assess

trait stability and genotype-by-environment interactions [30]. Selection typically targets yield components, plant architecture, maturity, and stress-related traits, although reliance on phenotypic evaluation alone can limit detection of underlying biochemical variation. Integration of molecular markers, high-throughput phenotyping, and mutation detection platforms (e.g., TILLING and sequencing-based approaches) plays an increasingly important role in improving selection accuracy and accelerating breeding progress.

The broad relevance of mutation breeding is demonstrated through its successful application across diverse crop species. Induced mutagenesis has contributed to improvements in yield components, lodging resistance, grain quality, and stress tolerance in cereals, legumes, and other crops [7,31,32]. These advances have led to the release and widespread adoption of numerous mutant-derived varieties [33]. A key advantage of this approach lies in its ability to modify specific traits without disrupting the overall genetic background of elite cultivars. Consequently, targeted improvements in nutritional quality, disease resistance, and environmental adaptability have been achieved across multiple crop systems. The relevance of mutation breeding is particularly high in crops with narrow genetic bases such as finger millet where conventional breeding options are limited.

Mutation breeding in finger millet has focused on generating variability for agronomic and nutritional traits. Mutant populations have shown variation in plant height, tillering capacity, grain yield, and maturity, indicating the potential of induced mutagenesis to enhance productivity [7,34,35]. Improvements in grain mineral content and protein composition further demonstrate its relevance for nutritional enhancement. Development of early-maturing and lodging-resistant genotypes has contributed to improved adaptation across production environments. However, progress in finger millet remains constrained by limited large-scale mutagenesis efforts, restricted research investment, and relatively small breeding populations compared to major cereals. Many studies rely on evaluations conducted under restricted environmental conditions, which reduces the robustness and generalizability of findings. The lack of integrative datasets linking genetic, phenotypic, and biochemical variation further limits the effective utilization of induced diversity.

Despite its potential, mutation breeding is constrained by several inherent limitations. The random distribution of induced mutations complicates prediction of phenotypic outcomes, and most mutations are neutral or deleterious. Extensive and resource-intensive screening is therefore required to identify useful variants. Phenotypic selection alone may fail to capture subtle biochemical and metabolic alterations associated with induced variation, and environmental effects often obscure genetic differences. These limitations are further amplified by long breeding cycles and limited population sizes in finger millet improvement programs. Molecular and biochemical profiling tools can improve detection of beneficial mutations and enhance selection precision. Continued methodological refinement, together with integration of advanced omics-based analytical techniques, will be critical for improving the effectiveness of mutation breeding in finger millet improvement.

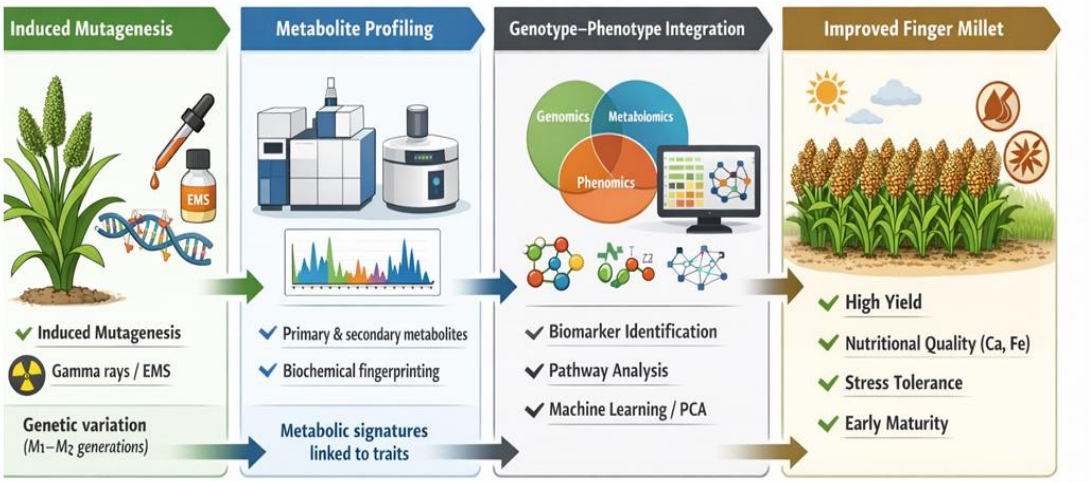

Figure 1: Conceptual Framework for Integrating Mutation Breeding and Metabolomics in Finger Millet Improvement.

