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Biochemical Pathways of Starch Synthesis in Cassava: Genetic Bases and Breeding Implications

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Abstract This systematic review aims to integrate current knowledge on the biochemical pathways of starch synthesis in cassava (*Manihot esculenta* Crantz), elucidate the genetic bases underlying these processes, and discuss the implications for breeding programs aimed at improving cassava starch quality. Recent studies have identified a total of 45 genes involved in starch biosynthesis in cassava, including key enzymes such as ADPG pyrophosphorylase (AGPase), granule bound starch synthase (GBSS), and starch branching enzyme (SBE). These genes play crucial roles in determining the content and structure of amylose and amylopectin, which are vital for the starch's unique properties in food processing and industrial applications. Additionally, 110 quantitative trait loci (QTLs) associated with starch content and pasting properties have been identified, offering valuable markers for breeding efforts. Comparative genomic analyses have revealed positive selection for genes related to photosynthesis and starch accumulation, as well as negative selection for genes involved in cell wall biosynthesis and secondary metabolism, including cyanogenic glucoside formation in cultivated cassava varieties. Furthermore, the reconstructed metabolic pathway of starch biosynthesis in cassava provides a framework for integrating omics data, which has demonstrated distinct activities of the pathway at different stages of root development. The findings from this review highlight the significant progress made in understanding the genetic and biochemical aspects of starch synthesis in cassava. These advancements not only contribute to the fundamental knowledge of cassava biology but also have the potential to significantly impact breeding programs by providing molecular tools and insights for the development of cassava varieties with improved starch quality.
Keywords Cassava (*Manihot esculenta* Crantz); Starch synthesis; Biochemical pathways; Genetic variation; Breeding; Quantitative trait loci (QTLs); Gene expression

1 Introduction

Cassava (*Manihot esculenta* Crantz) is a root crop of paramount importance in tropical and subtropical regions, serving as a staple food for millions of people. Its significance is attributed to its high carbohydrate content, adaptability to diverse environments, and potential for high carbohydrate production (Wang et al., 2014). As a staple, cassava plays a crucial role in food security and is a primary source of energy for populations in these regions. Beyond its role in human nutrition, cassava starch is a versatile material with a wide range of applications in food processing and industrial sectors due to its unique properties (Tappiban et al., 2019).

Starch, the most important carbohydrate source in plant species, consists of amylose and amylopectin, which determine its physicochemical properties and functionality (Tappiban et al., 2019). The economic and nutritional value of starch in cassava cannot be overstated, as it is the key determinant of the crop's quality for both consumption and industrial use. Understanding the biosynthesis of starch in cassava is therefore essential for improving its quality and tailoring it to specific needs.

The objective of this systematic review is to elucidate the biochemical pathways of starch synthesis in cassava, explore the genetic foundations underlying these processes, and discuss the implications for breeding programs. Recent advances have shed light on the identification of genes and enzymes involved in starch biosynthesis, as well as the mechanisms of gene regulation during root development (Tappiban et al., 2019). Comparative genomic analyses have revealed selection pressures on genes related to starch accumulation, which are a result of natural selection and domestication (Wang et al., 2014). Furthermore, genetic modification strategies have demonstrated

the potential to enhance starch production in cassava, providing a promising avenue for biofortification and yield improvement (Ihemere et al., 2006).

By integrating recent findings from genomic studies, pathway reconstructions, and transgenic approaches, this review aims to provide a comprehensive understanding of starch biosynthesis in cassava. Such knowledge is instrumental for the development of improved cassava varieties with enhanced starch quality, which can have significant impacts on food security and industrial applications.

2 Biochemical Pathways of Starch Synthesis in Cassava

2.1 Overview of starch synthesis: general steps in starch biosynthesis in plants

Starch biosynthesis in plants is a complex process involving the conversion of glucose units into starch polymers. This process begins with the synthesis of adenosine diphosphate glucose (ADP-glucose), which serves as the activated glucose donor for the synthesis of amylose and amylopectin, the two main components of starch. The biosynthesis pathway includes the action of several key enzymes such as ADP-glucose pyrophosphorylase (AGPase), starch synthase (SS), starch branching enzyme (SBE), and de-branching enzyme (DBE) (Li, 2024), which work in concert to form the highly organized structure of starch granules (Figure 1) (Tappiban et al., 2019).

