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Chapter 9

Recent Biotechnological Advances in the Improvement of Cassava

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http://dx.doi.org/10.5772/intechopen.70758

Abstract

Cassava (*Manihot esculenta* Crantz) is the fourth most important source of carbohydrates for human consumption in the tropics and thus occupies a uniquely important position as a food security crop for smallholder farmers. Consequently, cassava improvement is of high priority to most national agricultural research institutions in the tropics. With advances in functional genomics and genome editing approaches in this post genomics era, there are unprecedented opportunities and potential to accelerate the improvement of this important crop. These new technologies will need to be directed toward addressing major cassava production constraints, notably virus resistance, protein content, tolerance to drought and reduction of hydrogen cyanide content. Here, we discuss the important role novel functional genomics and genome editing technologies have and will continue to play in cassava improvement efforts. These approaches, including artificial miRNA (amiRNA), trans-acting small interfering RNA (tasiRNA), clustered regularly interspaced short palindromic repeat (CRISPR)-associated protein 9 (Cas9), and Targeting Induced Local Lesions IN Genomes (TILLING), have been shown to be effective in addressing major crop production constraints. In addition to reviewing specific applications of these technologies in cassava improvement, this chapter discusses specific examples being deployed in the amelioration of cassava or of other crops that could be applied to cassava in future.

Keywords: amiRNA, cassava, CRISPR, genetic engineering, tasiRNA, TILLING

1. Introduction

Cassava, *Manihot esculenta* Crantz, was transported to Africa by the Portuguese in the sixteenth century, and was initially grown in and around trading posts in the Gulf of Guinea in West Africa; it was subsequently introduced into East Africa from Madagascar in the later part of

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the eighteenth century [1]. Today, cassava is a staple food to an estimated 800 million people worldwide [2] and is grown almost exclusively by smallholder farmers (**Figure 1**) and in isolated areas where soils are poor and rainfall is low or unpredictable. Additional attributes of this crop include low-cost and readily planting material, tolerance to acid soils, forms a symbiotic association with soil fungi to help its roots absorb phosphorus and micronutrients. Thus, cassava production requires very low input and gives reasonable harvests where other crops would fail [2]. Cassava is also increasingly being adopted as a source of family income following the fall of coffee and cocoa in the world market. Consequently, improvement of this crop is of high priority to most national agricultural research institutions in Africa. Moreover, the recognition that cassava industrial starch-based products, especially in renewable energy, could enhance food security and livelihoods, makes this crop a potentially valuable source of economic growth on the African continent.



Figure 1. Africa produces more cassava than any other crop. (A) Cassava is a woody shrub that grows well in marginal lands; (B) a family in Southern Cameroons transporting cassava tuberous root harvest; (C) cassava tuberous roots are processed into many food types, here a family in Southern Cameroons preparing "garri," a flour produced from cassava tuberous roots.

http://dx.doi.org/10.5772/intechopen.70758

Cassava is cultivated principally for its tuberous roots, which are a good source of energy; additionally, in some parts of central Africa, leaves are also consumed as a source of protein, vitamins, and minerals. Cassava roots and leaves are deficient in sulfur-containing amino acids (methionine and cysteine) and some nutrients are not optimally distributed within the plant [3], leading to a deficiency in protein content, especially in roots. Cassava also contains antinutrients, most notably cyanogens, that can interfere with nutrient absorption and utilization and may have toxic side effects [4]. There are efforts to add nutritional value to cassava (biofortification) by increasing the contents of protein, minerals, starch, and β-carotene through biotechnological approaches [3]. Thus, nutritional content and production of cassava will benefit greatly from advances in genomics and biotechnological approaches.

