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REVIEW ARTICLE

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The future of cassava in the era of biotechnology in Southern Africa

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ABSTRACT

Cassava (*Manihot esculenta*) is a major staple food and the world's fourth source of calories. Biotechnological contributions to enhancing this crop, its advances, and present issues must be assessed regularly. Functional genomics, genomic-assisted breeding, molecular tools, and genome editing technologies, among other biotechnological approaches, have helped improve the potential of economically important crops like cassava by addressing some of its significant constraints, such as nutrient deficiency, toxicity, poor starch quality, disease susceptibility, low yield capacity, and postharvest deterioration. However, the development, improvement, and subsequent acceptance of the improved cultivars have been challenging and have required holistic approaches to solving them. This article provides an update of trends and gaps in cassava biotechnology, reviewing the relevant strategies used to improve cassava crops and highlighting the potential risk and acceptability of improved cultivars in Southern Africa.

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Introduction

It has been estimated that by 2050, the world's population will double to nine billion people [1]. However, the worldwide population could be less than that estimated by Bahar et al. [1] due to factors such as the COVID-19 pandemic and/or natural disasters, amongst others. Nevertheless, the limited resources and the worldwide growth might cause the food demand greater than supply [1]. As a result, cassava has been identified as an example of a staple crop that has the potential to reduce food insecurity, poverty, and hunger, particularly in developing African countries [2]. Cassava is thought to have originated in South America and, subsequently, spread to Asia and Africa [3]. Since then, it has become a staple food for more than 800 million people across the globe [4]. Cassava is grown primarily for the consumption of its starchy-dense roots [2], while in certain parts of Africa; its leaves are also eaten as a source of: protein, vitamins, and minerals [5]. Unfortunately, the roots lack major nutrients (protein and vitamins) [6], but they contain toxin (cyanogen glycoside) which, upon hydrolysis, produces hydrogen cyanide that is toxic to pathogens, animals, and humans, upon consumption [7,8]. Other limitations associated with this crop include (1) susceptibility to pests and diseases, (2) poor quality of starch content, and (3) post-harvest quality deterioration [2,9,10]. Some of these limitations have been targeted using several biotechnological approaches, as shown in Figure 1. Hence, this manuscript discusses the latest issues and developments related to cassava research in Southern Africa, as well as the research gaps within the region. The article is divided into three sections: (1) cassava improvement leveraging on biotechnological advances; (2) cassava biotechnology; success stories in Southern Africa; and (3) cassava biotechnology safety and associated risks; perceptions and acceptance of transgenic cassava. This work concludes by highlighting future perspectives and the biotechnological challenges ahead in cassava production in Southern Africa.

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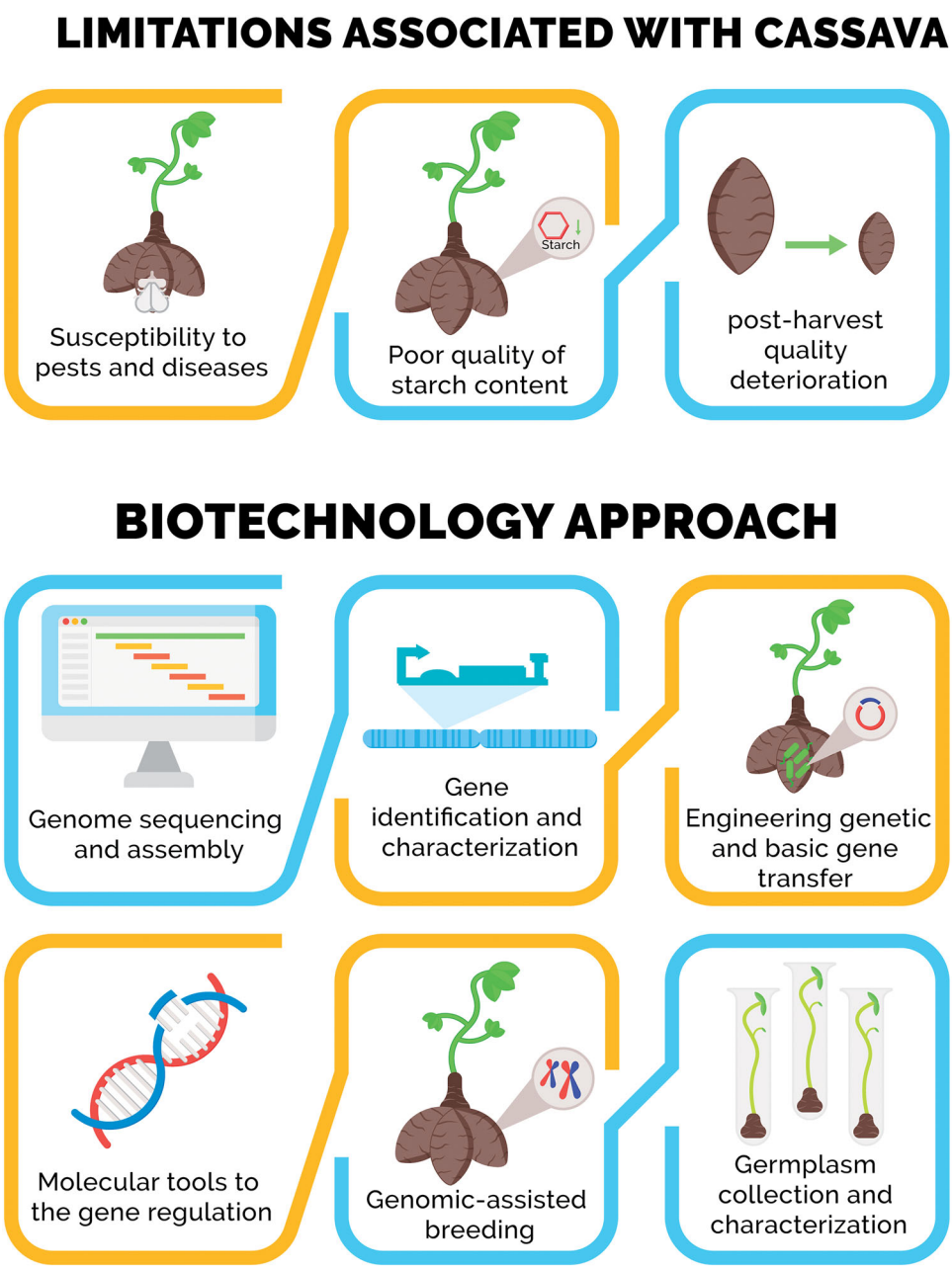

Figure 1. Biotechnology approaches to improve cassava. For cassava improvement, biotechnology has several approaches. The genome sequencing and assembly has allowed a better understanding of some of the cassava's genetics resource and its metabolism. Also, gene identification and characterization are prominent in studying the resistance and response mechanism to different cassava pathogens. With this knowledge, another popular approach is gene-editing technologies to create a new strain with specific characteristics. In the same way, genomic-assisted breeding and germplasm collection and characterization are traditional techniques for improving cassava and conservation.

Cassava improvement via *state-of-the-art molecular tools for gene expression regulation*

The cassava genome and the mechanisms involved in its interaction with the environment, pests, and diseases, have gained much attention in the last 15 years [11]. Several gene-regulation methods could be applied to improve its adaptability and quality [12]. Despite the challenges of genome mapping in this crop (allele diversity, polygenic traits, among others) [13], RNA interference (RNAi) and Targeting Induced Local Lesions IN Genome (Tilling) are just a few of the plant gene expression regulation tools. These tools have proven to be effective in the discovery of new traits as well as the development of cassava with high agricultural yields

and resilience to biotic and abiotic challenges. These different gene-regulation tools are detailed below:

I. RNAi: This is a type of post-transcriptional gene silencing that controls gene expression in a variety of ways. Small interfering RNAs (siRNAs), microRNAs (miRNAs), and PIWI-interacting RNAs (piRNAs) can all cause RNAi [14]. RNAi tools were used in controlling whitefly, vectors of cassava mosaic disease (CMD), and cassava brown streak disease (CBSD). The target is the inhibition of V-ATPase A, an enzyme that links the energy of ATP hydrolysis to proton transport across intracellular and plasma membranes of eukaryotic cells, as shown in Figure 2(a) [15]. siRNA and artificial miRNAs have been used for functional genomics and improving disease resistance in cassava [16]. For instance, the replacement of miR159 (a miRNA targeting the CBSV) precursor with artificial miRNAs from cassava brown streak viruses (Figure 2(b)), triggered a higher level of disease resistance in transgenic cassava [15].
   Furthermore, transacting siRNAs (ta-siRNA) and natural cis-antisense siRNAs (cis-nat-siRNAs) are two types of post-transcriptional gene silencing miRNAs that have recently been found [17]. Cassava has a total of 54 ta-siRNA loci, including a homolog of TAS3, the most studied plant ta-siRNA. Their functions in a variety of plant processes, such as pathogen response, are mostly unknown. An *in silico* study carried out by Quintero *et al*. [18] to detect ta-siRNAs and cis-nat-siRNAs in the cassava, comparing a wild-type cassava plant sRNA library with cassava plants infected with *Xanthomonas axonopodis PV. manihotis* library (Figure 2(c)). Fifteen of these loci were activated while 39 were suppressed.
II. Tilling: This is a reverse genetic method used in detecting induced mutations in a specific location. This method has been used to find alleles or characterize gene function in cassava as shown in Figure 2(d) [19]. However, its inability to detect mutations close to simple sequence repeats (SSRs) and the need for locus-specific polymerase chain reaction (PCR) products are some of its key drawbacks [15,19].