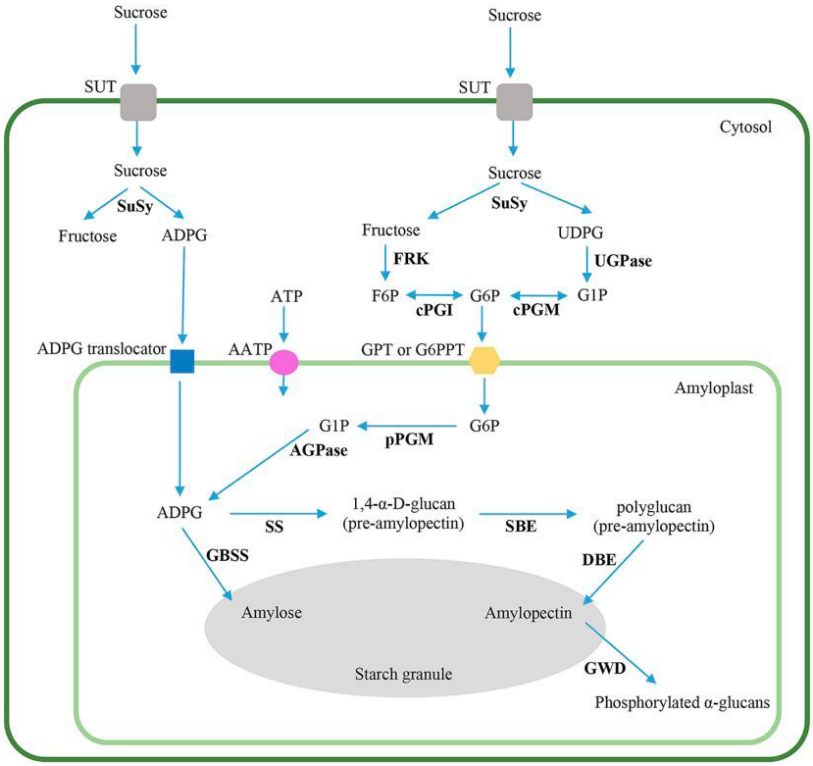

Figure 1 Schematic mechanism of starch biosynthesis in cassava storage root (Adopted from Tappiban et al., 2019)

Image caption: Abbreviations of enzymes are as follows: sucrose synthase (SuSy), UDPG pyrophosphorylase (UGPase), cytosolic phosphoglucomutase (cPGM), plastidial phosphoglucomutase (pPGM), fructokinase (FRK), cytosol phosphoglucose isomerase (cPGI), adenosine diphosphate glucose pyrophosphorylase (AGPase), granule bound starch synthase (GBSS), starch synthase (SS), starch branching enzyme (SBE), de-branching enzyme (DBE) and glucan, water dikinase (GWD). The membrane transportors are sucrose transporters (SUT), glucose 6-phosphate/phosphate transporter (GPT or G6PPT), ATP/ADP transporter (AATP) and ADPG translocator. Enzyme substrates and products including glucose 6-phosphate (G6P), adenosine Triphosphate (ATP), adenosine monophosphate (AMP), fructose 6-phosphate (F6P), uridine diphosphate (UDP), uridine diphosphate glucose (UDPG), pyrophosphate (PPi), orthophosphate (Pi), glucose1-phosphate (G1P), uridine triphosphate (UTP) and malto-oligosaccharide (MOS) (Adopted from Tappiban et al., 2019)

Figure 1 provides a detailed schematic representation of the starch biosynthesis pathway in the cassava storage root. This diagram includes various enzymatic steps and metabolic processes that occur within the plant's cells to convert basic sugar molecules into complex starches, which are stored in the roots.

