



Millet Analysis: Bridging Genetics and Health

Siddhant Bhandari^{1,2,3,4}, Surbhi Hirawat^{1,2,3,4}, Saravana Prakash Thirumuruganandham⁴

Sigma Xi, The Scientific Research Honor Society, NC, USA¹

World Institute for Scientific Exploration (WISE), Baltimore, USA²

Society for Universal Oneness (SFUO) (1World1Nation.org), NC, USA³

Siddha Vetha Multiversity, NJ, USA⁴

Abstract: This paper introduces a groundbreaking approach to optimize the health benefits of mil- lets as an alternative to vegan through the integration of advanced artificial intelligence (AI) technology. Millets, often overlooked in contemporary diets, are revealed to possess re-markable nutritional properties and disease-fighting capabilities, particularly when analysed through AI-driven methodologies. With inherent antioxidant-rich [1] and an alternative for the gluten-sensitive [2], providing a welcoming reprieve for anyone who has felt the discom- forting aftermath of a wheat-heavy [3] meal attributes, millets offer a versatile and nutritious dietary alternative. Leveraging AI, millets emerge as protagonists in the realm of health and wellness, transcending their traditional role and becoming indispensable components [4] of a balanced diet. This study underscores the transformative potential of combining ancient grains with AI technology, ushering in a new era of vitality and well-being. By harnessing the power of machine learning algorithms, we can unlock unprecedented insights into millet's nutritional profiles [5], tailoring superfood recommendations to individual health needs and dietary preferences. This fusion of tradition and technology paves the way for a revolution in nutritional science, making personalized health optimization not just a possibility, but a reality.

Keywords: Millets, Alternative to Vegan Diet, Artificial Intelligence in Nutrition, AI-Driven Nutritional Analysis, Antioxidant-Rich Grains, Gluten-Free Alternative, Nutritional Optimization, Machine Learning in Health, Superfood Recommendations, Personalized Health Optimization, Dietary Alternatives to Wheat, Ancient Grains and Modern Technology, Nutritional Science Revolution, Individualized Dietary Recommendations, Health Benefits of Millets, Disease Prevention through Diet, AI-Powered Dietary Insights, Wellness and Nutrition, Balanced Diet with Millets, Fusion of Tradition and Technology in Nutrition.

I. INTRODUCTION

Dive with me into the narrative of a tiny but formidable grain that's gently transforming the way we think about food, health, and our planet. In the vast tapestry of agriculture, where giants like wheat and rice have traditionally held sway, a quiet revolution is taking place. Millet, championed by countries such as India, are showing remarkable resilience, thriving where many crops would struggle, and emerging as heroes in our collective quest to combat malnutrition and ongoing health problems.

Let's wander through the vast and varied landscape of millet, where each type offers its own unique gifts of health and adaptability. Picture the resilient Pearl millet, thriving in the harshest of dry lands, alongside the nutrient-packed Finger millet, a staple in many time-honoured diets. These grains aren't just surviving; they're a testament to the strength found in diversity, playing a crucial role in ecological sustainability and enriching our meals with their nutritional abundance.

Truly, millets have made their place in the kitchens around the world. Their amazing versatility is reflected whether it is the home-cooked meal from the coziness of one's home or the creative dishes that world's best restaurants boast of. Whether comforting with a bowl of warm millet porridge on a winter morning or an elaborate millet pasta from fine dining, it starts to get going with flavour and becomes a chameleon with its adaptability.

Exploring millets is not just going around the search for a new ingredient; it's going around rethinking food. It caters to the celebration of the diversity our planet holds and making sustainable choices good for us and the environment. Such is the nature of mil- lets—indeed the best sustainable, nutritious, and delicious form of our cuisines. It would be connecting us with the wisdom of great ancestors and laying the stepping stones of pros- perous healthy futures. So here we are at one of the most awesome rides there is: one of honouring the earth, each other, and our bodies; one of exalting the incredible flavors that these grains bring to the table.

Nutrition-wise, millets are nothing short of impressive. They challenge our conventional ce- real hierarchies with their rich content in macronutrients and micronutrients, offering higher levels of calcium than dairy, more protein than many grains, and a fiber content that out- shines current superfoods. For those with dietary restrictions, such as gluten intolerance or diabetes, millets present a safe and nutritious option, thanks to their low gluten levels and beneficial amino acid profiles.



The potential of millets extends beyond just their nutritional value; it's about how they can be tailored to meet individual health needs, optimizing their inherent benefits to address specific dietary requirements. This approach doesn't just change how we think about food; it revolutionizes our entire dietary strategy, turning everyday meals into targeted interventions for better health.