Recent advances in the clustered regularly interspaced short palindromic repeats/CRISPR-associated protein9 (CRISPR/Cas9) genome editing process, which uses single-guide RNA for genome editing, have made it a: simple, stable, and effective tool for: targeted gene mutations, knockout, and knock-in/replacement to increase crop yield. The CRISPR/Cas technique is constantly evolving, and its applications have grown dramatically and help to create non-GM mutant plants by the delivery of gRNA and Cas9 reagents directly which can evade the strict regulations laid out for the GM crops harboring foreign genes. It may be used to change the genome sequence of any creature, including plants such as cassava, to produce the desired characteristic. CRISPR/Cas is now widely acknowledged as a game-changing tool in plant biology.

Cassava improvement via *genome editing tools*

In recent years, advances in genome editing technology have shown promise in crops, making it easier to develop new varieties [20]. Zink finger nucleases (ZFNs), transcriptional activator-like effector nucleases (TALENs), and more recently, CRISPR are important gene-specific genome editing technologies because they are fast, effective, and technically simple to use [21]. These tools have also encouraged the production of novel transgene-free crop varieties that are difficult to distinguish from those created through traditional breeding techniques [22]. Due to its ease, adaptability, and efficacy, the CRISPR/CRISPR-associated proteins genome editing technique has shown more promise in tackling agricultural difficulties than its predecessors [20]. However, when compared to other crops, such as rice, there is limited research on the effectiveness of CRISPR technology in cassava [23]. Several genome editing projects involving the CRISPR/Cas9 method have recently been completed to improve the yield of cassava, a drought reserve crop, by introducing: disease resistance, rapid flowering, herbicide tolerance, and reduced cyanide content in the leaves and roots [24–26].

Cassava improvement via CRISPR/Cas9

CRISPR-Cas9 is a rapid, inexpensive, precise, and efficient genome-editing tool. Odipio et al. [24] implemented for the first time this technology in Cassava. They developed a system to mutate the *MePDS* (Phytoene desaturase) in two cassava cultivars. For this, they design gRNAs targeting two sequences within MePDS exon 13. The gRNA and the CRISPR/Cas9 system were delivered using *Agrobacterium*. The result showed that 90–100% of the plant lines recovered had the albino phenotype resulting from the *MePDS* mutation. These results were confirmed by sequence analysis, in which 100% of the sequences analyzed show a mutation in *MePDS* (Figure 3(a)). Once verified the efficacy of

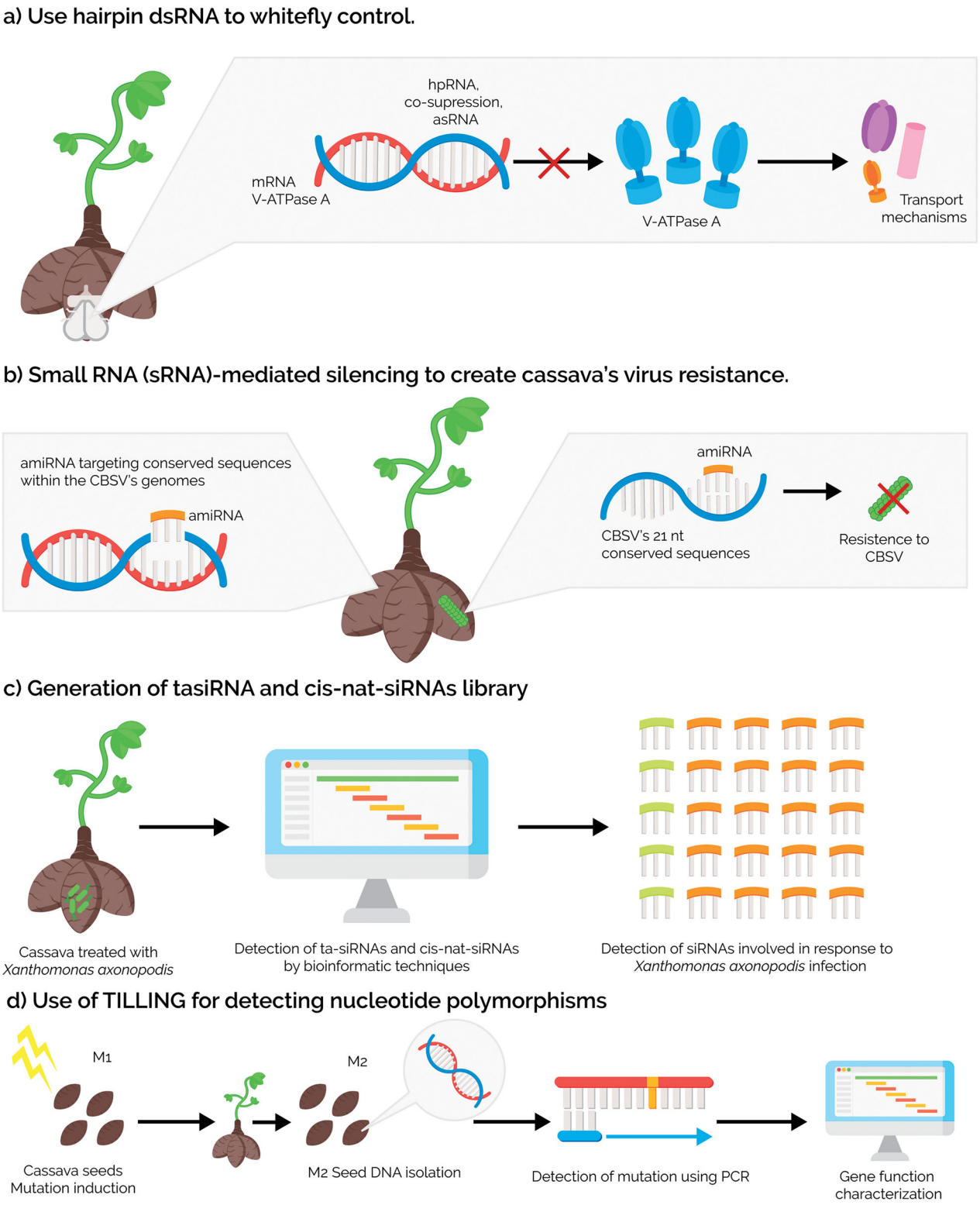

Figure 2. Molecular strategies to improve cassava. (a) Use hairpin dsRNA whitefly control. For the cassava mosaic, disease control could be used hairpin dsRNA. The hairpin dsRNA are directed to V-ATPase-A mRNA preventing its translation. The expression of V-ATPase-A activates other transport mechanisms causing the whitefly's death. (b) Small RNA (sRNA)-mediated silencing to create cassava's virus resistance. The sRNA can be used to create resistance to different cassava viruses. The expression of amiRNAs in cassava directed to CBSV's 21 nt conserved sequence generated resistance to these viruses. (c) Generation of tasiRNA and cis-nat-siRNAs library. In an infection by Xanthomonas cassava start, the expression of different siRNAs likes a response. A Library of these siRNAs was created through the cassava treated with *X. axonopodis* and bioinformatics tools to detect the siRNAs that over-express in this condition. (d) Use of TILLING for detecting nucleotide polymorphisms. TILLING is a tool to detect polymorphisms in a specific gene. The mutation induction and the use of this technique can help us to identify the function of a specific gene.