Starting with sucrose from the cytosol, the pathway utilizes enzymes like sucrose synthase (SuSy) to break down sucrose into fructose and uridine diphosphate glucose (UDPG). Other enzymes, such as UDPG pyrophosphorylase (UGPase), are involved in further transformations that form critical intermediates like glucose-1-phosphate (G1P). The figure also includes various other enzymes located in both the cytosol and plastid, such as phosphoglucomutases (cPGM and pPGM), fructokinase (FRK), and phosphoglucose isomerase (cPGI), highlighting their roles in manipulating glucose molecules into forms suitable for starch synthesis.

The core of the starch synthesis occurs in the plastid, where adenosine diphosphate glucose (ADPG) produced by ADP glucose pyrophosphorylase (AGPase) is polymerized into starch by enzymes such as granule-bound starch synthase (GBSS) and other starch synthases (SS). The pathway complexity is increased by the actions of starch branching enzymes (SBE) and de-branching enzymes (DBE), which modify the structure of the growing starch molecule (Li, 2024).

Additionally, the illustration includes membrane transporters such as sucrose transporters (SUT), glucose 6-phosphate/phosphate transporter (GPT or G6PPT), ATP/ADP transporter (AATP), and ADPG translocator, which are crucial for moving molecules like glucose 6-phosphate (G6P) and ADP across the plastid membrane, facilitating the flow of substrates necessary for starch biosynthesis.

2.2 Enzymatic roles and pathways

2.2.1 Specific enzymes involved in cassava starch synthesis

In cassava (*Manihot esculenta* Crantz), a total of 45 genes have been identified to participate in starch biosynthesis. These include isoforms of AGPase, granule-bound starch synthase (GBSS), SS, SBE, DBE, and glucan, water dikinase (GWD). Each of these enzymes plays a specific role in the synthesis and modification of starch, contributing to the unique properties of cassava starch that are important for food processing and industrial applications (Tappiban et al., 2019).

2.2.2 Pathway details from sucrose breakdown to starch assembly

The pathway of starch synthesis in cassava starts with the breakdown of sucrose into glucose and fructose. Glucose is then phosphorylated to glucose-6-phosphate and further isomerized to glucose-1-phosphate. AGPase catalyzes the conversion of glucose-1-phosphate to ADP-glucose, which is the substrate for starch synthases. GBSS is primarily responsible for amylose synthesis, while SS, SBE, and DBE are involved in amylopectin synthesis. The orchestrated action of these enzymes leads to the assembly of starch granules in the plastids (Ihemere et al., 2006; Tappiban et al., 2019).

2.3 Regulatory mechanisms: factors influencing enzyme activity and starch biosynthesis rate

The rate of starch biosynthesis in cassava is influenced by various factors, including gene regulation, enzyme activity, and environmental conditions. The expression of genes involved in starch biosynthesis is regulated throughout root development, and quantitative trait loci (QTLs) associated with starch content and pasting properties have been identified. Genetic modification techniques, such as CRISPR-Cas9 mediated targeted mutagenesis and transgenic breeding, have been used to alter the expression of key genes like *GBSS* and *PTST1*, thereby modifying the starch content and properties in cassava roots (Bull et al., 2018; Tappiban et al., 2019). Additionally, the domestication of cassava has led to the selection of genes that increase carbon flux towards starch accumulation, which is a reflection of the natural selection and breeding efforts to enhance starch production (Wang et al., 2014).

3 Genetic Bases of Starch Synthesis in Cassava

3.1 Genetic variability in starch synthesis genes

Cassava (*Manihot esculenta* Crantz) is a staple food crop whose starch composition is crucial for both consumption and industrial applications. The genetic variability in starch synthesis genes is significant, as evidenced by the identification of 110 quantitative trait loci (QTLs) associated with starch content and pasting properties (Tappiban et al., 2019). This genetic diversity is a valuable resource for breeding programs aimed at improving starch quality.