In a nutshell, it makes millets reflect the face of food's future, where what is eaten will not be something that may sustain a person but will rather be a prescription toward the very best management of health. With a multitude of antioxidants, phenols, fiber, and flavonoids, millets pack on to take chronic diseases head-on, offering hope in our never-ending health challenges. Thus, the sojourn into the world of millets is nothing but an open invitation to rethink our food systems, embrace diversity on our plates, and encourage making an informed choice in benefiting not only our health but also the planet. Millets, in which tradition and modernity are woven together in perfect harmony, offer a sustainable and nutritionally superior path.

These seven different varieties, each distinct from the other amidst diversity, were to imagine. Out of these seven different varieties, pearl millet is a predominant variety, with near-spherical grains, grown in India and Africa. Few preliminary research studies suggest that some of the millets may also have constituents targeting cellular deleterious entities selectively and efficiently [11]. Finger millets have distinct calcium content equal to that of very high calcium foods. Another variety considered to be of good content for protein is proso millet, which contributes a bright golden color. Foxtail, kodo, little, and barnyard millets are considered to add variety to the diet for their characteristic taste and nutritional profile. The richness of millets in fiber is a major contributor to satiety management, and therefore weight control, by supporting digestive health [12]. The method followed in this research extracted data from the FAO database for processing with a custom algorithm, which was aimed at synthesizing and corroborating the data points. Space mineral distribution, as well as comparative food nutrition, filled the research gaps for making dietary choices.

II. MILLETS: CHEMISTRY AND COMPOSITION

A. Nutritional Powerhouses

In the grand culinary theatre of the world, where grains play the starring roles, millets have emerged from the wings, ready to take centre stage as the Nutri-cereals of the future. With a script enriched by phytochemicals, nutrients, and antioxidants [13], these tiny titans are not just playing their parts; they're rewriting the playbook for healthy eating. Imagine millets as the unsung heroes, packed with a nutritional ensemble that includes carbohydrates doing the heavy lifting, proteins delivering punchy lines, fatty acids adding flavour, and a Chorus of vitamins and minerals providing the background score. Among this cast, Finger millet and Kodo millet shine as the stars, loaded with phenolics, tannins, and phytates—nature's own antioxidant ensemble, ready to combat oxidative stress with their natural flair.

B. Phenolic Profiling of Millets

Diving into the science, with high-performance liquid chromatography [14] and tandem mass spectrometry as the directors, we've identified over 50 phenolic compounds in these grains. It's like uncovering the secret ingredients of a blockbuster potion, with benzoic and cinnamic [15] derivatives playing the lead roles. Add to this a supporting cast of flavonoids, and you've got a nutritional drama rich in quercetin and flavan-3-ols, ready to steal the show.

C. Genomic Insights into Millet Adaptation

RNA-seq of 21-day Kodo millet seedlings subjected to PEG-induced dehydration revealed high reproducibility ($R \geq 0.78$) across triplicates. Sequencing yielded 351.3 million reads culminating in 239.1 million high-quality reads and 1,32,887 transcripts with an average 50.86 GC content, with 72,518 showing annotation upon UniProt database alignment. Differential expression analysis uncovered extensive gene regulation variations post-treatment, with thousands of genes differentially expressed across time points, reflecting the stress response's complexity. Key DEGs related to root architecture and osmotic stress were identified, suggesting roles in drought adaptation and growth regulation in Kodo millet. Figures 1, 2, and 3 collectively present a comprehensive genomic and nutritional analysis of millets. Figure 1 uses RNA-seq and CRISPR-Cas9 simulations, combined with GO and KEGG pathways, to map gene functions associated with nutrient synthesis in the Foxtail Millet Genome. Figures 2 and 3 visualize the distribution of nutritional and functional benefits across different millet types, employing kernel density analysis to highlight variations in nutrient profiles. Together, these figures underscore the genetic and nutritional diversity within millets, revealing key insights into their health-promoting properties.

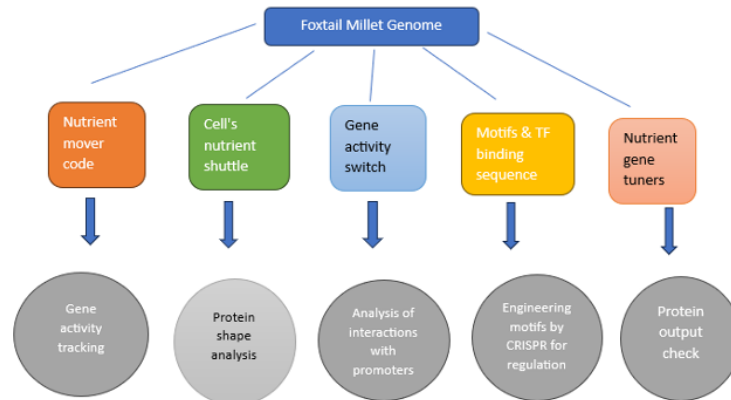


Figure 1: Advanced genomic analyses, including RNA-seq and CRISPR-Cas9 simulations, were implemented for gene function elucidation as depicted in Figure 1 complemented by GO and KEG pathways for comprehensive mapping. We interrogated the Gramene database, applying R and Python for statistical validation, STRING for protein-protein interaction analysis, and MEME for motif identification, aiming to elucidate the genetic underpinnings of nutrient synthesis in the Foxtail Millet Genome.

