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Harnessing Genomic Tools for Cassava Improvement: Advances and Prospects

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Abstract As an important staple crop in tropical and subtropical regions, cassava (*Manihot esculenta*) faces an urgent need to improve yield, nutritional value, and environmental adaptability. In recent years, the rapid development of genomic tools has brought unprecedented opportunities for cassava improvement, greatly improving the efficiency and accuracy of breeding. This research explores the integrated application of genomic tools such as high-throughput sequencing, gene editing techniques, and omics methods in cassava breeding, and their profound impact on accelerating specific trait improvement. This research discusses the implementation of high-throughput sequencing and gene editing techniques such as CRISPR/Cas9 and TALENs, which have led to precise gene modifications for disease resistance and yield improvement. In addition, it also highlights how transcriptomics, proteomics, and metabolomics can help deepen understanding of the physiological response and metabolic pathways of cassava. However, the application of these technologies faces multiple challenges, including high costs, complexity of genomic data, ethical issues of genetic modification, and unpredictability of global environmental change. Despite these obstacles, genomic tools still have promising prospects in transforming cassava breeding, providing possible solutions for food safety in developing regions. This research emphasizes the necessity of collaborative efforts to effectively utilize genomic tools and ensure that the benefits of these technologies can be widely and beneficially applied in different agricultural environments.

Keywords Cassava (*Manihot esculenta*); Cassava improvement; Genomic tools; High-throughput sequencing; Gene editing; Omics technologies

Cassava (*Manihot esculenta*) is a root crop that has become a staple food for millions of people across the tropics, particularly in Africa, Asia, and Latin America. It is the third-most important source of calories in the tropics after rice and maize. Due to its resilience and adaptability to various environmental conditions, cassava plays a pivotal role in global agriculture and food security. Its importance is further underscored in regions where it serves as a primary food source and contributes significantly to the livelihoods of smallholder farmers (Mbanjo et al., 2021).

Despite its significance, cassava faces several challenges that impede its production and quality. These include a long growth cycle, genetic heterozygosity, and susceptibility to diseases and pests. Traditional breeding methods have been somewhat limited in addressing these challenges due to the crop's complex genetic makeup and the lack of a large set of DNA markers and genetic maps.

The advent of genomic tools has revolutionized the field of crop improvement. Genomic tools such as molecular markers, genome sequencing, and genetic transformation are now being harnessed to overcome the breeding obstacles in cassava. These tools have enabled the development of a more saturated genetic linkage map of cassava, combining simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers, which is crucial for the genetic analysis of economically important traits (Hu et al., 2021). Furthermore, the sequencing of wild and cultivated cassava and related species has revealed extensive interspecific hybridization and genetic diversity, providing a rich resource for breeding programs (Adu e al., 2021).

Recent biotechnological advances, including functional genomics and genome editing approaches such as CRISPR-Cas9 and TILLING, offer unprecedented opportunities to accelerate the improvement of cassava. These technologies are being directed toward addressing major production constraints, such as virus resistance, protein content, tolerance to drought, and reduction of cyanogenic glucosides.

The integration of genomic tools into cassava breeding programs is essential for the improvement of this vital crop. The research aims to explore the current status and future prospects of genomic tools in cassava improvement, with a focus on how these tools can be applied to enhance yield, quality, and resistance to biotic and abiotic stresses.

1 Genetic Background and Genomic Characteristics of Cassava

1.1 Genetic diversity and genome structure of cassava

Cassava, a staple crop in many tropical and subtropical regions, is renowned for its remarkable adaptability to diverse environments and its ability to thrive on marginal soils. The genetic diversity of cassava is vast, reflecting its cultivation by various cultures across numerous agroecological zones for centuries. This diversity is crucial for breeding as it encompasses a range of traits like drought tolerance, disease resistance, and varying root qualities.

The cassava genome is diploid, containing 18 chromosomes, and has been estimated to be about 770 megabases in size. It is characterized by a high level of heterozygosity and significant presence of repetitive elements, which together account for nearly two-thirds of the genome. These genomic characteristics pose challenges in genome assembly and sequence analysis but are vital for understanding the genetic basis of its phenotypic traits (Elias et al., 2017).

1.2 History and current status of cassava genome research

Cassava genome research has progressed significantly over the past decade. The first draft of the cassava genome was published in 2009, marking a pivotal step in cassava genomics. This initial effort provided a foundation for identifying genes associated with important traits such as starch production and pest resistance. Since then, several refined versions of the genome have been released, utilizing advances in sequencing technologies which have enabled higher resolutions of genomic structure and variation.