3. Metabolomics

Metabolomics represents a comprehensive analytical approach focused on the identification and quantification of small-molecule metabolites within biological systems. These metabolites include primary compounds such as sugars, amino acids, and organic acids, as well as secondary compounds such as phenolics and flavonoids [14]. Metabolite profiles provide a direct reflection of cellular biochemical activity and physiological state. Advanced analytical platforms, including GC–MS, LC–MS and NMR, enable high-resolution detection and quantification of diverse metabolites [12]. Both targeted and untargeted approaches are used to investigate metabolic variation. Targeted metabolomics focuses on a predefined set of compounds or metabolites, whereas untargeted profiling captures a broad spectrum of metabolites

[36,37]. The resulting datasets are highly complex and require advanced statistical and computational tools for interpretation [38]. Multivariate analysis methods such as principal component analysis are commonly used to identify patterns and relationships among samples. Metabolomics has become an essential tool in plant science for linking biochemical processes to phenotypic traits.

Applications of metabolomics in crop research have expanded rapidly due to its ability to link biochemical variation with phenotypic traits. Metabolomic profiling has been used to identify biomarkers associated with yield and stress tolerance [39]. Changes in metabolite accumulation reflect alterations in key metabolic pathways under different environmental conditions. Carbon and nitrogen metabolism, phenylpropanoid pathways, and energy metabolism are frequently studied in relation to plant performance. Metabolomics enables detection of subtle biochemical differences that are not visible at the morphological level. This improves understanding of genotype–phenotype relationships and physiological adaptation under varying environmental conditions. Crop studies have demonstrated that metabolic signatures can be used to classify genotypes and predict performance [38,40]. Integration with other omics approaches, including genomics and transcriptomics, further strengthens the ability to connect genetic variation with biochemical outcomes. Metabolomics contributes to a systems-level understanding of plant biology and supports modern precision breeding approaches.

Finger millet contains a diverse range of metabolites that contribute to its nutritional and functional properties. High levels of phenolic compounds and flavonoids are associated with antioxidant activity and health benefits. Mineral content, including calcium and iron, contributes to its nutritional value. Metabolite profiling studies have identified variation in these compounds across different genotypes. Primary metabolites play essential roles in growth, development, and grain filling, whereas secondary metabolites contribute to defense mechanisms and stress responses [41]. Environmental factors strongly influence metabolite accumulation, leading to significant variation in biochemical composition across environments. Existing studies provide initial insights into the metabolic composition of finger millet; however, they remain limited in scale and coverage. Limited metabolomic coverage in finger millet necessitates expanded high-resolution profiling to fully characterize its biochemical diversity.

Analytical challenges in metabolomics include variability in sample preparation, instrument sensitivity, and data interpretation. Standardization of methodologies is required to ensure reproducibility of results. Large datasets generated from metabolomic studies require advanced bioinformatics tools for analysis. Identification of metabolites is often limited by incomplete reference databases. Quantification can be affected by ion suppression and matrix effects. Integration of metabolomic data with phenotypic and genomic datasets requires careful experimental design and robust statistical frameworks. Despite these challenges, technological advancements continue to improve analytical capabilities. High-throughput platforms enable large-scale metabolite profiling across multiple samples, while development of metabolite databases supports improved identification and annotation.

Metabolomics offers significant potential for improving crop breeding through the identification of biochemical markers associated with desirable traits. These biomarkers facilitate early selection of superior genotypes, thereby reducing reliance on long-term and resource-intensive field evaluations. As a result, metabolomics enhances the efficiency and precision of breeding programs, positioning it as a valuable component of modern crop improvement strategies. Metabolite profiling also facilitates detection of physiological responses to environmental stress, improving understanding of adaptation mechanisms. Integration with genomic and transcriptomic data further enhances interpretation of genotype–phenotype relationships. However, application in underutilized crops such as finger millet remains limited. Expansion of metabolomic research in this crop will support the development of improved varieties. Improved analytical infrastructure, increased accessibility of platforms, and strengthened computational capacity will be essential for broader adoption. Overall, metabolomics represents a critical component of modern crop improvement strategies and provides a foundation for integrative breeding approaches.

4. Integration of Metabolomics and Mutation Breeding

Mutation breeding and metabolomics represent complementary approaches that can be integrated into a unified pipeline for improving crop selection and accelerating trait discovery. This integration follows a sequential yet iterative framework linking induced genetic variation to metabolite-assisted selection (Figure 1). Mutation breeding generates genetic diversity through induced DNA changes, producing M1 and M2 populations with a wide range of phenotypic variation [7-10]. Whereas metabolomics enables detailed characterization of downstream biochemical responses associated with this variation [12,16,42]. Integration therefore establishes a functional connection between induced genetic variation and metabolic pathway regulation, enabling direct interpretation of genotype–phenotype relationships.