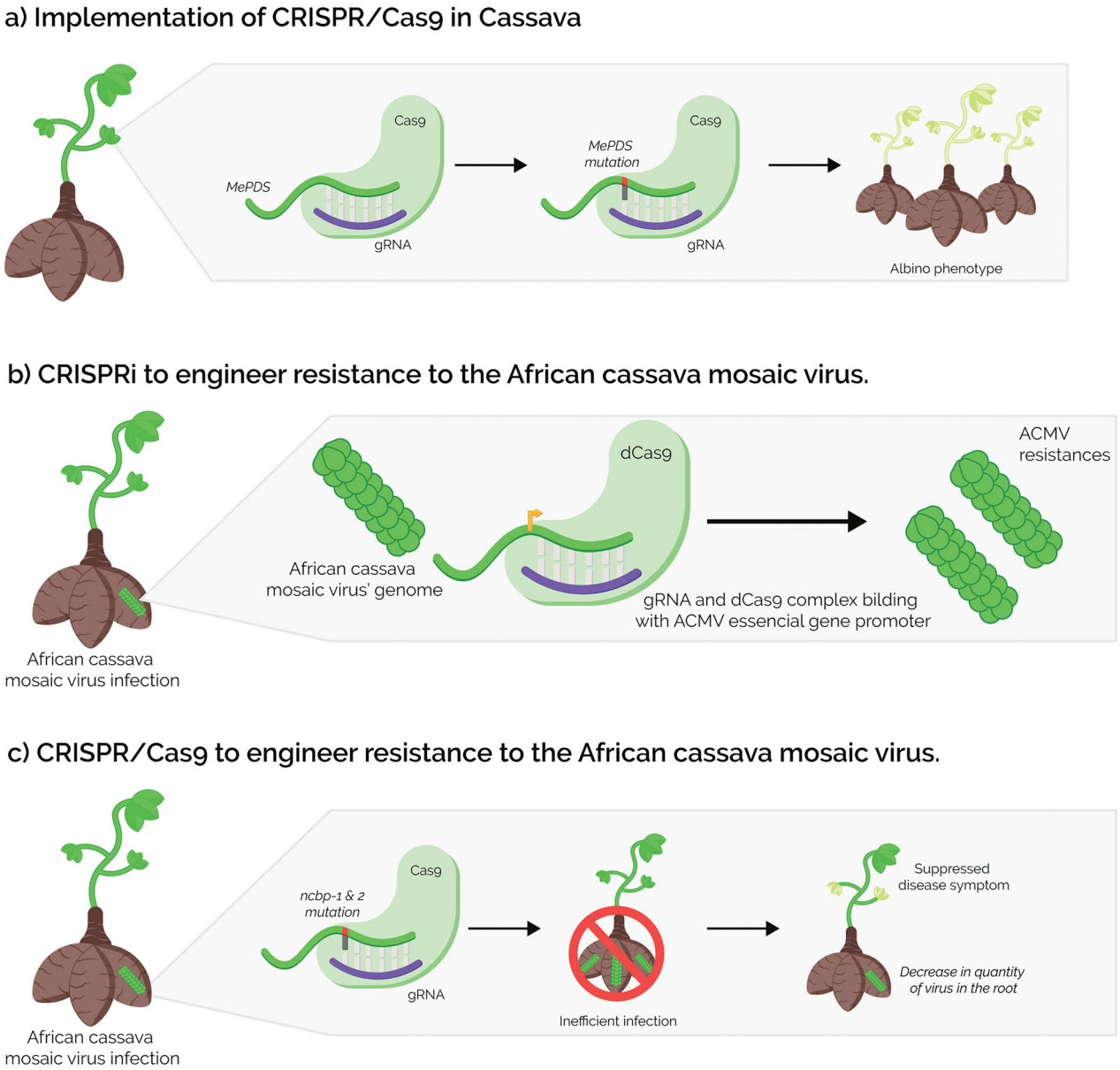

Figure 3. CRISPRi to engineer resistance to the African cassava mosaic virus. The CRISPRi is a promising tool to generate resistance to different cassava viruses like the African cassava mosaic virus. This technique consists of the design of a gRNA that binds with the virus' genome and a dCas. However, the studies published using this technique report an inefficient resistance.

this technology in Cassava, Mehta et al. [22] used CRISPR-Cas9 interference (CRISPRi) to engineer resistance in cassava to the African cassava mosaic virus (ACMV). The result revealed that during glasshouse inductions, the virus evolved and acquired resistance to the CRISPR system. Hence, the transgenic cassava expressing Cas9 was not resistant to the viral infection (Figure 3(b)). However, more studies were designed to create cassava strains with resistance to the ACMV. Gomez et al. [27], used CRISPR/Cas9 to create a cassava strain with a mutation in the genes ncbp-1 and ncbp-2. These genes have a critical role in the ACMV infection process. The strains with the mutations expose suppressed disease symptoms and fewer viruses in the root in comparison with the control (Figure 3(c)). Herbicide-resistant cassava cultivar was created using CRISPR/Cas9-mediated gene insertion and substitution. This strategy produced glyphosate-tolerant cassava that was phenotypically normal, demonstrating the value of gene editing in cassava improvement [28].

Although CRISPR/Cas9-based genome editing technology has advanced significantly in recent years, it still confronts some hurdles, including off-target effects, CRISPR/Cas9 delivery systems, side effects on nearby genes, and regulatory concerns. Although the

Table 1. The nutrient value of the top 10 staple crops in the world (per 100 g dry weight).

Nutrient	Maize	Rice	Wheat	Potatoes	Cassava	Soybeans	Sweet potatoes	Yams	Sorghum	Plantain
Water content (%)	10	12	13	79	60	68	77	70	9	65
Energy (kJ)	1698	1736	1574	1533	1675	1922	1565	1647	1559	1460
Protein (g)	10.4	8.1	14.5	9.5	3.5	40.6	7.0	5.0	12.4	3.7
Fat (g)	5.3	0.8	1.8	0.4	0.7	21.6	0.2	0.6	3.6	1.1
Carbohydrates (g)	82	91	82	81	95	34	87	93	82	91
Fiber (g)	8.1	1.5	14.0	10.5	4.5	13.1	13.0	13.7	6.9	6.6
Sugar (g)	0.7	0.1	0.5	3.7	4.3	0.0	18.2	1.7	0.0	42.9
Calcium (mg)	8	32	33	57	40	616	130	57	31	9
Iron (mg)	3.01	0.91	3.67	3.71	0.68	11.09	2.65	1.80	4.84	1.71
Magnesium (mg)	141	28	145	110	53	203	109	70	0	106
Phosphorus (mg)	233	131	331	271	68	606	204	183	315	97
Potassium (mg)	319	131	417	2005	678	1938	1465	2720	385	1426
Sodium (mg)	39	6	2	29	35	47	239	30	7	11
Zinc (mg)	2.46	1.24	3.05	1.38	0.85	3.09	1.30	0.80	0.00	0.40
Selenium (µg)	17.2	17.2	81.3	1.4	1.8	4.7	2.6	2.3	0.0	4.3
Vitamin C (mg)	0.0	0.0	0.0	93.8	51.5	90.6	10.4	57.0	0.0	52.6
Thiamine (B1) (mg)	0.43	0.08	0.34	0.38	0.23	1.38	0.35	0.37	0.26	0.14
Riboflavin (B2) (mg)	0.22	0.06	0.14	0.14	0.13	0.56	0.26	0.10	0.15	0.14
Niacin (B3) (mg)	4.03	1.82	6.28	5.00	2.13	5.16	2.43	1.83	3.22	1.97
Folate Total (B9) (µg)	21	9	44	76	68	516	48	77	0	63
Vitamin A	238	0	10	10	33	563	4178	460	0	3220
Vitamin E, alpha-tocopherol (mg)	0.54	0.13	1.16	0.05	0.48	0.00	1.13	1.30	0.00	0.40
Vitamin K1 (µg)	0.3	0.1	2.2	9.0	4.8	0.0	7.8	8.7	0.0	2.0
Beta-carotene (µg)	108	0	6	5	20	0	36996	277	0	1306
Saturated fatty acids (g)	0.74	0.20	0.30	0.14	0.18	2.47	0.09	0.13	0.51	0.40
Monounsaturated fatty acids (g)	1.39	0.24	0.23	0.00	0.20	4.00	0.00	0.03	1.09	0.09
Polyunsaturated fatty acids (g)	2.40	0.20	0.72	0.19	0.13	10.00	0.04	0.27	1.51	0.20

Source:[29]

CRISPR/Cas9 system is still afflicted by these problems, it will surely revolutionize and solve the bulk of them.