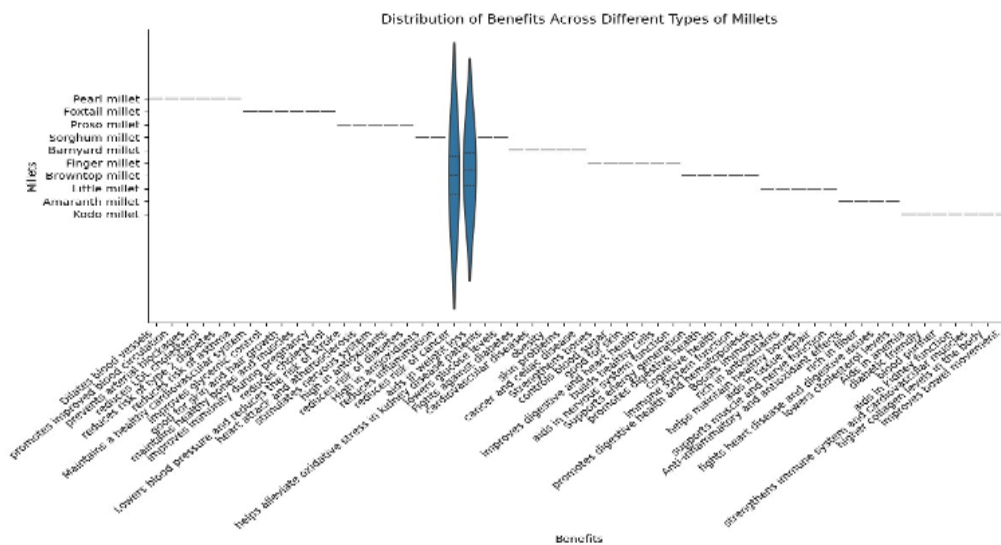


Figure 2: Illustrates the distribution of nutritional and functional benefits across diverse millet types, with kernel density analysis highlighting variations.

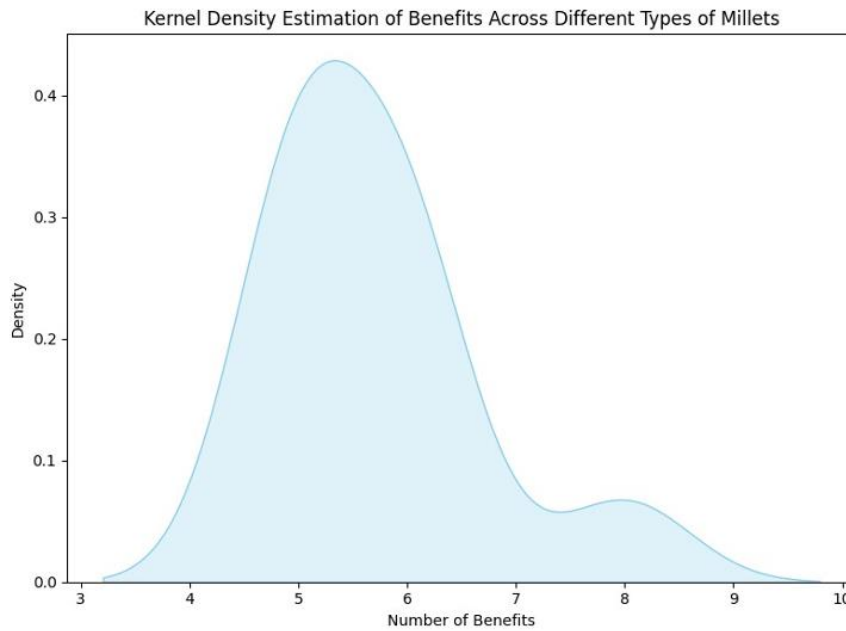


Figure 3: Illustrates the distribution of nutritional and functional benefits across diverse millet types, with kernel density analysis highlighting variations.

