

Comprehensive Genomic Analysis Unveils Genetic Architecture of Seed Vigour Traits in Millet

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Abstract

Particularly in regions with poor growing conditions, seed vigour is essential to millet cultivation since it impacts germination rates, seedling emergence, and crop output. New genomics research has allowed us to learn more about genetic factors affecting seed vigour. This study aims to provide a comprehensive genomic analysis of millet to identify critical genetic loci and underlying biochemical mechanisms that regulate seed vigour. Using advanced methods from comparative genome sequencing, gene expression profiling, and genome-comprehensive association studies (GWAS), we identify the genes and structures of regulation crucial for enhancing seed vigour in this research. The findings provide valuable information that breeding programs may utilize to improve millet seed quality and yield.

Keywords: Seed vigour, Millet, GWAS, Genomic analysis, Seed germination, Crop yield, Molecular pathways, Breeding

1. Introduction

In semi-arid and tropical climates, especially in Asia and Africa, food security is ensured by millets, a family of small-seeded cereal plants. Millets are an essential staple crop in areas where other grains, such as rice and wheat, may not do well due to their low water requirements and ability to flourish in poor soils. Nevertheless, millet farmers face tremendous obstacles in maintaining high seed vigour, which profoundly impacts germination, seedling creation, and total harvest productivity.

How quickly and evenly seeds germinate in different environments is a measure of their vigour, affecting how well they grow seedlings. Strong seed vigour is essential for improved crop stand in challenging environments including drought, heat stress, and soils lacking nutrients. While the seed's physiological state and genetic composition influence seed vigour, pinpointing the exact genetic components that control seed vigour has proven difficult, especially in crops that have received less attention, such as millet.

Now that genomic technology has advanced, we can use methods like transcriptome analysis and genome-wide association studies (GWAS) to break down complicated features like seed vigour. Breeding programs may use these methods to find millet varieties with the best possible performance by identifying genetic markers and molecular mechanisms that regulate seed vigour.

This in-depth genomic investigation aims to reveal the millet seed vigour genetic architecture. We seek to provide complete knowledge of the genetic variables affecting seed vigour by using GWAS to identify significant genetic loci and transcriptome data to understand gene expression patterns during seed

germination. Doing so would hopefully pave the way for more precise breeding techniques to increase millet seed output and improve seed quality.

2. Materials and Methods

2.1 Plant Genetics and Characterisation

Millet germplasm, consisting of 200 diverse accessions, was sourced from national and international gene banks. These accessions were grown under controlled conditions, and seed vigour traits such as germination rate, seedling emergence, and seedling dry weight were evaluated following standardized protocols. Environmental conditions such as temperature, soil moisture, and nutrient levels were closely monitored to minimize variability.

2.2 Genotyping and Sequencing

Millet accessions were gathered, and DNA was extracted. High-density single nucleotide polymorphism (SNP) arrays were used for genotyping. Whole-genome sequencing was done to provide a clear picture of the genetic diversity for some accessions. We performed a quality control filter on the generated data and removed SNPs having a minor allele frequency (MAF) < 5%.

2.3 Genome-Wide Association Study (GWAS)

GWAS was performed to find the genetic loci linked to seed vigour characteristics. Mixed linear models (MLM) were used to link the genotypic and phenotypic data to account for factors including population structure and familial relationships. Significant SNPs were found using a criterion based on FDR correction. The findings were visualized using Manhattan and QQ plots.

2.4 Transcriptomic Analysis

To complement the GWAS results, transcriptomic analysis was conducted on seeds at various stages of germination. RNA was extracted, sequenced, and analyzed for differential gene expression. Genes showing significant upregulation or downregulation during germination were identified and mapped to seed vigour-associated SNPs.

2.5 Functional Annotation and Pathway Analysis

The significant SNPs and differentially expressed genes were annotated using publicly available millet genome databases. Gene ontology (GO) and pathway enrichment analyses were conducted to identify biological processes and molecular functions associated with seed vigour. Comparative genomics was also performed to identify conserved genes and pathways in other cereal crops.

3. Results

3.1 Phenotypic Variation in Seed Vigour Traits

When it came to seed vigour features, there was much phenotypic heterogeneity among the millet accessions. There was considerable variation in the germination rate (65% to 95%) and the rate of seedling emergence (70% to 90%). A range of 15 mg to 35 mg was recorded for the seedlings' dry weight. Next, genetic analysis was based on these variants.

3.2 SNPs Associated with Seed Vigour

GWAS identified several significant SNPs associated with seed vigour traits. The most notable loci were found on chromosomes 3, 5, and 8, harbouring SNPs significantly correlated with germination rate and seedling emergence. The Manhattan plots showed clear peaks corresponding to these loci, suggesting substantial genetic control over these traits.

3.3 Differential Gene Expression During Germination

During germination, transcriptomic analysis showed that many genes showed differential expression. There was a notable upregulation of genes in high-vigour seeds involved in hormone signalling pathways, such as gibberellin and abscisic acid. There were additional differences in the expression of genes that were involved in glucose metabolism, cell wall alterations, and stress response.

3.4 Functional Annotation and Pathway Enrichment

Pathways, including hormone control, energy metabolism, and oxidative stress response, were enriched in the critical genes, according to gene ontology analysis. The abscisic acid signalling pathway stood out due to its enrichment, suggesting its crucial function in controlling seed dormancy and germination. Genomic comparisons across maize, rice, and sorghum showed that many genes involved in seed vigour are conserved.

4. Discussion

By illuminating several significant loci and pathways involved in the genetic control of millet seed vigour features, our work offers significant new information for advancing this crop. We found substantial single nucleotide polymorphisms (SNPs) linked to seed vigour characteristics such as germination rate, seedling emergence, and seedling dry weight using genome-wide association studies (GWAS). Significant correlations with these parameters were found at many loci on chromosomes 3, 5, and 8, which may indicate that these areas include essential genes that control millet seed vigour.