Cassava improvement, either through conventional breeding or through genetic engineering, is challenging. The most reliable regeneration system cassava so far is through somatic embryogenesis (**Figure 2**) [5]. In the case of conventional breeding, which so far is the most routinely used approach to improve this crop is challenging due to several factors associated with several factors, include: (1) Lack of useful genes in the core cassava germplasm collections; (2) Heterozygosity and allopolyploidy of the cassava genomes; (3) Irregular flowering;

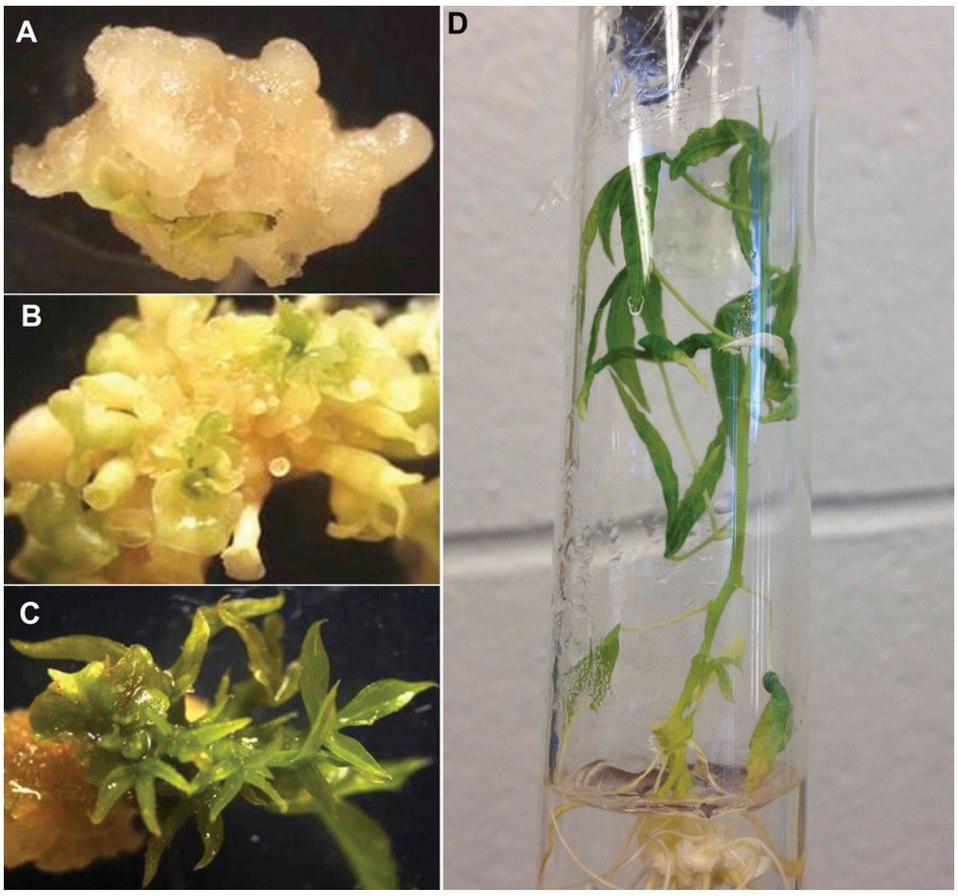


Figure 2. Regeneration of cassava cultivars from Cameroon [5]. Callus with proembryogenic masses (A); clusters of organized embryogenic structures consisting of globular, heart and torpedo structures, early cotyledonary stage, asynchronous development of somatic embryos (B); organogenic callus with green cotyledons developed clusters of shoot buds (C); shoot buds rooted and developed into whole plantlets in vitro (D).

and (4) Low fertility, seed set, and germination rates. As for genetic engineering and gene transfer, over the past few decades, this approach has been used to complement conventional breeding [6]. Undoubtedly, advances in modern technologies such as transcriptomics, proteomics, and metabolomics are likely to benefit breeding and genetic engineering strategies from an understanding of plant metabolic pathways and the role of key genes associated with their regulation. In this chapter, we identify some of the most important nutritional characteristics of cassava and production constraints that can benefit from advances in genome editing and functional genomics approaches are discussed in this section.