Cassava improvement, either through conventional breeding or through molecular/genome alteration biotechnologies, is challenging. The major limitation is a lack of appropriate genetic data to address the sequence specificities for various genome editing tools. Therefore, the creation of a "genome editing" database including experimental references and *in-silico* model organism prediction data could be very useful. In summary, with the utilization of this set of genome-editing techniques, some breakthroughs in cassava improvement, such as: biofortification, removal of toxin, improving starch quality, combating diseases, improving yield, and minimizing postharvest loss, have been reported. There is substantial potential for more novel discoveries to improve the cassava crop and promote its global cultivation and consumption.

Improving cassava nutrient content (biofortification)

The protein content of cassava roots is poor compared to other common staple crops globally (Table 1). Its protein content is approximately 3.5 g/100 g of dry weight on average, compared to 10.4 g for maize grain [6,29]. In general, a 500 g cassava meal provides only 30% of the daily protein requirement and processing further reduces its protein content [2,6]. Consequently, people who eat cassava exclusively suffer from protein deficiency symptoms, such as Kwashiorkor, stunted growth in children, amongst others [13,30]. Identified targets for cassava's nutrient improvement include biofortification of cassava with protein, fat, essential minerals, and vitamins.

Cassava biofortification via biotechnological techniques

A multidisciplinary review from agricultural experts, nutritionists, public health experts, consumers, and breeders, is the first step in the biofortification process [31]. Plant breeders must be able to produce traits that meet nutritional needs and customer preferences (taste, color, and cooking time) [32]. Critical nutritional deficiencies in cassava diets include: protein, fat, zinc, iron, vitamins A, and vitamin B6 (Table 1). However, efforts are being made to increase them in transgenic cultivars [17,33,34].

One of the first attempts to store proteins in cassava roots, for example, was the synthesis of artificial storage proteins and their accumulation in the cytoplasm. The aspartic proteinase (*ASP1*) gene, which is an artificial storage protein and the CaMV 35S promoter were used to control transgenic protein expression in leaves and roots [35] (Figure 4(a)). Similarly, Telengech et al. [36] created transgenic beta-carotene cassava roots by the

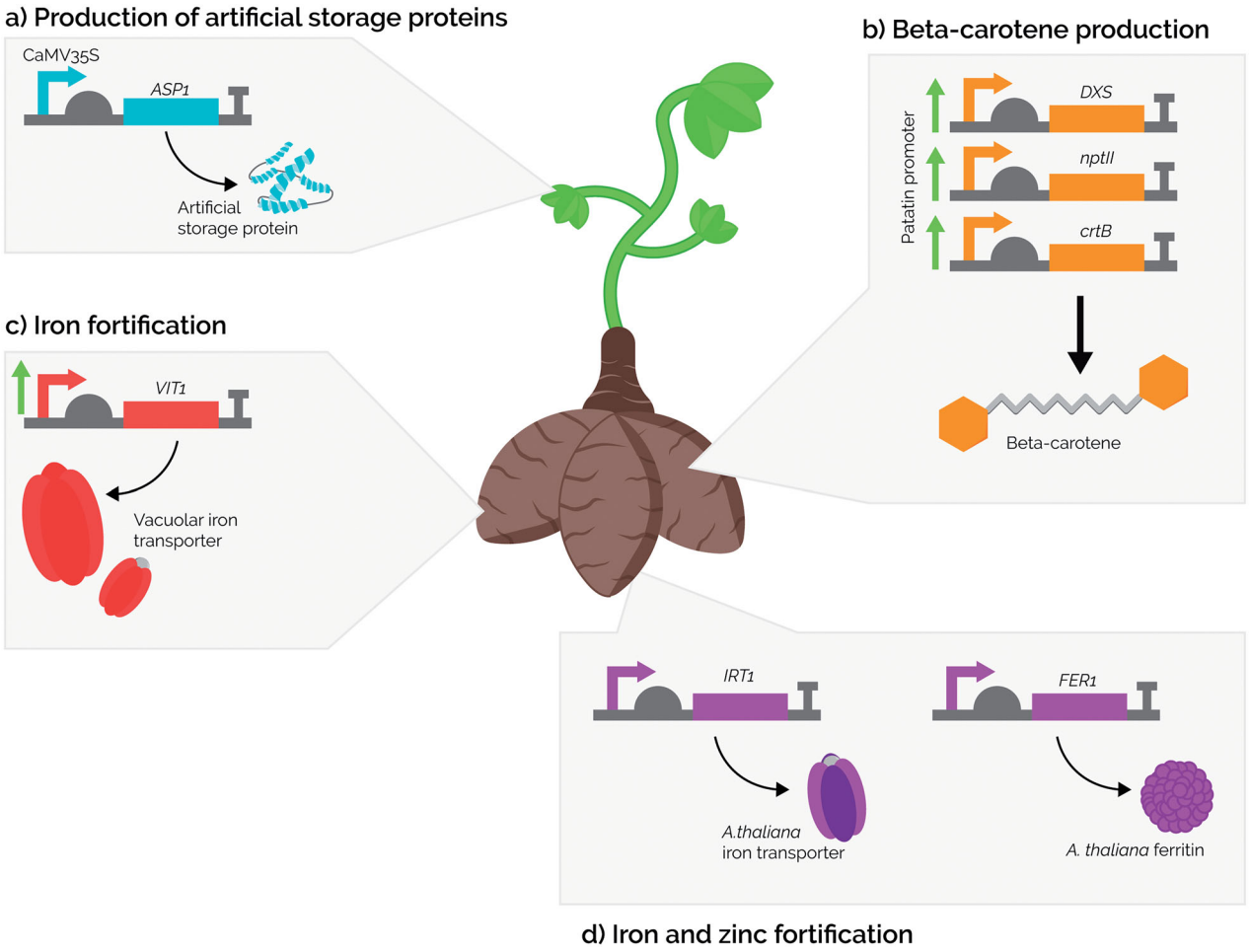

Figure 4. Biotechnology approaches to cassava biofortification. (a) Production of artificial storage proteins. The production of proteins with a high level of essential amino acids is one of the main objectives of the biofortification approach. To complete this objective transgenic cassava that produces ASP1 was created. (b) Beta-carotene production. Another approach is the increase of beta-carotene concentration in the root. For this DXS, nptII and crtB were overexpress using a root-specific promoter. (c) Iron fortification. For the Iron fortification, the gene VIT1 was overexpressed. This gene encodes to a vacuolar iron transporter, generating the accumulation of iron in cassava storage roots.

overexpression of genes, 1-deoxy-D-xylulose-5-phosphate synthase (*DXS*), and bacterial phytoene synthase (*crtB)* in cassava leaves (Figure 4(b)). Beyene et al. [37], also quantified β-carotene concentrations in biofortified transgenic cassava roots, which were improved by expressing transgenes such as bacterial phytoene synthase (*crtB*) that were upregulated by the *patatin*-type 1 promoter CYP and *DXS*. Harvested biofortified cassava roots had a 15- to 20-fold increase in carotenoids, reaching up to 50 g/g DW compared to wild-type cassava roots.

As mentioned previously, cassava yields are limited by several diseases. Narayanan et al. [38] have recently developed a cassava cultivar with high levels of disease resistance to these viruses as well as biofortified levels of iron and zinc to improve consumer health. In restricted field trials in Puerto Rico, transgenic cassava roots with overexpression of the *Arabidopsis thaliana* vacuolar iron transporter VIT1 accumulated three to seven times more iron than non-transgenic controls (Figure 4(c)).

While plants co-expressing a mutant *A. thaliana* iron transporter (IRT1) and *A. thaliana* ferritin (FER1) in the field gathered 7–18 times more iron and 3–10 times more zinc than non-transgenic controls. The fortification was unaffected by storage root yield or growth.