3.2 Key genes and alleles: description of major genes involved and their known variants

A total of 45 genes have been identified as participants in the starch biosynthesis pathway in cassava. These include ADPG pyrophosphorylase (AGPase), granule bound starch synthase (GBSS), starch synthase (SS), starch branching enzyme (SBE), de-branching enzyme (DBE), and glucan, water dikinase (GWD) (Tappiban et al., 2019). Among these, GBSS is particularly noteworthy as it is directly involved in amylose biosynthesis. CRISPR-Cas9 mediated targeted mutagenesis of GBSS, as well as PROTEIN TARGETING TO STARCH (PTST1), has been shown to reduce or eliminate amylose content, significantly altering the physicochemical properties of starch (Figure 2) (Bull et al., 2018). These findings highlight the potential of gene editing techniques in developing cassava varieties with modified starch characteristics for specific applications.

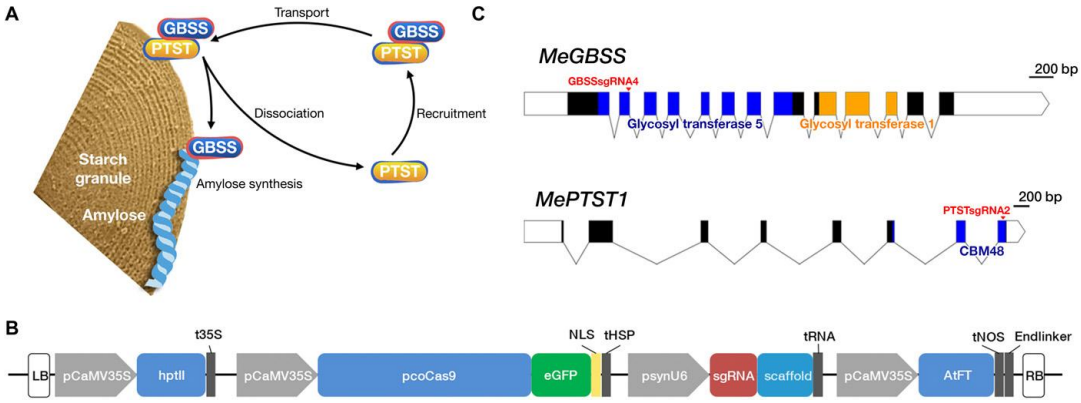

Figure 2 Genome editing of *MeGBSS* and *MePTST1* (Adopted from Bull et al., 2018)

Image caption: (A) Model for association between GBSS, PTST1, and starch granules as proposed by Seung et al. (2015). Image recreated with permission. (B) Binary expression construct containing *hptII* (hygromycin B resistance) for plant selection; *Cas9* codon optimized for cassava usage (pcoCas9) and fused to the *eGFP* reporter gene and directed to the nucleus via a nucleoplasmin nuclear localization signal (NLS); transcription terminated by sequence from a heat shock protein (tHSP) (Nagaya et al., 2010). A synthetic pU6 promoter [(psynU6; (Nekrasov et al., 2013)] used to drive expression of the protospacer sequence [single guide RNA (sgRNA)] and synthetic scaffold (Jinek et al., 2012) to generate the desired sgRNA. *AtFT* for early flowering is constitutively expressed by the *CaMV*35S promoter and terminated by nopaline synthase sequence (tNOS). Binary expression constructs named *pCas9-sgGBSS-FT* and *pCas9-sgPTST-FT*. Left and right borders (LB and RB) are shown. Diagram not to scale. (C) Gene maps of *MeGBSS* and *MePTST1*. Exons are depicted as blocks, and regions encoding functional domains are shaded. The target sites for the sgRNAs are indicated. Scale bars are shown (Adopted from Bull et al., 2018)

Figure 2 presents the genome editing strategy used to modify the *MeGBSS* and *MePTST1* genes in cassava. The illustration details a model of how GBSS and PTST1 associate with starch granules, as well as the construction of binary expression constructs used for plant transformation. This approach utilizes CRISPR/Cas9 technology, optimized for cassava, to target specific gene sequences responsible for starch biosynthesis and transport, which are crucial for understanding and manipulating starch accumulation and properties in cassava roots. By employing this targeted editing, the study aims to elucidate the roles of these genes in starch granule formation and potentially improve cassava's starch yield and quality.