The first stage of the integrated framework involves mutation induction and population development. Physical and chemical mutagens generate heritable genetic variation, which becomes segregated and phenotypically expressed in subsequent generations. These mutant populations exhibit complex biochemical and physiological changes that are not fully captured through morphological evaluation alone, highlighting the need for higher-resolution analytical approaches. The second stage involves phenotypic screening to identify preliminary trait variation within mutant populations. Conventional evaluation focuses on agronomic traits such as yield components, growth characteristics, and stress responses. However, phenotypic expression alone often fails to reveal underlying biochemical

mechanisms, particularly for complex traits influenced by multiple metabolic pathways. The third stage introduces metabolomic profiling as a high-resolution analytical layer. Metabolite extraction and analysis using platforms such as GC–MS, LC–MS, and NMR enable detection of primary and secondary metabolites associated with induced variation. Metabolomic profiling therefore captures hidden biochemical variation and enables identification of metabolic shifts resulting from induced mutations. Induced mutations alter enzymatic activities, regulatory networks, and metabolic pathways, leading to measurable changes in metabolite accumulation. Alterations in amino acid composition, sugar metabolism, and phenylpropanoid pathways reflect variation associated with stress tolerance, yield potential, and nutritional quality [8,14,42].

The fourth stage involves data integration and analysis, where metabolomic and phenotypic datasets are combined using multivariate statistical approaches such as principal component analysis, partial least squares discriminant analysis and correlation-based network modelling. These approaches enable identification of metabolite–trait associations and functional biomarkers [16]. Integration with machine learning algorithms further enhances predictive modelling by linking metabolic signatures with agronomic performance. This stage represents a critical transition from descriptive profiling to predictive, data-driven breeding frameworks.

The fifth stage focuses on metabolite-assisted selection, where identified biomarkers are used to screen and select superior genotypes at early breeding stages. This approach reduces reliance on multi-generation phenotypic evaluation and significantly improves selection efficiency and accuracy. Early identification of desirable genotypes accelerates breeding cycles and supports targeted improvement of complex traits. Despite these advantages, implementation of this integrated pipeline remains limited in finger millet. A major constraint is the lack of large-scale datasets linking induced genetic variation with metabolic outcomes. Limited access to metabolomic platforms and analytical infrastructure further restricts application in key production regions. Restricted bioinformatics capacity constrains integration and interpretation of complex multi-omics datasets. Fragmented research efforts and weak coordination between mutation breeding programs and metabolomics-based studies further limit progress.

Integration of mutation breeding and metabolomics also enables improved understanding of pleiotropic effects of induced mutations. Single mutations may influence multiple metabolic pathways, resulting in complex and often non-linear trait expression patterns. Metabolomic profiling enables detection of these systemic responses and facilitates identification of unintended biochemical consequences of mutagenesis. Monitoring metabolite profiles across generations further supports confirmation of trait stability and genetic fixation. This integrated framework aligns with modern precision breeding and systems biology approaches by linking genetic variation, metabolic function, and phenotypic expression within a unified analytical system. Metabolite-based markers derived from integrated analyses can be used for indirect selection, reducing breeding cycle duration and improving prediction accuracy for complex traits. This framework strengthens precision breeding by linking genetic, metabolic, and phenotypic information within a unified system.

Current research in finger millet remains largely fragmented, with mutation breeding and metabolomics typically applied independently rather than within integrated frameworks. A clear gap exists in the availability of large-scale, high-resolution datasets linking induced genetic variation with metabolic responses. Addressing these gaps requires development of standardized metabolomic workflows, expanded germplasm characterization, multi-environment validation of mutant populations, and integration with genomics and phenomics datasets. Strengthened analytical infrastructure, improved bioinformatics capacity, and coordinated interdisciplinary research efforts will be essential for enabling predictive and mechanism-based crop improvement strategies in finger millet.

5. Conclusion

Mutation breeding and metabolomics are complementary tools for improving finger millet through genetic and biochemical insights. Mutation breeding expands genetic variability and enables development of novel phenotypes, while metabolomics offers detailed insight into biochemical processes underlying trait expression. Integration of these approaches enhances understanding of genotype–phenotype relationships and provides a more functional basis for selection. Finger millet remains insufficiently explored in terms of integrative breeding strategies, and linkage between induced genetic variation and metabolic profiling is still largely unexplored. This limitation slows the development of mechanism-based breeding strategies. Development of coordinated research frameworks is required to support effective integration of mutation breeding and metabolomics. Improved analytical capacity, standardized metabolomic workflows, and expanded metabolite reference databases will enhance research reproducibility and application. Strengthened bioinformatics infrastructure will further support interpretation of complex multi-omics datasets. Metabolomics-assisted mutation breeding offers strong potential for improving selection efficiency and accelerating development of nutritionally superior and climate-resilient finger millet varieties. Advancing multi-omics integration and metabolite-guided selection will be essential for sustained genetic gain in finger millet improvement.

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Author Contributions

All authors contributed equally from the conceptualization, writing and editing of the manuscript. All authors reviewed and approved the final manuscript.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationship that could have appeared to influence the work reported in this paper.

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