D. Ensuring Nutritional Security and Transcriptomic Analysis of Millets

Millets assure nutritional security due to their richness in bioactive compounds, micronutrients like calcium, iron, zinc, and favourable amino acid profiling. The low glycaemic index (GI) makes it suitable for diabetic individuals and serves as an alternative to celiac diseases, as it is free from gluten. Economically, millets require minimal investment and are resilient to climate variability, bolstering food and economic security through value addition and sustainable farmer income. Their resistance to stress, pests, and diseases further cements their role in sustainable food systems and combating global hunger amidst changing climates. AI is at the forefront of unlocking the full potential of millets, grains teeming with nutrients including B vitamins, and key minerals such as calcium, phosphorus, iron, and zinc [16]. Delving deeper, millets harbor considerable quantities of proteins, essential amino acids, and beneficial lipids. However, culinary practices can influence their nutritional integrity; for instance, boiling may impinge on antioxidant levels, while mechanical processes might impede fiber content. Figure 4 presents transcriptomic data analysis, showcasing Pearson correlation of triplicates over dehydration time points (0, 3, and 6 hours), along with DEG profiling that quantifies up- and down-regulated genes, and Venn diagrams illustrating overlapping and distinct DEGs across conditions.

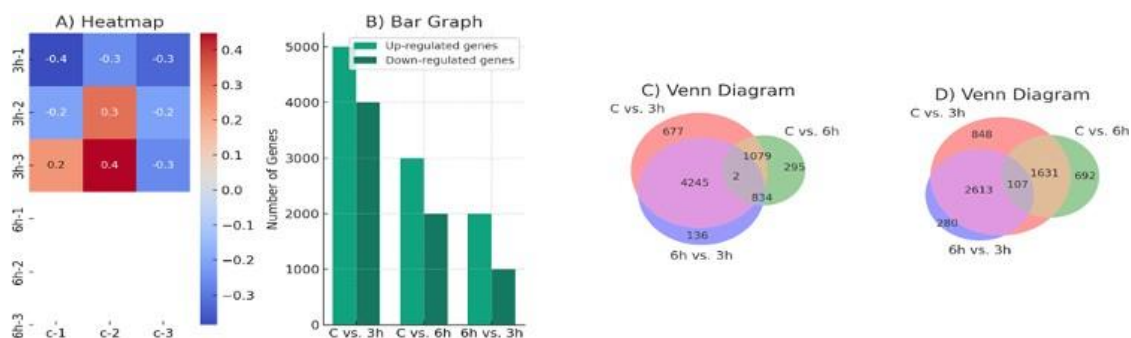


Figure 4: Transcriptomic Data Analysis and DEG Profiling. (A) Pearson correlation of triplicates at 0-, 3-, and 6-hours post-dehydration. (B) Quantitation of DEGs (up regulated/down regulated) for C vs. 3h, C vs. 6h, and 6h vs. 3h comparisons. Venn diagrams show overlapping and distinct (C) up regulated and (D) down regulated DEGs across conditions.

E. Optimizing Millet Nutrition through Culinary Practices

In contrast, germination [17] and fermentation [18] have been documented to enhance bioavailability and amplify protein, enzymatic activity, and flavonoid concentration, despite possible reductions in mineral availability. Yet, the resilience of



millet-derived polyphenols [19] against varying pH levels assures enduring health advantages. Acknowledged for their essential amino acids [20] and dietary fiber [21], millets' nutritional journey within the human body is a testament to their capacity to regulate glycemia, foster gut microbiota, and offer defence against chronic conditions. Employing AI, researchers are fine-tuning food preparation techniques [22] and probing into fermented millet products [23], aiming to optimize antioxidant efficacy and nutritional value, illustrating that these ancient grains are not merely sustenance but catalysts for well-being, with every component from kernel to compound contributing to a holistic health narrative [24, 25].

III. BIOAVAILABILITY OF MILLET NUTRIENTS

When we consume millets, the journey begins with absorption [26]. Millets are rich in phenolic compounds, antioxidants that must navigate the complex environment of our digestive system. The fiber in millets plays a dual role here, slowing down digestion to allow a more gradual absorption of sugars, thus providing a steady release of energy and aiding in the management of blood sugar levels. Once absorbed, the nutrients and bioactive compounds in millets distribute throughout the body. The phenolic compounds, now entering the bloodstream, travel to various tissues, offering their antioxidant protection far and wide. This distribution is not just about delivering nutrients but ensuring that every cell gets its share of millets' protective benefits [27]. The metabolism of millets involves a fascinating interplay between our own enzymes and our gut microbiota. The bound phenolic compounds in millets are transformed into more accessible forms, allowing our body to utilize their antioxidant properties fully. This process underscores the importance of the food matrix and processing methods, which can significantly affect the bioavailability and impact of these compounds. Figure 5 illustrates KEGG pathway enrichment analysis, mapping 1,982 differentially expressed genes (DEGs) across 103 pathways. The top 20 enriched pathways include metabolic (268 DEGs) and secondary metabolite biosynthesis (205 DEGs), with notable representation in plant-pathogen interaction, phenylpropanoid, and flavonoid biosynthesis pathways.