3.3 Gene expression patterns: how genetic factors influence the expression and functionality of enzymes in different cassava varieties

The expression of starch biosynthesis genes is regulated during root development, which determines the functionality of the enzymes and ultimately the starch properties of the cassava roots (Tappiban et al., 2019). Comparative genomic analyses have revealed that genes involved in starch accumulation have been positively selected in domesticated cassava varieties (Wang et al., 2014). Additionally, transgenic approaches have shown that increasing the expression of genes like *AGPase* in cassava roots can lead to a substantial increase in starch production (Ihemere et al., 2006). The expression profiles of genes such as those in the UGPase family also vary across different tissues, which may influence the distribution and quantity of starch synthesis within the plant (Ihemere et al., 2006). Understanding these expression patterns is crucial for the development of cassava varieties with desired starch qualities through breeding and genetic modification.

4 Case Studies

4.1 Successful breeding programs

Cassava breeding programs have made significant strides in improving starch yield and quality by leveraging genetic insights. A comprehensive review highlighted the identification of 45 genes involved in starch biosynthesis in cassava, including key enzymes like ADPG pyrophosphorylase (AGPase) and granule bound starch synthase (GBSS). These genetic discoveries have facilitated the development of 110 quantitative trait loci (QTLs) for starch content and pasting properties, which are instrumental for breeders to enhance cassava starch through biotechnologies such as transgenic breeding and molecular marker-assisted selection (Tappiban et al., 2019).

4.2 Genetic modification successes

Genetic modification has been a powerful tool in the enhancement of starch synthesis in cassava. CRISPR-Cas9 mediated targeted mutagenesis of genes involved in amylose biosynthesis, such as PROTEIN TARGETING TO STARCH (PTST1) and GRANULE BOUND STARCH SYNTHASE (GBSS), has been shown to reduce or eliminate amylose content in root starch. This modification not only impacts the physicochemical properties of starch but also accelerates flowering, which is beneficial for breeding programs. The resulting transgene-free progeny inherits these edited genes, demonstrating the potential of genome editing in creating novel cassava varieties with modified starch suitable for both food and industrial applications (Figure 3) (Bull et al., 2018).

Figure 3 provides a comparative overview of traditional breeding versus New Plant Breeding Techniques (NPBTs) for trait improvement in crops. It illustrates the lengthy and complex process required for breeding recessive traits using conventional methods, which often involves multiple generations of crossing and selection to introgress desired traits from wild relatives or mutagenized plants into preferred crop genotypes. In contrast, the diagram depicts how NPBTs, such as genome editing with CRISPR/Cas9, can significantly accelerate this process. By using Agrobacterium-mediated transformation to introduce specific mutations, breeders can achieve faster flowering, simplify the segregation of edited lines, and potentially bypass some of the more time-consuming steps associated with traditional breeding, such as repeated backcrossing and selfing.

4.3 Field trials and outcomes

Field trials are critical for assessing the practical impacts of genetic and biochemical knowledge on cassava cultivation. Genetic modification of cassava to enhance starch production has been tested with promising results. Transgenic plants expressing a modified bacterial *glgC* gene, which encodes a more active form of AGPase, showed up to a 2.6-fold increase in total tuberous root biomass. This increase in sink strength for carbohydrate led to significant increases in both tuberous and above-ground biomass, suggesting that modifying enzymes that regulate source-sink relationships can be an effective strategy for increasing carbohydrate yields in cassava (Ihemere et al., 2006). Additionally, the integration of omics data into the metabolic pathway of starch biosynthesis has provided insights into the distinct activities of the pathway during different stages of root development, which is crucial for the genetic improvement of cassava (Saithong et al., 2013).