Currently, the focus of cassava genome research is on applying genomic tools to enhance breeding efficiency. Technologies such as genome-wide association studies (GWAS) and genomic selection have become integral in identifying genetic markers linked to desirable traits. This approach facilitates the rapid selection of superior genotypes without the need for extensive field testing (Tuo et al., 2023).

Moreover, the integration of functional genomics, through transcriptomic and proteomic studies, has started to elucidate the molecular mechanisms underlying cassava's responses to environmental stresses and nutritional content. This comprehensive genomic knowledge not only accelerates the traditional breeding processes but also opens up possibilities for the application of modern biotechnological techniques such as CRISPR/Cas9 for targeted gene editing.

The current status of cassava genome research is therefore marked by a transition from basic genomic characterization to more applied genomic-assisted breeding and genetic engineering. This transition is supported by an ever-growing body of genomic resources and tools, promising to unlock further potential of cassava as a crop vital for food security and economic development in many developing countries.

2 Currently Applied Genomic Tools and Technologies

2.1 High-throughput sequencing technology

High-throughput sequencing (HTS) technologies, including next-generation sequencing (NGS) and single-cell sequencing, have revolutionized genomic research in cassava by providing extensive data on genetic variation, gene expression, and molecular interactions. NGS platforms such as Illumina and PacBio have enabled the sequencing of entire cassava genomes and transcriptomes at a significantly reduced cost and time, facilitating a detailed understanding of the genetic makeup and variability within different cassava strains. Single-cell sequencing, although less commonly used in plants, offers unique insights into the cellular responses of cassava under various stress conditions, potentially isolating novel adaptive genetic traits (Veley et al., 2021).

2.2 Gene editing technologies

Gene editing technologies, notably CRISPR/Cas9 and TALENs (Transcription Activator-Like Effector Nucleases), have opened new avenues for precise alterations in the cassava genome, aimed at improving traits such as yield, nutritional value, and resistance to diseases and pests. CRISPR/Cas9 has been particularly transformative due to its simplicity and efficiency. It has been successfully used to target and modify genes responsible for starch biosynthesis pathways, enhancing the quality and quantity of cassava starch. TALENs also offer a robust method for targeting specific genomic sequences, though they are generally more complex and less versatile than CRISPR/Cas9 (Lyons et al., 2021).

2.3 Applications of transcriptomics, proteomics, and metabolomics

The integration of omics technologies—transcriptomics, proteomics, and metabolomics—into cassava research has provided comprehensive insights into the plant's functional biology. Transcriptomics analyses help in understanding gene expression patterns and regulatory mechanisms under different environmental conditions. Proteomics approaches are crucial for studying protein profiles that dictate phenotypic traits and stress responses. Metabolomics, which examines the chemical fingerprints that cellular processes leave behind, is particularly useful in optimizing cassava's nutritional content and taste, by identifying key metabolic pathways that can be targeted for enhancement or modification (Hu et al., 2023).

2.4 Genome selection and marker-assisted breeding

Genome selection (GS) and marker-assisted breeding (MAB) are pivotal in modern cassava breeding programs. GS allows breeders to predict breeding values of offspring by analyzing genome-wide genetic markers, significantly accelerating the breeding cycle. This approach is highly effective in cassava due to its complex genetic architecture and long growth cycle. MAB, on the other hand, utilizes specific markers linked to desirable traits (like disease resistance or drought tolerance) to facilitate the selection of superior plants early in their developmental stage. This method has proven successful in developing new cassava varieties that are robust against biotic and abiotic stresses (Adu e al., 2021).

These genomic tools and technologies are collectively enhancing the precision and efficiency of cassava breeding, promising not only to boost its yield and quality but also to ensure the crop's resilience against changing climate and evolving pests and diseases.

3 Examples of Application of Genomic Tools in Cassava Improvement

3.1 Improve yield and stress resistance

The application of genomic tools in cassava improvement has been pivotal in addressing the challenges posed by its biological characteristics, such as a long growth cycle and a heterozygous genetic background. These challenges have historically slowed breeding goals, including yield increases and disease resistance (Juma et al., 2022). However, the integration of genomics with traditional breeding has shown promise in enhancing abiotic stress adaptation, such as drought and aluminum toxicity. For instance, the use of genotyping-by-sequencing (GBS) has facilitated the identification of single nucleotide polymorphisms (SNPs) associated with traits like resistance to cassava mosaic disease (CMD) and yield under CMD pressure. Additionally, the International Institute of Tropical Agriculture (IITA) has evaluated cassava genotypes for yield components and adaptation to different environments, which is crucial for genetic enhancement aimed at increasing production and productivity (Figure 1) (Veley et al., 2023).