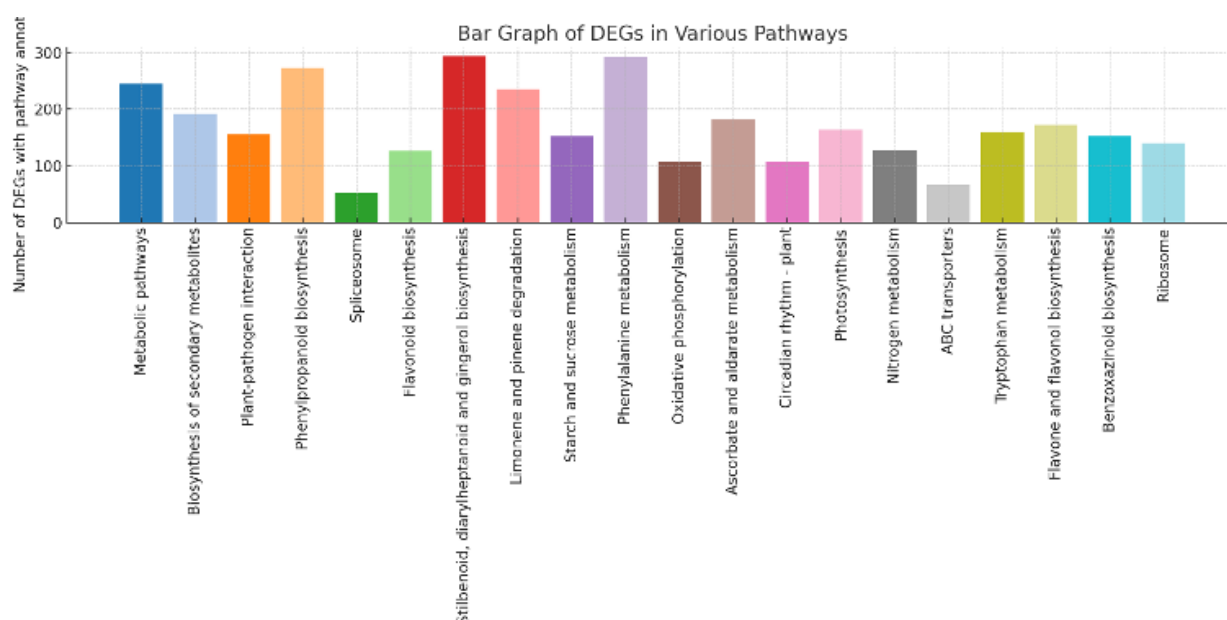


Figure 5: KEGG pathway enrichment analysis allocated 1982 DEGs across 103 pathways; the top 20, DEGs enriched metabolic (268 DEGs) and secondary metabolite biosynthesis pathways (205 DEGs), alongside significant representation in plant-pathogen interaction, phenylpropanoid, and flavonoid biosynthesis pathways

IV. MILLETS AND GENOME ANALYSIS

Millet dB is a treasure trove for millet researchers and breeders, thoughtfully designed to fuel advances in functional genomics and crop improvement for this hardy group of grains. Recognizing the critical role millets play in securing nutrition in climates where other crops falter, Millet dB collates a vast array of genomic and multi-omics data. It's a digital space where one can navigate through genomes of six millet species and related varieties, delve into pearl millet's pan-genome, or sift through troves of data detailing how these plants cope with stress—a true one-stop-shop for anyone



looking to unravel the genetic secrets of these resilient crops. The strength of Millet dB lies not just in its extensive data repository but in its accessibility. This platform is inviting—from a seasoned bioinformatician to a biologist making his first steps into the big world of big data. Tools like gene mapping and co-expression analysis will assist in easily searching for stress-related genes and their further detailed investigations of functions. This database played a key role in pinpointing genes such as PMA1G03779.1, which holds promise for enhancing yield under stress, showcasing how Millet dB can act as a springboard for discoveries that bridge the gap between lab research and field applications. This methodological step is critical for quantitative gene expression studies using tools like qPCR. The specific database source was derived from large-scale RNA sequencing efforts. This approach is vital for minimizing experimental error and enhancing data accuracy. Figure 6 displays a bar chart of pairwise variation (V) analysis, identifying the optimal number of reference genes required to ensure accurate transcript normalization across four experimental datasets.

Pairwise variation (V) analysis to determine the optimal number of control genes for accurate transcript normalization in all the four experimental sets.

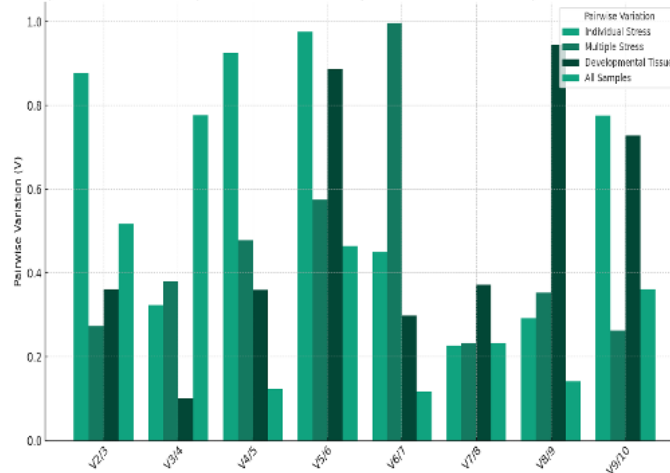


Figure 6: The bar chart likely represents a pairwise variation (V) analysis to establish the ideal count of reference genes needed for accurate transcript normalization across four experimental data sets.