Elimination of hydrogen-cyanide content in cassava

The hydrolyzation of two cyanogenic glycosides, "Linamarin" and "Lotaustralin" present in cassava, creates "hydrogen cyanide" (Figure 5(a)), which is toxic to parasites, herbivores, and humans, causing muscle and

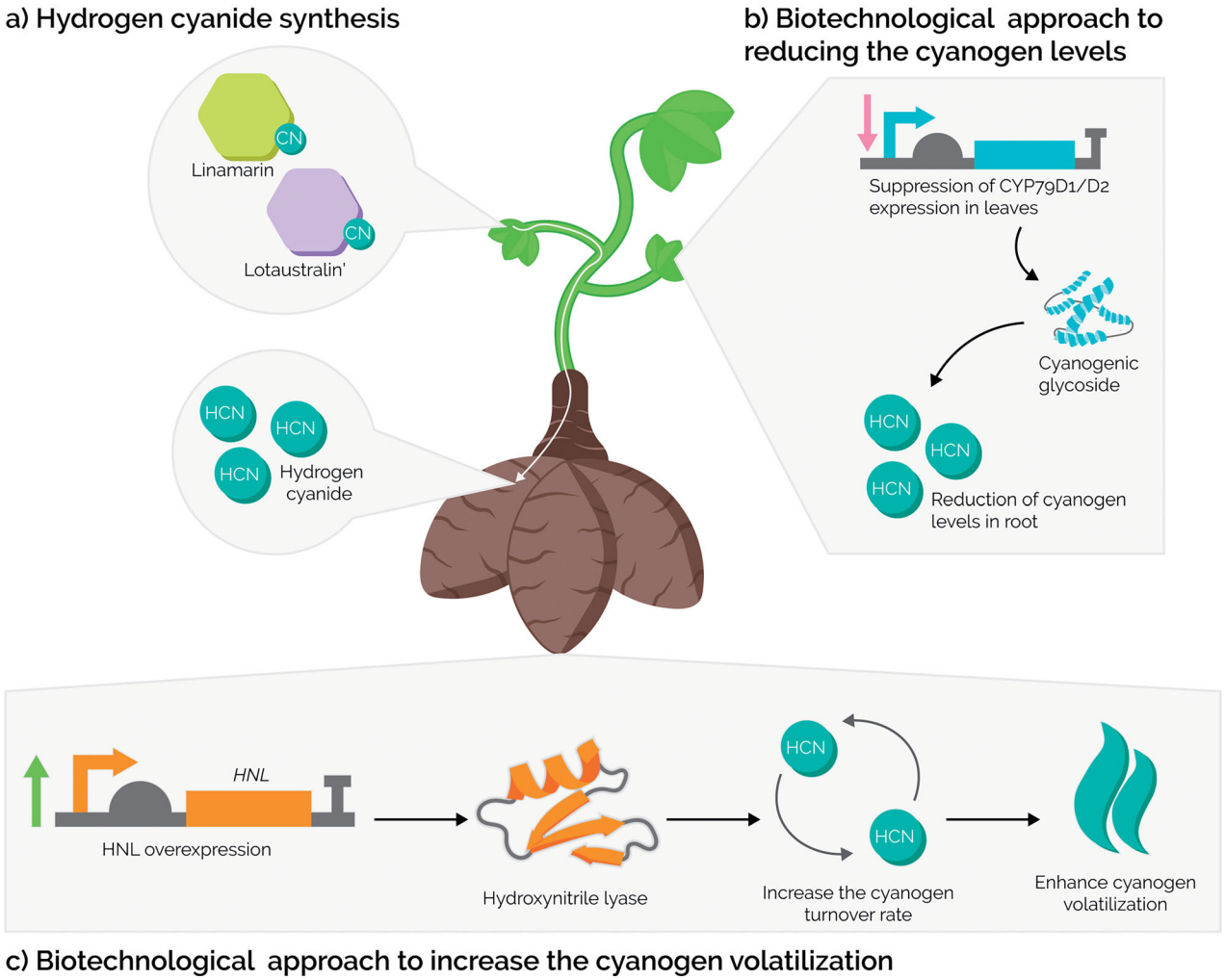

Figure 5. Biotechnology approach to hydrogen-cyanide elimination. (a) Hydrogen-cyanide synthesis. The hydrogen cyanide is synthesized through the degradation of linamarin and lotaustralin. (b) Biotechnological approach to reducing the cyanogen levels. The expression of CYP79D1/D2 in leaves was suppressed. These genes codified to the cyanogenic glycoside, which has an important role in linamarin degradation. The suppression of this enzyme caused a reduction of cyanogen levels in the roots. (c) Biotechnological approach to increase cyanogen volatilization. Another approach to reducing the cyanide levels is the enhance cyanogen volatilization. For this, NHL was overexpressed. The NHL codified hydroxynitrile lyase that increases the cyanogen turnover rate and the cyanogen volatilization.

brain paralysis [39,40]. This toxicity is a significant factor limiting cassava's use as food or feed [7]. Cassava cultivars are categorized as sweet or sour depending on the amounts of cyanogenic glucosides present. The sweet cultivars can produce as low as 20 mg of cyanide per kilogram of fresh roots, whereas bitter cultivars can produce approximately 50 times more (1 g/kg), of which Linamarin accounts for more than 80% of the total cyanogenic content [39,41]. The amount of cyanogenic glycosides in cassava is determined by several factors: poor soil fertility, water availability, high nitrogen fertilization, and amongst others [42]. However, chronic cassava cyanide toxicity symptoms are tropical ataxic neuropathy, goiter, cretinism, retarded growth, and sometimes death. They are prevalent in some African regions, where poorly processed cassava products are highly consumed, and their protein intake is very minimal [2,39]. As a result, many conventional processing methods (fermentation, cooking, drying, steaming, baking, and frying) have been employed to minimize cyanide levels in cassava products [7,43]. The main areas identified for cassava's toxins elimination are (1) development of cyanogen-free cassava cultivars and (2) development of rapid screening methods for the cyanogenic glucoside content of cassava products that are easily applicable under field conditions.

Removal of cassava's toxins via biotechnological techniques

Reduced cyanogen levels in cassava foods must be achieved before being considered safe for consumption [7]. Conventional processing techniques such as

Figure 6. Cassava diseases. (a) Symptoms of cassava mosaic disease, cassava brown streak disease on cassava farm. (b) Infected plants showing severe stunting, distortion of leaves, and twisted leaflets with mosaic and mottling symptoms. (c) Symptoms of whiteflies and mealybugs.

cooking or fermentation can help to achieve this goal but they have a negative effect regarding the nutrient value. So far, two strategies have been developed to reduce cyanogen toxicity in food [44]. One of these is the generation of cyanogen-free cassava plants using conventional breeding. The selective suppression of *CYP79D1/D2* (valine N-monooxygenase 1-like) gene expression was used to create cyanogen-free cultivars in 2003 (Figure 5(b)). The tissue-specific suppression of CYP79D1/D2 expression in leaves resulted in a 99% reduction in root cyanogen levels, demonstrating that linamarin, a cyanogenic glycoside, is produced in leaves and delivered to roots [45]. The other is the development of transgenic cassava plants that speed up cyanogen turnover and volatilization during food processing. The enzyme hydroxynitrile lyase (HNL), which catalyzes the final step in cyanogenesis, was overexpressed in roots to create cultivars that enhance fast cyanide volatilization (Figure 5(c)). The result indicated that increased HNL activity resulted in a three-fold increase in the cyanogen turnover rate [46]. The cytochrome P450 genes CYP79D1 and CYP79D2, whose protein products accelerate the initial stage in cyanogenic glucoside biosynthesis, were disrupted using CRISPR-mediated mutagenesis to reduce cyanide levels in cassava. Cassava accession 60444 and the West African farmer-preferred cultivar TME 419 had cyanide in their leaves and store roots after knocking out both genes. Although CYP79D2 deletion resulted in a considerable reduction in cyanide, mutation of CYP79D1 did not, showing that the functions of these paralogs have separated [26].

Furthermore, the development of a rapid cyanide content screening protocol is essential in managing this challenge [7]. Once the mRNAs that regulate the development of key enzymes have been isolated, DNA probes can be established to test cassava cultivars for their glucoside content [41]. Although this is ongoing research, it is not readily available or easily applicable in the field. Yet, DNA probes could provide a fast and sensitive cyanogenic detection in cassava before and after processing [47].