5 Breeding Implications

5.1 Breeding objectives: traits related to starch content and quality that are targeted in breeding programs

Breeding programs for cassava (*Manihot esculenta* Crantz) have been primarily focused on improving starch content and quality due to its significance as a staple food and its industrial applications. The starch in cassava is composed of amylose and amylopectin, which determine its unique properties for food processing and industrial uses (Tappiban et al., 2019). The identification of 110 quantitative trait loci (QTLs) for starch content and pasting properties has provided breeders with valuable genetic markers for selecting desirable traits (Tappiban et al., 2019). Additionally, the discovery of genes specific to wild and domesticated varieties of cassava, such as those involved in photosynthesis and starch accumulation, has highlighted the potential for selecting traits that enhance carbohydrate production (Wang et al., 2014).

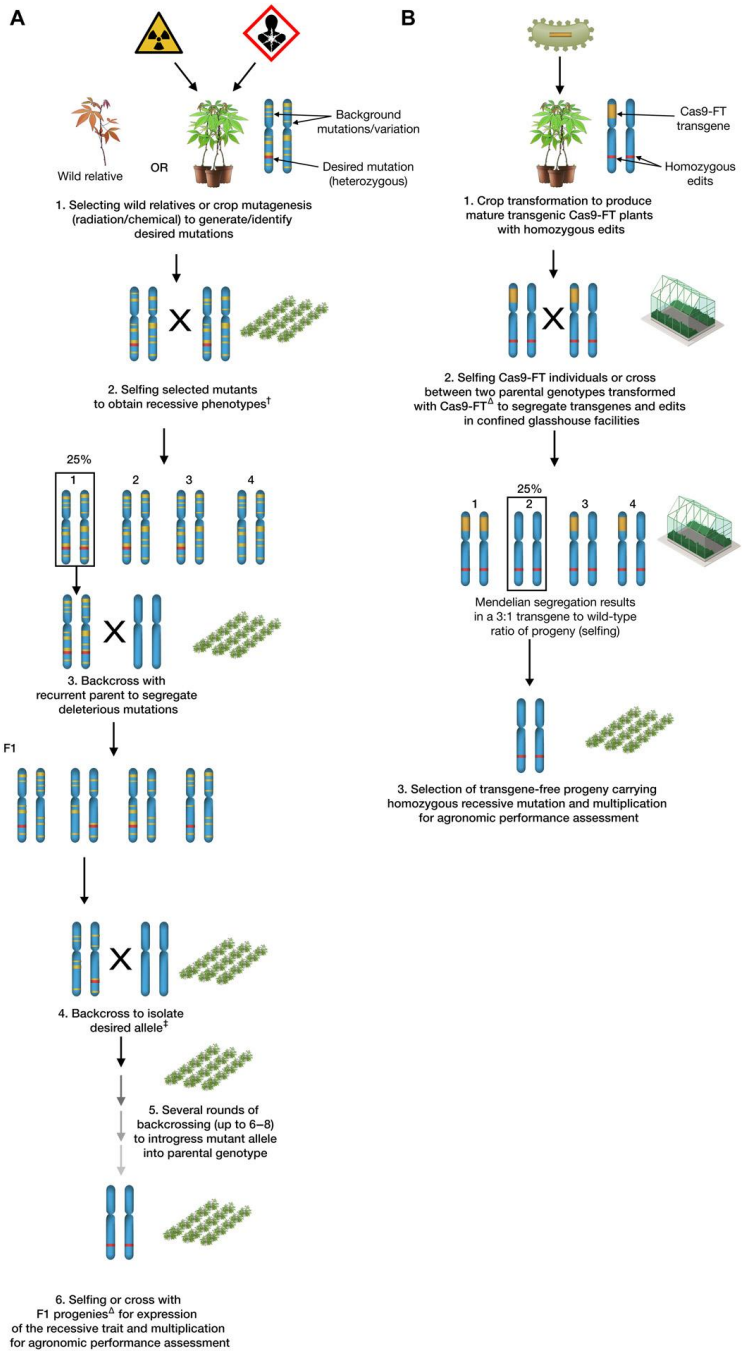

Figure 3 Schematic representation of conventional breeding and the designed NPBT for trait improvement (Adopted from Bull et al., 2018)