To help breeders select high-vigour characteristics more effectively, discovering specific SNPs presents an opportunity to create molecular markers for use in marker-assisted selection (MAS). Particularly in areas where high seed vigour is crucial for surviving environmental challenges like drought and poor soil conditions, these results might directly affect millet breeding efforts.

During seed germination, our transcriptome analysis identified significant genetic loci and showed that many genes related to hormone signalling, glucose metabolism, and stress response showed differential expression. Important hormones that control seed germination and dormancy include gibberellins (GA) and abscisic acid (ABA). We observed that genes linked with gibberellin production and signalling, which promote germination, were more highly expressed in high-vigour seeds compared to low-vigour seeds and that genes related to ABA, which are typically associated with seed dormancy, were more highly expressed in low-vigour seeds.

Evidence from other cereal crops, including wheat and rice, suggests that ABA and GA signalling play a significant role in regulating seed vigour, consistent with the enrichment of these hormonal pathways. This further proves that seed vigor-related mechanisms are conserved across cereal crops, opening the door to options to improve millet breeding via cross-species comparative genomics.

The study also discovered that high-vigour seeds have elevated genes related to carbohydrate metabolism, including genes that control starch breakdown. The energy needed for quick germination and early seedling growth aligns with this. It would be helpful to understand these metabolic pathways to improve vigour during germination so that seed therapies may be developed.

Millet has many genes with other grains, including maize and sorghum, which is an intriguing finding from our comparative genomics study. This paves the way for future research into hybrid breeding techniques or the possibility of genetic engineering or CRISPR-based methods for transferring desirable features.

The study's shortcomings should be considered even though the findings provide substantial insights. Environmental variables, seed management procedures, and genetics all play a role in the complicated vigour of seeds. More studies should centre on the interplay between genotype and environment to further understand how various millet varieties operate under varied environmental circumstances (GxE). The molecular processes underlying seed vigour may be better understood if proteomics and metabolomics data are integrated.

5. Conclusion

This work used extensive genomic analysis to reveal the genetic architecture of millet seed vigour features. Breeders may now improve millet seed vigour features thanks to the discovery of novel molecular targets for marker-assisted selection, made possible by identifying significant SNPs on chromosomes 3, 5, and 8. Transcriptome research also showed that hormone modulation, especially gibberellin and abscisic acid signalling, is critical for seed vigour. Consistent with previous studies in cereal crops, our results highlight the importance of these pathways in enhancing seed quality in all species.

Seed vigour is a complicated attribute, and identifying differentially expressed genes in stress response and glucose metabolism only adds to that complexity. These molecular insights provide the groundwork for future breeding programs that might raise millet yields by improving seed quality, crop establishment, and overall yield, particularly in areas with difficult growing circumstances.

The future of understanding the processes influencing seed vigour hinges on integrating multi-omics technologies like metabolomics and proteomics with environmental interaction research. By focusing on these areas for the future, we may improve our breeding efforts and produce millet varieties that can withstand the effects of climate change and other agricultural threats, ensuring that the world's food supply remains stable.

Genomic techniques to boost seed vigour may ensure that millet remains a robust crop, which will help with sustainable agriculture and food security in areas vulnerable to environmental pressures.

6. References

1. Choudhary, P., Shukla, P., & Muthamilarasan, M. (2023). Genetic enhancement of climate-resilient traits in small millets: A review. In *Heliyon* (Vol. 9, Issue 4, p. e14502). Elsevier BV. <https://doi.org/10.1016/j.heliyon.2023.e14502>
2. Huang, C.-T., Klos, K. E., & Huang, Y.-F. (2020). Genome-Wide Association Study Reveals the Genetic Architecture of Seed Vigor in Oats. In *G3 Genes|Genomes|Genetics* (Vol. 10, Issue 12, pp. 4489–4503). Oxford University Press (OUP). <https://doi.org/10.1534/g3.120.401602>
3. Maharajan, T., Krishna, T. P. A., Krishnakumar, N. M., Vetriventhan, M., Kudapa, H., & Ceasar, S. A. (2024). Role of Genome Sequences of Major and Minor Millets in Strengthening Food and Nutritional Security for Future Generations. In *Agriculture* (Vol. 14, Issue 5, p. 670). MDPI AG. <https://doi.org/10.3390/agriculture14050670>
4. Dwivedi, S. L., Spillane, C., Lopez, F., Ayele, B. T., & Ortiz, R. (2021). First the seed: Genomic advances in seed science for improved crop productivity and food security. In *Crop Science* (Vol. 61, Issue 3, pp. 1501–1526). Wiley. <https://doi.org/10.1002/csc2.20402>
5. Choudhary, P., Shukla, P., & Muthamilarasan, M. (2023). Genetic enhancement of climate-resilient traits in small millets: A review. In *Heliyon* (Vol. 9, Issue 4, p. e14502). Elsevier BV. <https://doi.org/10.1016/j.heliyon.2023.e14502>
6. Wang, A., Guo, W., Wang, S. et al. Transcriptome analysis unveiled the genetic basis of rapid seed germination strategies in alpine plant *Rheum pumilum*. *Sci Rep* 14, 19194 (2024). <https://doi.org/10.1038/s41598-024-70320-x>
7. Huang CT, Klos KE, Huang YF. Genome-Wide Association Study Reveals the Genetic Architecture of Seed Vigor in Oats. *G3* (Bethesda). 2020 Dec 3;10(12):4489-4503. doi: 10.1534/g3.120.401602. PMID: 33028627; PMCID: PMC7718755.