A. Development and Utility of Millet dB

Developing Millet dB was a meticulous process, involving the assembly of genomic sequences, transcriptomic profiles, and reams of phenotypic data, all sewn together with sophisticated bioinformatics tools. But it's more than a static collection; it's a living, growing resource with updates that keep pace with the latest discoveries. Milletdb epitomizes the marriage of data science with plant genetics, offering the global scientific community a window into how to breed hardy varieties of millet that can. Theirs is a forward effort to zero in on millets, not just as the minor grains of yesteryears but as great staples of a greener tomorrow.

B. Statistical Significance and Expression Analysis

Statistical significance and expression of top 30 dehydration stress responsive DEGs. Sequencing generated 351.3 million reads, producing 239.1 million clean reads post-quality filtering (Q 30), and assembled 1,32,887 transcripts with an average GC content of 50.86. Transcriptome alignment to the UniProt database, with BlastX (E-value 10^{-3}), annotated 72,518 transcripts, showing high similarity to various species. Venn analysis was performed on cis-acting elements contained in more than 10 genes to further analyze the significance of these elements. For example, among the environmental stress-related elements, the three SiSPL genes (SiSPL9, SiSPL16, and SiSPL2) contained ARE (AAACCA), GC-motif (CCCCCG), LTR (CCGAAA), and MBS (CAACTG) cis-acting elements. Figures 7 and 8 provide an in-depth analysis of dehydration-responsive gene expression and stress-related cis-acting elements in Kodo millet. Figure 7 illustrates the statistical significance and expression patterns of the top 30 dehydration-responsive DEGs through differential expression analysis using de novo RNA-seq, with volcano plots (A-C) comparing conditions (C vs. 3h, C vs. 6h, and 6h vs. 3h) and a heat map (D) detailing expression across conditions. Figure 8 expands this analysis by performing a Venn analysis to identify significant cis-acting elements, highlighting environmental stress-related elements like ARE (AAACCA), GC-motif (CCCCCG), LTR (CCGAAA), and MBS (CAACTG) found in the SiSPL genes (SiSPL9, SiSPL16, and SiSPL2), providing insights into their potential regulatory roles under stress conditions.