Improving cassava starch quality

Cassava is a source of dietary carbohydrate for an estimated 800 million people worldwide due to its high starch content amongst other benefits [48]. Cassava roots have a higher dry matter content (30−40%) than other roots like yam, potato, etc. [49]. Cassava is also suitable for the production of both industrial starch and bioethanol, but its fundamental properties, as well as its resilience under different stress conditions, are poorly understood [50]. The identified main area for improvement is the development of cassava varieties with better starch quality.

Improving cassava starch quality via biotechnological techniques

To improve starch efficiency, researchers isolated and characterized cassava's gene homologs involved in processes such as assimilated carbon conversion to sucrose in photosynthetic cells, sucrose transportation through the phloem to storage organs, sucrose conversion to starch, and starch degradation into simple sugars [50–52]. By modifying gene activities through gene-editing techniques, the molecular and functional characterization of the genes involved in these processes can significantly increase cassava's starch content [51–53].

Furthermore, advances in the knowledge of starch biosynthesis and the isolation of particular genes involved have made it possible to genetically modify cassava roots to produce more and better quality starch

[54,55]. Lastly, Zhou et al. [53] reported the development of transgenic cassava with starches containing up to 50% amylose due to the constitutive expression of hairpin dsRNAs targeting the 1,4-alpha-glucan-branching enzyme (*be1*) genes. Using the CRISPR/Cas9 technology to construct MESSIII-1 and MESSIII-2 mutants obtained from MESSIII genes of the cassava crop, researchers were able to produce cassava with edited genes related to the starch synthesis pathway [56]. The involvement of genes in the control of amylopectin glucan production in cassava was investigated as a result of this study. Bull et al. [57] demonstrated that CRISPR/Cas9-mediated targeted mutation of the two amylose synthesis genes, PTST1 and GBSS, can diminish or delete amylose from root starch.

Combating diseases in cassava

Although cassava is the fourth largest source of calories in the world, it is subject to yield losses due to both abiotic (temperature, climate change, etc.) and biotic (pest and pathogens) stress [58]. The biotic stresses include bacterial, viral diseases – CMD, CBSD [16,59–61] and pests (whiteflies and mealybugs) being the most important stresses, causing production losses and yield reduction [9,62] as shown in Figure 6. The pests and pathogens involved in transmitting these diseases usually knock down their host using an arsenal of weaponry; cell wall degrading enzymes, effectors, and oxalic acid during pathogenicity [63].

The following are the primary areas for development that have been identified:

- Improvements in resistance to multiple biotic stress cultivars.
- Biological management systems for large pests should be developed.
- Virus and bacterial disease screening approaches should be improved.
- Fundamental research on resistance mechanisms should be expanded.

Improving cassava's disease tolerance via biotechnological techniques

Molecular methods and next-generation sequencing have significantly advanced our understanding of cassava virus diversity and genome molecular functions, especially within the past decade [9,64]. The two most prominent biotic restrictions influencing cassava production in Southern Africa are CMD and CBSD. The most effective and realistic strategy to reduce losses for African farmers is to deploy cassava cultivars that are disease resistant. Hence, to develop dually resistant F1 progenies, researchers crossed the Tanzanian local cassava variety Namikonga (CBSD resistant/CMD susceptible) with an introduced cassava germplasm AR37-80 (CBSD susceptible/CMD resistant) from South America. They were evaluated for two seasons at Naliendele in Southern Tanzania, a CMD and CBSD hotspot area. CMD-resistant progenies had foliar severities similar to the CMD-resistant parent (1.8 on a five-point scale). CBSD resistant progenies exhibited minimal foliar severity (2.0) and root necrosis (1.2), which were identical to the CBSD resistant parent, but CBSD tolerant progenies had significant foliar severity (up to 3.3) but minor root severity (1.2). The progenies *Namar 050* and *Namar 371* showed high root weights of 27.5 t/ha and 28.2 t/ha, respectively, with substantial genetic gains of 56.1 percent and 58.5 percent. *Namar 050*, *Namar 100*, *Namar 130*, Namar 200, Namar 334, Namar 371, and Namar 479 were discovered as dual resistance progenies with minimum CMD and CBSD symptoms severity (2.0) and could be used to create superior cassava varieties [65].

Furthermore, the capacity of a transgene-derived RNA hairpin is identical to an overlapping region of the CMD replication-related protein and probable virus suppressor of silence protein (AC1/AC4) to provide tolerance in the CMD-susceptible model cassava cultivar 60444 was reported. Compared to untransformed control plants, three of the fourteen transgenic lines expressing CMD AC1/AC4 hairpin-derived siRNAs displayed lower symptoms and viral loads. The expression of CMD AC1/AC4 homologous siRNAs revealed that this resistance is likely linked to the virus's post-transcriptional gene silencing [15].

By inducing RNA silencing, the expression of double-stranded RNA (dsRNA) homologous to viral sequences can effectively interfere with RNA virus infection in plant cells [17]. This method was used to combat the ACMV in its natural host, cassava. siRNA from a CaMV 35S promoter-controlled, intron-containing dsRNA homologous to the common region containing the bidirectional promoter of ACMV DNA-A were expressed in transgenic cassava plants. Accelerated plant recovery following ACMV-NOg infection was found in two of three independent transgenic lines, which correlates with the presence of transgene-derived siRNAs 21–24 nt in length [17]. Overall, the symptoms of CMD were significantly reduced in these two lines, and viral DNA build-up in their leaves was significantly lower than in wild-type plants' leaves. The build-up of viral single-stranded DNA was dramatically decreased in a transient replication assay employing leaf disks from the two transgenic lines. It was recorded that in transgenic plants expressing dsRNA cognate to the viral

promoter and common area, a natural RNA silencing mechanism targeting DNA viruses *via* the synthesis of virus-derived siRNAs is turned on sooner and more efficiently [66].

Furthermore, Liu et al. [67], in collaboration with Allie et al. [61], investigated the interaction of cassava geminivirus and host plants using high-throughput sequencing. Several photosynthesis-related genes are down-regulated in virus-infected cassava, resulting in chlorophyll-b and light-harvesting complex II (LHCII) deficiency [67]. It was reported that infected leaves with chlorosis had fewer grana lamellae. This research identified photosynthesis-related genes that may play a role in cassava disease growth, as well as the critical roles of chlorophyll degradation and LHCII inhibition during ACMV infection [61]. The findings not only provided a link between gene expression and the CMD phenotype for the first time, but they also allowed for further research into the molecular mechanisms of viral pathogenesis. Despite several attempts to generate disease-resistant cultivars, Gomez et al. [27] were the first to use Cas9/gRNA to employ targeted mutation. The two eIF4E isoforms, nCBP-1 and nCBP-2, were altered at the same time, resulting in heritably delayed and suppressed CBSD aerial symptoms, as well as a reduction in the severity and frequency of storage root necrosis.

Improving cassava yield capacity

Most national and international cassava research programs emphasize the production of high-yielding cassava cultivars [2]. Low yields are often caused by: weeds, pest infestation, disease pressure, poor soil, and climatic conditions [68,69]. The main area for improvement identified is the development of high-yielding cultivars for specific regional and farming conditions.

Improving cassava's yield via biotechnology

Due to the polygenic nature of cassava, improving root yield under stress conditions with biotechnological techniques is difficult [12,70]. Naconsie et al. [71] used two-dimensional gel electrophoresis and liquid chromatography-mass spectrometry to compare the proteomes of roots at different stages of development and discovered that secondary growth is needed for cassava root development. According to comparative proteomes, metabolic and regulatory processes in cassava leaves can also affect root formation [72]. Using amplified fragment length polymorphism-based transcript profiling, Sojikul et al. [73] discovered that four genes in the MeKD family were expressed in the initiation and early stages of cassava storage root development. However, more research is required to understand its mechanism of action.

Cassava flowering was further enhanced by adding the Arabidopsis FLOWERING LOCUS T gene into the genome-editing cassette, which is rare in glasshouse settings [74]. Odipio et al. [24] were able to achieve triggered flowering acceleration in cassava *via* CRISPR/Cas9-mediated disruption of Multiple TFL-like floral repressors.

Minimizing postharvest deterioration of cassava roots

Postharvest physiological degradation (PPD) occurs rapidly in harvested cassava roots [75,76]. PPD is caused by mechanical damage, which occurs most frequently during the harvesting of tuberous roots and progresses from the proximal site of damage to the distal end, rendering the roots unpalatable within 72 h [10]. Fresh cassava roots can be stored for up to 2 weeks if they are treated with fungicide and kept in perforated plastic bags [77]. While studies on storage of freshly harvested cassava roots are limited, some successful prevention methods are known including, but not limited to, good sanitary measures, such as washing and disinfecting warehouses before restocking and easily removing infested material [10,78].