Image caption: Panel (A) shows the traditional pathway for breeding recessive traits, requiring multiple cross-generations to achieve homozygosity in a farmer-preferred genotype, often taking several years. Panel (B) illustrates the application of NPBTs, specifically genome editing for accelerated flowering and segregation in crop improvement. This includes steps like Agrobacterium-mediated transformation and screening of mutant populations in controlled environments, highlighting the efficiency and speed of achieving T-DNA-free progeny with desired mutations (Adapted from Bull et al., 2018)

5.2 Genetic engineering and CRISPR/Cas9: advances in genetic modifications to enhance starch yield and quality

Recent advances in genetic engineering, particularly CRISPR/Cas9-mediated targeted mutagenesis, have opened new avenues for modifying starch biosynthesis pathways to enhance yield and quality. For instance, the targeted modification of genes like PROTEIN TARGETING TO STARCH (PTST1) and GRANULE BOUND STARCH SYNTHASE (GBSS) has been shown to significantly alter amylose content in cassava starch (Bull et al., 2018).

Moreover, the integration of genes such as the Arabidopsis *FLOWERING LOCUS T* into the genome-editing cassette has accelerated flowering and, consequently, the breeding cycle in cassava (Bull et al., 2018). These techniques offer a promising approach to rapidly introduce beneficial traits into cassava varieties.

5.3 Conventional breeding vs. molecular breeding: comparisons, advantages, and challenges

Conventional breeding in cassava faces challenges such as long breeding cycles and limited genetic variability within cultivated varieties. In contrast, molecular breeding approaches, including transgenic breeding and molecular marker-assisted selection, leverage the knowledge of genes and enzymes involved in starch biosynthesis to improve cassava starch quality (Tappiban et al., 2019). For example, increasing the sink strength for carbohydrate by expressing a modified bacterial ADP-glucose pyrophosphorylase (AGPase) gene has led to increased starch production in cassava roots (Ihemere et al., 2006). However, molecular breeding techniques require a deep understanding of the genetic basis of starch biosynthesis, as well as the integration of omics data to elucidate the dynamic regulation of these pathways during root development (Saithong et al., 2013). Despite the potential for higher precision and faster breeding cycles, molecular breeding must also address regulatory, biosafety, and public acceptance issues associated with genetically modified organisms.

6 Challenges and Opportunities

6.1 Current limitations in understanding and manipulating starch synthesis pathways

Despite significant progress in understanding the starch biosynthesis in cassava, there are still limitations in fully comprehending and manipulating these pathways. The complexity of starch biosynthesis is evident from the identification of 45 genes involved in the process, including isoforms of enzymes like ADPG pyrophosphorylase (AGPase), granule bound starch synthase (GBSS), and others (Tappiban et al., 2019). While the functions of these genes have been characterized to some extent, the intricate regulation of their expression during root development and the interaction between different enzymes in the pathway are not completely understood (Tappiban et al., 2019). Additionally, the genetic modification of cassava to enhance starch production has shown potential, but the translation of these findings to field conditions remains a challenge (Ihemere et al., 2006). The use of CRISPR-Cas9 mediated targeted mutagenesis has been successful in modifying genes involved in amylose biosynthesis, but the long-term effects and stability of these modifications need further investigation (Bull et al., 2018).