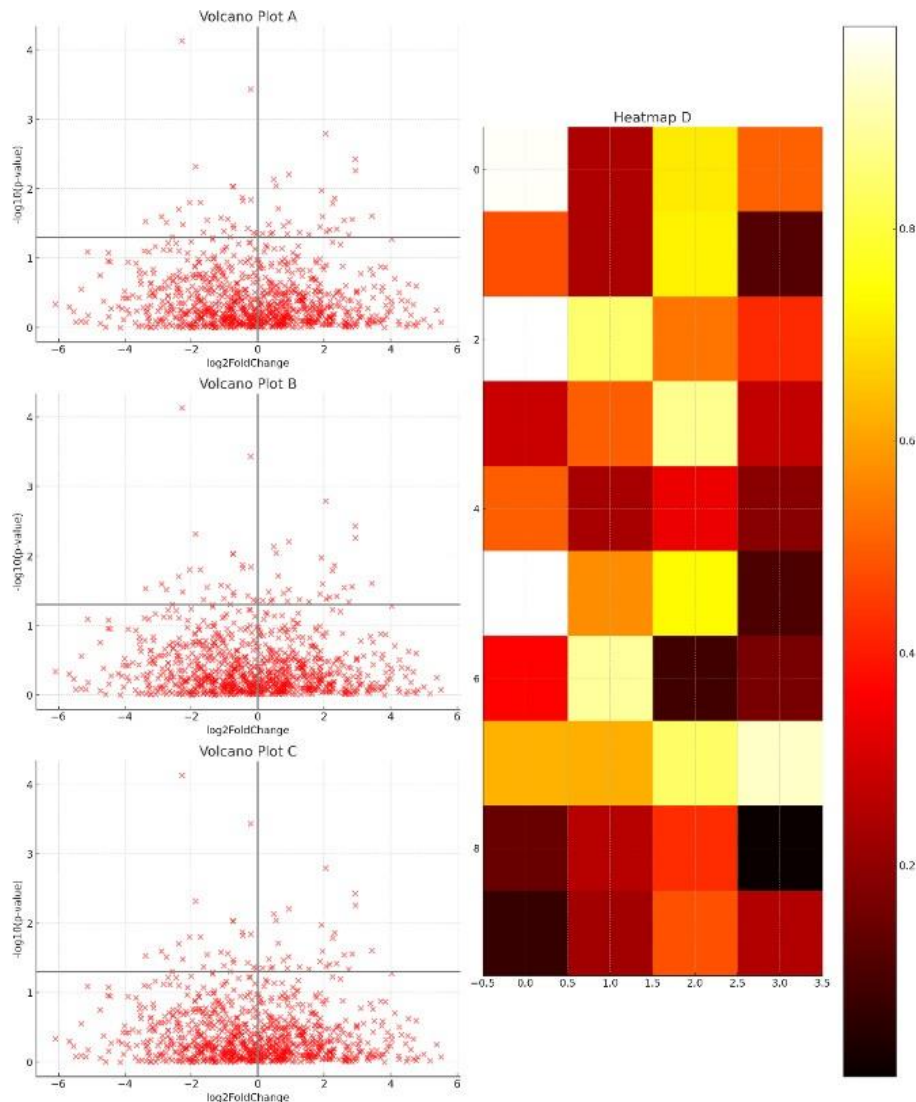


Figure 7: Portrays the statistical significance and expression levels of the top 30 dehydration responsive DEGs in Kodo millet. Differential expression analysis, via de novoRNA-seq, led to volcano plots (A-C) for comparisons C vs. 3h, C vs. 6h, and 6hvs. 3h, highlighting DEGs' significance. A heat map (D) details these DEGs' expression patterns across the conditions-seq on 21-day-old Kodo millet seedlings, treated with PEG, yielded high-reproducibility (Pearson R ≥ 0.78) across biological replicates.

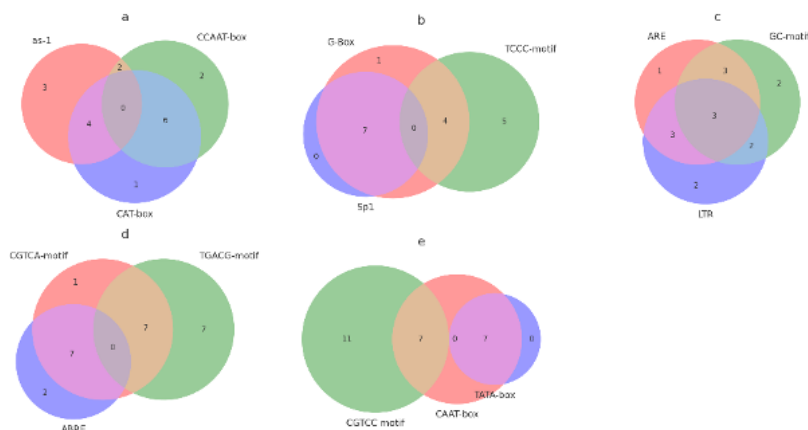


Figure 8: Venn analysis was performed confiscating elements contained in more than 10 genes (to further analyze the significance of these elements. For example, among the environmental stress-related elements, the three SiSPL genes



(SiSPL9, SiSPL16, and SiSPL2) contained ARE (AAACCA), GC-motif (CCCCCG), LTR (CCGAAA), and MBS (CAACTG) cis-acting elements.

V. METHODOLOGY

A. Investigating Pearl Millet Adaptation

For the first time, we comprehensively investigated the adaptive strategies of pearl millet (*Pennisetum glaucum*) to abiotic stresses through genomic, transcriptomic, and epigenetic cross-experiment data that have been intertwined with phenotypic analysis. The primary objective of this research was to unravel the complexities of pearl millet responses to environmental adversities with the main aim of enhancing comprehensions on mechanisms of its survival. Whole-genome sequence data of 11 selected pearl millet accessions were therefore retrieved from the NCBI Assembly database [28]. This endeavor was broadened through the inclusion of genomic sequences from related millet species, facilitating a comparative analysis. The construction of the pan-genome was achieved through the application of Ortho MCL and MUMmer software, which enabled the identification and categorization of core, dispensable, and unique genes across the collected genomes.

B. Genetic Analysis and GWAS

Advancing our genetic analysis, we incorporated data from 378 whole-genome resequencing efforts. These datasets were meticulously aligned to our constructed pan-genome using vg tools. This alignment served as the foundation for conducting comprehensive genome-wide association studies (GWAS) [29], leveraging GEMMA[30] software to identify genetic markers linked to stress resilience.

C. Transcriptomic Investigation

Our transcriptomic investigation spanned 192 samples, capturing the gene expression profiles of pearl millet under various abiotic stresses. Using Kallisto for expression level analysis and Hmisc software[31] for co-expression studies, we aimed to illuminate the gene regulatory networks activated in response to environmental stimuli.