The following are the identified key areas to limit the postharvest deterioration of cassava: (1) Slowing down physiological degradation processes to extend the storage time of fresh cassava roots. (2) Improved pest control methods during storage.

Minimizing cassava's postharvest deterioration via biotechnological techniques

Genetic improvement can potentially delay or inhibit PPD in cassava, as the PPD process differs among cassava genotypes [75]. It was reported that the PPDsusceptible *HMC-1* variety and PPD-tolerant experimental hybrid *AM206-5* were significantly different in PPD level and secondary metabolic synthesis [75]. A better understanding of the mechanism of PPD processes might enable the engineering of cassava storage roots with prolonged shelf-life. The major visual symptom of PPD is blue/black discoloration, which is caused by diverse enzymes (scopoletin, scopolin, esculin, and esculetin), all linked in a hydroxycoumarin pathway [79]. Based on starch catabolism, biochemical analysis revealed that acidic polysaccharides could delay PPD [77]. Hence, the co-expression of antioxidant enzymes, superoxide dismutase and catalase, in transgenic cassava results in a synergistic effect that decreases reactive oxygen species (ROS) levels meanwhile delaying cassava PPD [76].

Improvements in the susceptibility of cassava to abiotic stress

Improvements have been reported in combating natural environmental stresses such as drought, low and high temperature, and floods among others, in cassava [80–83]. Besides the use of conventional methods, sequencing and assembly of the cassava genome has paved a way for the improvement of the crop against abiotic stresses. Genes and proteins that impart or confer resistance/tolerance to single or multiple abiotic stress responses have been reported [72,82,84,85]. For example, in a study by Ou et al. [85], KUP family genes were identified as candidates for improving resistance to multiple abiotic stress in cassava. Furthermore, Fan et al. [82] reported candidate ethylene response factor (*MeERF*) genes that were identified for genetic improvement of abiotic stress in cassava. These family genes have been reported to play a major role in response to biotic and abiotic stresses in cassava. One of the largest families of plant-specific transcription factors is NAC (no apical meristem [NAM], Arabidopsis transcription activation factor [ATAF1/2], and cup-shaped cotyledon [CUC2]) proteins, which play a critical role in plant growth, development, and adaptation to the environment. In the cassava genome, 96 NAC genes (MeNACs) were identified by Hu et al. [86] and the expression of 12 NAC genes (MeNAC) was studied in response to: osmotic, salt, cold, ABA, and H_2O_2 treatments, revealing that cassava NACs may serve as an intersection for several signaling pathways including the JA-signaling pathway. These findings revealed the complexities of MeNAC gene transcriptional control and back up the idea that NACs are vital for: plant growth, development, and environmental adaptation [86].

With continued advancements in cassava biotechnology, cassava has the potential to become the most important staple crop shortly. Moreover, the economic impact of cassava and cassava-based goods, especially in industrial starch and renewable energy, will boost food security and livelihoods, making this crop a potentially lucrative source of economic growth, especially on the African continent. As a result, most agricultural research organizations in Africa, particularly Southern Africa, emphasize improving this crop.

Cassava biotechnology; success stories in Southern Africa

Over the past 15 years, cassava biotechnology in Southern Africa has exceeded timelines with the development of more than 51 publicly bred cultivars [87]. Transferring knowledge and technology to African laboratories and farmers are critical for achieving food security and long-term crop development within the region [88]. Since the first reports of transgenic cassava [89], cassava genetic transformation systems have advanced significantly from the model cultivar to the development of "improved" cultivars, especially disease-resistant cultivars [54]. Another aspect of their contribution includes biofortification potentials and addressing other socio-economic issues affecting the cassava industry in the region [35,90]. Although there is room for improvement, some contributions to cassava biotechnology research and its applications in Southern Africa are listed in Table 2.

A bottleneck for Southern African research institutes in the implementation of gene-editing technology is the difficulty in obtaining laboratory supplies, as well as the need for consistent and ample funding. Similarly, as in many African countries, the legal and regulatory frameworks needed to guide the use of this technology are still missing, obstructing the movement of plants from the laboratory to the field [69]. Furthermore, public concern about biosafety problems persist, hence, consumer acceptance and trust are crucial for the successful implementation of cassava biotechnology and the realization of its full potential.

Biosafety and associated risks; perceptions and acceptance of cassava biotechnology

Biotechnological techniques have grown over the last few decades because of their potential to improve crops such as cassava [91]. The literature on biosafety aspects of the selectable and scorable markers currently used in cassava biotechnology was surveyed to assist research planning and regulatory submission. Hence, selectable and scorable markers are essential for successful transformation technology, and they must be assessed for biosafety, performance, and cost-effectiveness [33].

Unfortunately, many risks may be associated with the introduction of transgenic crops [92,93]. Some of the identified risks include but are not limited to:

1. Invasion of new, transformed crops may bring about large ecological changes [93].
2. The introduction of genetically engineered crops may lead to genetic erosion [69].
3. Transformed crops with introduced disease and pest resistance may be more susceptible to other pests and diseases [92].
4. The introduction of improved crops in marginal areas may lead to loss of soil fertility or erosion problems [94].

Table 2. Notable contributions to cassava biotechnology in Southern Africa.

Contributions	References
1. The increased number of genome-wide studies has been attributed to the joint initiatives involving International Universities, National Agricultural Research Systems (NARS), and significant support from the donor communities. Several genes/QTLs underlying important cassava traits have been identified, and trait-linked markers, which are required for marker-assisted selection, have been created.	[85]
2. In terms of promoting cassava production and commercialization, the Southern Africa Root Crops Research Network (SARRNET), in collaboration with national research programs in the Southern Africa Development Community (SADC), has had a significant impact in the SADC region. They are actively involved in germplasm development study, which resulted in the release of 51 improved cassava cultivars. These cultivars have been distributed to farmers through accelerated plant material multiplication and distribution programs in at least 10 of the 14 SADC countries. Also, they provide funding for postgraduate/graduate studies, short-term training courses, workshops, and symposia. SARRNET has had a significant impact on skill development and knowledge in root crops research for development in the SADC region.	[89]
3. Southern African Value-Added Cassava (SAVUCA), funded by the Swiss National Research Foundation and National Research Foundation (South Africa), is involved in the genetic engineering of virus-resistant cassava and investigation of molecular determinants for natural virus resistance/tolerance in South Africa.	[88]
4. The African Center for Crop Improvement is investigating the feasibility of industrial-scale cassava production; i.e., producing200,000 seedlings from relatively 10 parent plants.	www.acci.org.za.
5. The National Working Group (NWG) is involved in evaluating and breeding cassava varieties that meet South Africa's industrial and local needs. So far, they have bred a new cassava cultivar in seven-month rather than the conventional 18 months breeding time. Unfortunately, the cultivar could not survive through the winter season. More research is still ongoing.	[85]
6. At the Agricultural Research Council (ARC), Industrial Crops Institute, the investigation into the mechanization of cassava production in the eastern part of South Africa is ongoing. They also provide training in mechanized production techniques, including the best planting techniques for mechanical harvesting.	www.arc.agric.za.
7. At the University of KwaZulu-Natal, African Center for Crop Improvement, research was conducted to enhance bulking and root carotene in cassava's earliness. Their effort to improve pests and disease resistance was reported. They combined the mosaic-resistant genes of the Ceara rubber x cassava hybrid 58,308, with genes from local and exotic cultivars with high yield, good root quality, low cyanogen, and resistance to lodging.	[85]
8. Professor Chrissie Rey and her research team at the University of the Witwatersrand School of Molecular and Cell Biology, for over 15 years, have been researching to find a solution to the crippling cassava mosaic disease (CMD). Their excellent results with promising transgenic lines currently are being tested for resistance to one of the cassava viruses in greenhouse trials. They also registered a South African and US patent for developing RNA hairpin duplexes to engineer plants for stable virus resistance, studying the biodiversity, evolution, and epidemiology of cassava begomoviruses and whitefly vectors in southern Africa.	[9,59,88,90]
9. After maize, cassava is Zambia's second most important staple food crop, and it is currently being field-tested in cassava-eating communities in the Luapula, Western, North-Western, and Northern provinces. The HarvestPlus biofortification program in collaboration with the Consultative Group on International Agricultural Research (CGIAR) centers, aimed to make staple crops, including cassava, rich in vitamin A, iron, and zinc. The sole goal of this project is to grow micronutrient-rich crops through breeding and distribution in developing countries where micronutrient malnutrition is a major problem	[34,85]
10. South Africa's efforts to kick-start the African Center had supported large-scale cassava production for Crop Improvement (ACCI). Since the ACCI began training plant breeders in 2002, at least nine of its Ph.D. graduates have worked on breeding improved cassava varieties and a variety of these improved varieties have been published in their home countries in Southern Africa.	[85]
11. For the first time on the African continent, a robust cassava transformation cultivar was created. T200, a high-starch commercially grown cultivar, has higher transformation and regeneration efficiencies when compared with TMS 60444 (model cultivar).	[85]
12. The Technical Innovation Agency, the National Research Foundation, and Casquip Starch Manufacturing Pty. Ltd, the Gauteng Department of Agriculture and Rural Development (GDARD) are all funding partners for cassava development research in South Africa. They are supporting several research groups such as Prof Rey's team at the University of Wits amongst others.	[91]

5. The newly introduced information may be transmitted to related wild species, extending the risk of acquired properties to the wild plants [95,96].