6.2 Research gaps: areas needing further research based on current findings

There are several research gaps that need to be addressed to advance our understanding of starch synthesis in cassava. Firstly, the identification of quantitative trait loci (QTLs) and candidate genes associated with starch quality traits is a significant step forward, but the functional validation of these QTLs and their use in breeding programs requires more research (Tappiban et al., 2019). Secondly, the comparative analysis of the cassava genome between wild ancestors and cultivated varieties has revealed positive selection for genes involved in starch accumulation, but the underlying mechanisms of this selection and its impact on starch biosynthesis are not fully explored (Wang et al., 2014). Thirdly, the expression profiles of genes related to starch metabolism, such as those encoding uridine diphosphate glucose pyrophosphorylase (UGPase), have been analyzed, but the environmental and developmental factors influencing these profiles are still to be determined (Ha et al., 2019).

6.3 Future prospects in cassava breeding with genetic insights

The future of cassava breeding looks promising with the integration of genetic insights into breeding programs. The use of genome editing techniques, such as CRISPR-Cas9, offers the possibility of creating cassava varieties with modified starch properties tailored for specific industrial applications or improved cooking qualities (Bull et al., 2018). The acceleration of flowering in cassava through genetic modification could also reduce breeding times, allowing for faster development of new varieties (Bull et al., 2018). Furthermore, the reconstruction of the starch biosynthesis pathway using genome information provides a framework for integrating omics data, which can lead to a better understanding of the dynamic regulation of this pathway and identify key targets for genetic improvement (Saithong et al., 2013). The exploitation of the starch biosynthesis pathway for data integration

could also facilitate the identification of novel regulatory mechanisms and metabolic bottlenecks, which could be addressed through targeted breeding or genetic modification (Saithong et al., 2013).

7 Concluding Remarks

The systematic review has highlighted significant advancements in understanding the biochemical pathways of starch synthesis in cassava (*Manihot esculenta* Crantz), which is a staple food and industrial crop in tropical and sub-tropical regions. A total of 45 genes have been identified as participants in the starch biosynthesis pathway, including key enzymes such as ADPG pyrophosphorylase (AGPase), granule bound starch synthase (GBSS), starch synthase (SS), starch branching enzyme (SBE), de-branching enzyme (DBE), and glucan, water dikinase (GWD). The expression patterns of these genes vary across different organs and developmental stages, with a higher activity toward the development of mature storage roots. Additionally, 110 quantitative trait loci (QTLs) associated with starch content and pasting properties have been identified, which are crucial for the improvement of starch quality through biotechnologies like transgenic breeding and molecular marker-assisted selection.

Transgenic approaches have successfully increased starch production in cassava by enhancing the sink strength for carbohydrate through the expression of a modified bacterial *AGPase* gene. Furthermore, the regulation of the AGPase gene by transcription factors such as MeSAUR1 has been elucidated, providing insights into the molecular mechanisms of starch accumulation. The onset of storage root formation has been associated with specific gene expressions, including sulfite reductase and calcium-dependent protein kinase, which may play roles in signaling pathways and sulfur-containing protein biosynthesis.

Future research should focus on the integration of omics data to further elucidate the dynamic regulation of starch biosynthesis in cassava. This includes the use of transcriptomic, proteomic, and metabolomic approaches to understand the complex interactions between genes, proteins, and metabolites during root development. Additionally, the role of postharvest physiological deterioration (PPD) in starch metabolism warrants further investigation, as it has implications for the shelf-life and commercial value of cassava. The exploration of natural genetic variation and the identification of novel SNPs in starch pathway genes could also provide new opportunities for breeding cassava varieties with improved starch quality and yield.

The findings from this review have profound implications for food security, economic development, and sustainable agricultural practices. Cassava is a vital source of calories for millions of people, and improvements in starch quality and yield can significantly enhance the nutritional status and livelihoods of subsistence farmers. The development of cassava varieties with higher starch content and better resistance to PPD can reduce postharvest losses, increase the shelf-life of cassava products, and expand their use in the food and industrial sectors. Moreover, understanding the genetic bases of starch synthesis can facilitate the breeding of cassava varieties that are better adapted to climate change, thereby contributing to the sustainability of agricultural systems in vulnerable regions.

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Conflict of Interest Disclosure

The author affirms 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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