D. Epigenetic Analysis

Complementing our genetic and transcriptomic insights, we delved into the epigenetic landscape of pearl millet. ChIPseq [32] data from selected tissues were analyzed using MACS software for peak calling, revealing the role of histone modifications in the regulation of stress-responsive genes.

E. Development of Millet dB

The culmination of our efforts is the development of Millet dB, a comprehensive database hosting the integrated genomic, transcriptomic, epigenetic, and phenotypic data, designed to support interactive queries and further research.

VI. RESULTS

A. Insights into Pearl Millet Adaptation

In the current study, we attempt to bring this complexity of pearl millet adaptation to abiotic stresses to the fore, and we report an important set of findings made across the genomic, transcriptomic, and epigenetic landscapes. This has indeed been disclosed through pan-genomic analysis to establish a rich mosaic of genetic diversity, proving that the wide spectrum of genes continues to contribute toward the adaptability and resilience factors of these plants. Our resequencing data were thus subjected to GWAS [29] with the sole aim of discovering genetic markers that can be associated with stress tolerance. Such markers represent promising targets for pearl millet through breeding programs addressing better resistance of pearl millet to hostile environment conditions.

B. Transcriptomic Dynamics

In the field of transcriptomics, our analysis came out with an open dynamic regulatory landscape and a significant change in gene expression in response to stress. This has shown the presence of the intricate gene networks poised to mediate survival strategies of the plant under challenging conditions.

C. Epigenetic Regulation

Furthermore, the regulation of histone modification turned out to be our exploration of the mechanisms at work within epigenetics for gene expression under stress. This thus adds a new epigenetic dimension to mechanistic understanding of plant response mechanisms and opens novel avenues to be furthered in research for crop improvement.

D. Value of Integrated Data Streams

Such integrated data streams are invaluable resources to the scientific community in Millet dB, the latter of which should facilitate not only ongoing but also future investigations of the genetic and epigenetic[33] bases of stress tolerance in



pearl millet. The results presented herein advance not only plant genomics but also open new opportunities for developing more resilient crop varieties that can cope with the increasing challenges placed by climate change.

VII. CONCLUSION

The research on pearl millet adaptation mechanisms to various abiotic stresses has greatly enhanced our understanding of the genetic, transcriptomic, and epigenetic frameworks that contribute to plant resilience. Through comprehensive data analysis, including genomic, transcriptomic, epigenetic, and phenotypic information, this study unveils the intricate biological processes that enable pearl millet to survive in harsh environmental conditions. The discovery of the pearl millet pan-genome highlights significant genetic diversity and evolutionary traits that contribute to the plant's robustness, opening new avenues for resilience-focused breeding. Transcriptomic insights reveal dynamic gene regulation in response to stress, while epigenetic findings underscore the role of histone modifications in stress adaptation, suggesting novel targets for resilience enhancement. Additionally, the creation of Millet dB provides a valuable database for future research, promoting an integrative approach to understanding plant resilience. Beyond millet, these findings offer a model for studying stress tolerance in other crops, with implications for global food security amid climate change. Finally, this work advocates for sustainable dietary practices, encouraging the inclusion of millets as a nutritious and eco-friendly alternative aligned with vegan principles. Together, these insights underscore the critical need for multidisciplinary research to advance resilient, sustainable agriculture.

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BIOGRAPHY



Siddhant Bhandari is an accomplished technology professional with a strong background in cybersecurity, cloud infrastructure, and technology strategy. He holds a Master's in Cybersecurity from the New York Institute of Technology and a bachelor's in computer science engineering. Currently, Siddhant is a Software Engineer at Akamai, where he focuses on developing secure, scalable systems and enhancing risk management processes. In addition, he serves in a voluntary leadership role as the Head of IT Infrastructure and Security at 1World1Nation, managing platform development and data security for the organization's UNY architecture. Siddhant's experience spans roles in software development, system architecture, and IT project management, with a commitment to driving innovation and operational efficiency.



Surbhi Hirawat is a seasoned data science and AI professional with over 14 years of comprehensive experience. She holds a CFA charter and a Master's in Data Science from NYIT, complemented by a postgraduate degree in Artificial Intelligence and Machine Learning from the University of Texas. As an Adjunct Faculty member at Siddha Vetha Multiversity and the Head of Development at 1World1Nation, Surbhi combines academic rigor with practical expertise. Her versatile skill set and collaborative nature have made her a trusted leader in data science, AI, and applied research, consistently driving impactful solutions across various domains.



Dr Saravana Prakash is a computational physicist with a robust academic background. He holds a DEA in Chemical and Process Engineering and a Ph.D. in Physics. Saravana has likely engaged in extensive research and academic endeavours throughout his career, focusing on computational methods and their applications in physics. His interdisciplinary education bridges engineering and physics, equipping him with a diverse skill set in both theoretical and applied sciences.