To reduce these risks, careful examination of transgenic plants in the field during several cropping cycles has to occur [8,69].

Transgenic cassava, while beneficial, is linked to negative public perception and extensive domestic and international regulation. In most environmental risk assessments of genetically altered crops, the likelihood and implications of gene flow to wild relatives are taken into account. Hokanson et al. [97] utilized a problem formulation technique to analyze current data for a risk assessment of gene flow from genetically engineered for viral resistance cassava (*Manihot esculenta*) to its "wild" (naturalized) relative *M. glaziovii* in East Africa. In this research, two environmental risks were considered: (1) loss of genetic variation in the germplasm pool and (2) loss of valuable species, ecosystem resources, or crop productivity and quality due to weediness or invasiveness of wild relatives. Gene flow was expected (increase in *M. glaziovii*), however, it did not reduce the genetic diversity in the germplasm pool and this would not necessarily result in the environmental consequences reported [97]. Furthermore, the toxicity, allergenicity, pleiotropic effects, horizontal gene transfer, and their impact on food or feed safety, as well as environmental safety, were all assessed by Petersen *et al*. [98]. According to their research, selectable marker genes

nptII (Neomycin phosphotransferase II), *hpt* (Hygromycin phosphotransferase), *bar/pat* (Phosphinothricin acetyltransferase), and *manA* (phosphomannose isomerase), as well as the scorable marker gene *uidA* (β-glucuronidase, GUS), all pose a low danger of biosafety, hence, they appear to be the safest cassava biotechnology solutions [98].

In Table 3, improved cassava characteristics were evaluated for their impact assessment in connection to biotechnological improvements. Assessment areas

Table 3. Acceptance, research capacity, and risk associated with biotechnologically improved cassava cultivars.

Improved areas		Impact assessment (Adoption/Research Capacity/Associated Risk)	References
Propagation	Adoption by local farmers:	Micropropagation can increase farmers' reliance on seed companies or local institutes.	[85,97]
	Local research and extension capacity:	A simple plant tissue laboratory, as well as a modest infrastructural and research capability, would suffice for the application of micropropagation. A medium-level infrastructure and research capability are necessary for the development of true seeds.	[97,98]
	Associated risks:	There are possibilities that the progeny plants may be susceptible to other diseases, hence, resulting in a lack of overall disease resilience.	[99]
Germplasm	Adoption by local farmers:	Although farmers have no control over germplasm storage at institutes, however, the adoption by local farmers is poor due to the cost and accessibility.	[68,100]
	Local research and extension capacity:	Maintaining a germplasm bank necessitates a moderate level of research capability. Cryopreservation would necessitate a well-developed infrastructure and medium-level study.	[101]
	Associated risks:	Plant genetic resources are collected, kept, and distributed by research institutes globally, however, when germplasm is moved, the risk of accidentally introducing plant pests along with the host plant is inescapable.	[102]
Drought-tolerant cultivar	Adoption by local farmers:	Farmers in drought-prone areas can readily adopt cassava varieties that give good yield stability under drought conditions or are early maturing.	[8,93]
	Local research and extension capacity:	Fundamental studies on the genetic characterization of drought tolerance in cassava would necessitate a significant amount of time and resources.	[76,103]
	Associated risks	Drought-tolerant cassava varieties can lead to an increase in cassava cultivation in drought-prone areas, especially on soils prone to erosion. As a result, anti-erosion control should be given special attention.	[76,104,105]
Disease-resistant cassava cultivar	Adoption by local farmers:	Small and large farmers would benefit from the production of disease-resistant varieties and improved screening methods. Improved screening methods could lead to the rapid distribution of disease-free material, allowing small farmers to use improved varieties on a larger scale.	[13,92]
	Local research and extension capacity:	Fundamental studies on the detection of resistance genes and the production of improved screening methods need high-grade research and capital inputs. When standard transformation or screening procedures are available, less advanced laboratories and medium-level research and development may benefit.	[65,106]
	Associated risks:	The dangers of introducing transformed cassava plants into the natural world have yet to be assessed. It is essential to assess the risk of vertical resistance (e.g., cross protein resistance), which is easily broken. Priority should be given to multilevel resistances.	[13,58]
Pest control	Adoption by local farmers:	The cost and availability might discourage farmers' acceptance.	[60]
	Local research and extension capacity:	It is also difficult to evaluate research and infrastructure needs.	[3]
	Associated risks:	This form of vertical resistance poses a significant risk of being quickly broken down.	[9,67]
Yield capacity	Adoption by local farmers:	Cassava varieties with a decent yield, yield stability, and secondary qualities would be adopted and easily implemented in all farming systems.	[2,95]
	Local research and extension capacity:	On-going research	[95]
	Associated risks:	Increased cultivation in marginal areas caused by the introduction of varieties with high yield ability under stress conditions can hasten soil erosion.	[95]
Cassava root quality	Adoption by local farmers:	While low cyanogenic cultivars are often preferred for human health reasons, farmers who grow cassava as a food crop can find it challenging to adopt acyanogenic cassava varieties.	[2,52]
	Local research and extension capacity:	Cassava starch consistency and protein content could be improved using recombinant DNA techniques. In conjunction with fundamental research, the application of these techniques necessitates a high level of research and infrastructure growth.	[35,38,53]
	Associated risks:	Cyanogenic cassava varieties may be more susceptible to pests and diseases. Also, the safe level of exposure to cyanogenic glycosides is set at 10 ppm.	[39,69]
Postharvest storage	Adoption by local farmers:	Farmers who produce fresh cassava for the local market will accept the introduction of new varieties that can be stored for longer periods.	[74]
	Local research and extension capacity:	More fundamental knowledge about root degradation is needed before biotechnology can be implemented. This necessitates a significant amount of study and infrastructure.	[73]
	Associated risks:	Postharvest produce is at significant risk for over-ripening, decay, deterioration, pathogen attack, and physiological abnormalities due to a burst in 'stress ethylene' synthesis.	[107]

include (1) adoption by local farmers, (2) local research and extension capacity, and (3) associated risks.

Concluding remarks: way forward and challenges ahead

Cassava is an important food and industrial crop with much potential for reducing hunger and poverty by creating jobs (directly and indirectly). Although Africa is the largest producer of this remarkable crop, it is mainly consumed locally, with limited exportation due to physiochemical, biological and socio-economic limiting factors mostly discussed in this article. Cassava transgenics have been created to withstand both biotic and abiotic stressors. In addition, cassava that has been biofortified with vitamins and vital minerals has been developed. However, before such transgenics are introduced into the market, they must be field-tested and their safety and bioefficacy assessed.

In the future, the transfer of this biotechnological knowledge and expertise to other Southern African countries would face significant technological and infrastructural obstacles. However, this manuscript embodies the different strategies and current state of the literature toward developing endogenous transgenic technology capacity in these nations as it serves as a critical starting point for the application of transgenic technologies to address cassava production constraints, such as: disease resistance, starch modification, nutritional enhancement, and non-food genetically modified cassava for biofuel and biodiesel production. Lastly, governments of Southern Africa countries should make policy decisions to make transgenic cassava available to farmers and the general population to reduce malnutrition, hunger, and poverty in their region.

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