Veley et al. (2023) highlights the potential of genetic modifications to enhance disease resistance in crops, reducing reliance on chemical treatments and improving agricultural sustainability. The figure illustrates a study on plant disease resistance, focusing on the interaction between the TAL20 effector and the *MeSWEET10a* gene in cassava. Panel A shows cassava leaves, highlighting the characteristic lesions caused by disease (white arrows). Panel B describes the molecular mechanism: the top section depicts the TAL20 effector binding to the *MeSWEET10a* promoter, leading to gene activation and susceptibility, as evidenced by the diseased leaf. The bottom section shows a modified DNA sequence where mutations (indicated by orange circles) in the TAL20 binding site prevent the effector from binding. This results in resistance to the disease, as shown by the healthy leaf.

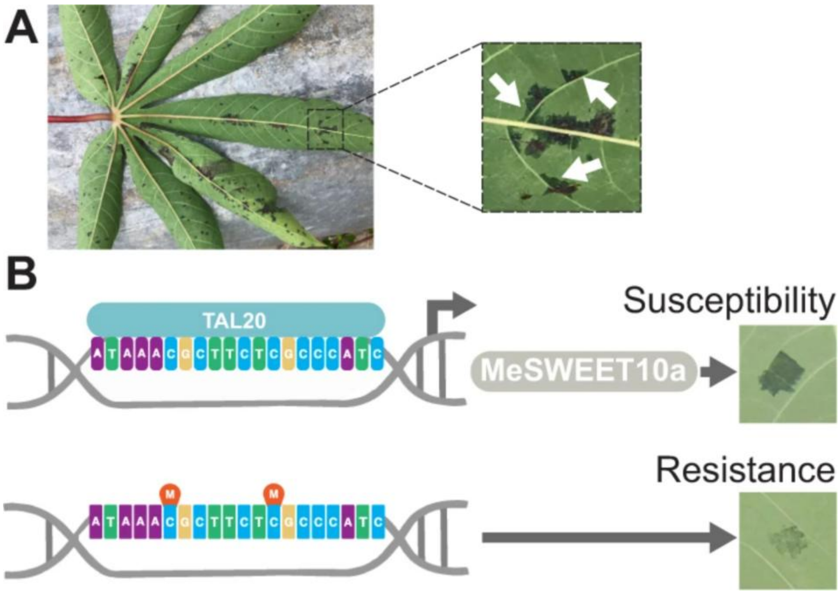

Figure 1 Cas9/gRNA Agrobacterium-mediated cassava transformation and recovery of transgenic cassava plants (Adopted from Veley et al., 2023)

Note: A Left: Example of CBB-infected cassava in a field. Right: selected region from leaf on the left. Water-soaked regions are indicated with white arrows. B Graphical representation of epigenetic CBB disease resistance strategy. Top: In WT plants, TAL20 from Xam binds a specific sequence (EBE) present in the promoter of the S gene *MeSWEET10a*. Upon binding, TAL20 induces the ectopic expression of *MeSWEET10a*, a sugar transporter, which is required for establishment of disease. A typical ‘water-soaked’ lesion is shown to the right, an early indicator of CBB. Bottom: DNA methylation prevents TAL20 from binding the EBE. *MeSWEET10a* expression is not induced, and disease symptoms are reduced. Source data are provided as a Source Data file (Adopted from Veley et al., 2023)

3.2 Improving nutritional quality

Improving the nutritional quality of cassava, particularly its provitamin A carotenoid content, is another area where genomic tools have been effectively employed. Genomic selection (GS) has been tested for its effectiveness in rapidly improving cassava for total carotenoids content, with promising results indicating that GS can be a useful tool for genetic improvement in this aspect (Esuma et al., 2021). The identification of quantitative trait loci (QTL) associated with carotenoids content has also been facilitated by genomic approaches, which can lead to the development of cassava varieties with enhanced nutritional profiles (Yonis et al., 2019).

3.3 Case studies: successful improvement projects and practical application of technology

The next-generation cassava breeding project, led by Cornell University and implemented in collaboration with IITA and the National Root Crop Research Institute (NRCRI) of Nigeria, is an exemplary international cooperation project. This project has innovatively accelerated the breeding process of cassava by utilizing genome selection and genotyping techniques. By accurately identifying genetic markers that control key traits such as high yield and disease resistance, researchers are able to efficiently screen plants with excellent characteristics, greatly shortening the multi-year field trial cycle required in traditional breeding. This technological innovation has successfully nurtured five high-yield and disease resistant new varieties, including "Game Changer", "Hope", "Obasanjo 2", "Baba 70", and "Poundable". These varieties not only have high yields and strong adaptability, but also have been customized according to the preferences of local consumers, indicating that their promotion in Nigeria and even the entire African region will have a profound impact on improving food security levels and promoting income growth for farmers (Improving Crops, 2023, https://annualreport.iita.org/2023/03/27/molecular-breeding-comes-of-age-in-cassava-improvement/).

IITA's molecular breeding program further deepens the application of genomics in cassava improvement. This plan provides a strong foundation for precise localization of genes related to key agronomic traits by constructing high-quality reference genomes and haplotype maps (Divya et al., 2024). Specifically, the project team identified specific chromosomal regions associated with cassava mosaic disease resistance and reduced tuber blueing (an adverse phenomenon affecting quality), laying a solid foundation for targeted cultivation of new varieties with these ideal traits. Using genome selection methods, IITA successfully shortened the breeding cycle of cassava from the traditional 5 years to only 2 years, greatly improving breeding efficiency. This progress has enabled the rapid introduction of more cassava varieties that are adaptable to a wide range of environmental conditions, high-yield, and have excellent consumer quality, which is of great significance for alleviating food shortages in Africa and enhancing the sustainability of agricultural production systems (Bakum, 2020, https://mel.cgiar.org/projects/411/381/nigeria-releases-five-cassava-varieties-developed-with-genomics-assisted-breeding-and-consumer-preference-studies).

4 Challenges and Limitations

4.1 Complexity and cost of technology implementation

While genomic tools offer transformative potential for cassava improvement, their implementation is not without challenges. The complexity and cost of advanced genomic technologies, such as CRISPR/Cas9 and high-throughput sequencing, can be prohibitive, especially for research institutions in developing countries where cassava is most vital. These technologies require not only expensive equipment and reagents but also highly specialized knowledge and facilities. The maintenance of such technologies and training personnel to operate them efficiently adds additional layers of difficulty, making it challenging for less-funded programs to adopt these advancements fully.

4.2 Acceptability and ethical issues of genetic resources

The use of genomic tools in cassava breeding also raises significant acceptability and ethical issues. There are concerns about the ownership and control of genetic resources, particularly in regions where cassava is a staple and cultural keystone. The prospect of genetically modified (GM) cassava varieties, although potentially beneficial in terms of yield and resistance to disease, often faces skepticism and resistance from both the public and various regulatory bodies. This resistance is due in part to fears about potential impacts on biodiversity, health, and local farming practices. Ethical considerations must also address the equitable distribution of benefits derived from genetic improvements, ensuring that advancements in cassava genomics do not disproportionately favor wealthier farmers over smaller, resource-poor farmers (Wang et al., 2022).

4.3 Effects of global change and environmental stress on breeding strategies

Global environmental changes, including climate change, soil degradation, and water scarcity, pose additional challenges to cassava breeding strategies. These changes can alter the expression of genetic traits in unpredictable ways, complicating the task of breeding varieties suited to future conditions. The dynamic nature of environmental stresses requires breeding strategies that not only address current conditions but are also adaptable to future changes. This necessitates a more profound understanding of the interactions between genetic traits and environmental factors, which can be complex and expensive to study. Moreover, breeding for resistance to one stress might inadvertently reduce resistance to another or affect other important traits such as yield or nutritional content, demonstrating the intricate balance required in the genetic improvement of cassava (Sonnewald et al., 2020).

5 Outlook and Prospects

The integration of emerging technologies such as artificial intelligence (AI) and machine learning in genomic data analysis is poised to revolutionize cassava improvement programs. These technologies can enhance the interpretation of complex genomic data, leading to more precise breeding decisions and the acceleration of genetic gain in cassava breeding programs.

The development of policy and regulatory frameworks is essential to support the application of genomic technologies in cassava improvement. Such frameworks can facilitate the adoption of new breeding techniques, ensure biosafety, and promote the equitable distribution of benefits arising from genetic advancements.

International cooperation and resource sharing are critical for the advancement of cassava genomics. Collaborative efforts can lead to the pooling of financial, human, and technical resources, fostering the exchange of germplasm, genomic information, and breeding materials. This can significantly contribute to the global effort to improve cassava productivity and resilience.

The achievements in cassava genomics have been substantial, with the development of molecular markers, genome mapping, and the identification of genes associated with key agronomic traits. These advancements have provided powerful tools for understanding the genetic basis of crop performance and have laid the groundwork for further improvement..

However, the need for continuous research and technological development cannot be overstated. As the genomic landscape of cassava becomes increasingly understood, it is imperative to maintain momentum in research to harness the full potential of genomic tools. This includes expanding genomic resources, improving genomic prediction models, and developing varieties that meet the changing needs of farmers and consumers. The future of cassava improvement is bright, with genomics playing a pivotal role in ensuring food security and economic development in regions dependent on this vital crop.

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Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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