2. Important characteristics of cassava requiring improvement

2.1. Protein content

Cassava tuberous roots have relatively low protein content, which on the average ranges from 2 to 3% dry weight [7], compared with 9–11% for maize grain [8]. Indeed, a 500-g cassava meal provides only 30% of the daily protein requirement. Added to the low protein content, is the fact that roots are processed and the processed product is essentially protein-free. Consequently, individuals consuming exclusively or predominantly cassava usually suffer from protein-deficiency symptoms [9]. There is evidence suggesting that protein content in the roots can be considerably higher (6–8%) in some landraces [7] and such an important attribute can be introgressed into cassava through classical breeding. However, as indicated above, cassava breeding is rather challenging. Thus, fortification via genetic engineering is a more feasible option in efforts aimed at improving cassava protein content. For example, cyanide derived from linamarin is a major cause of reduced nitrogen for cassava root protein synthesis, thus disruption of linamarin transport from leaves to the roots through gene silencing-mediated inhibition of the two cytochrome P450s genes, CYP79D1/D2, resulted in an increase in nitrogen levels in cassava roots and higher levels of root protein content [10]. There have also been attempts to increase protein root content by producing transgenic cassava expressing genes that enhance protein root accumulation, including an artificial storage protein gene, *ASP1* [11]. Thus, advances in genomics and transcriptomics will undoubtedly identify genes and pathways that will provide new opportunities to increase protein content using genetic engineering approaches.

2.2. Hydrogen cyanide content

Consumption of residual cyanogens (linamarin and lotaustralin) in incompletely processed cassava roots can cause various health disorders that render a person unsteady and uncoordinated [12]. Hydroxynitrile lyase (HNL) catalyzes the conversion of acetone cyanohydrin to cyanide and is expressed predominantly in the cell walls and laticifers of leaves, compared with tuberous roots, which exhibit very low [10]. Transgenic cassava over-expressing HNL was shown to display significantly reduced acetone cyanohydrin levels and exhibited increased cyanide volatilization in processed or homogenized roots [12]. It has been shown

that the genomic region surrounding the cytochrome P450, CYP79D3, contains all genes required for cyanogenic glucoside biosynthesis in cassava [13]. As indicated above, this provides an additional opportunity to reduce cyanogen content in cassava by for example tissue specific suppression of two P450 genes, CYP79D1/D2, that catalyze the first-dedicated step in cyanogen synthesis [14]. Thus, at the molecular level, cyanogen detoxification can either be achieved by gene overexpression or through gene suppression, either of which can be achieved through genome editing techniques.

2.3. Starch quality

Due to its high starch content, cassava provides a source of dietary carbohydrate to an estimated 800 million people worldwide [2]. Insight in cassava development and starch biosynthesis is necessary to improve cassava starch quality and quantity [15]. Isolation and characterization of cassava gene homologs implicated in processes affecting the conversion of assimilated carbon to sucrose in photosynthetic cells, the phloem transport of sucrose to storage organs, the transition of sucrose to starch, and the degradation of starch into simple sugars, could be exploited to improve starch quality. Molecular and functional characterization of the genes involved in these processes will greatly enhance cassava varietal improvement by altering the gene activities either via genetic manipulation or through gene editing. Also, application of advanced systemic-based computational techniques to understand the physiological regulation and control of starch metabolism in plant plastids would be the basis for understanding these processes in cassava.

Cassava is also a good source of industrial starch and bioethanol [16, 17]; in both situations, the quality of starch is important. Starch consists of two glucan polymers: amylose and amylopectin. Amylopectin is extremely soluble in water whereas amylose has a strong tendency to recrystallize after dispersion in water, a property referred to as retrogradation. Retrogradation is undesirable for many applications of starch in which a defined and stable viscosity is required. Therefore, for industrial purposes, starch is often treated with chemicals in order to make the amylose less sensitive to crystallization [18]. As retrogradation is caused mainly by the amylose fraction in starch, amylose-free starches do not have to be treated with chemicals [19]. There are therefore efforts to generate amylose-free cassava through genetic engineering; for example, starch-free cassava was obtained by silencing *GBSSI*, the granule-bound starch synthase gene, which is required for the synthesis of amylose [20].

2.4. Postharvest physiological deterioration and storage

Harvested cassava tuberous roots undergo rapid postharvest physiological deterioration (PPD) [21, 22]. PPD is initiated by mechanical damage, which typically occurs during tuberous roots harvesting and progresses from the proximal site of damage to the distal end, making the roots unpalatable within 72 h [22, 23]. Reactive oxygen species (ROS) production has been identified as one of the earliest events in PPD [21, 22, 24]. Under conditions of stress, the equilibrium between the production and scavenging of ROS is

disturbed, resulting in a rapid increase in the buildup of ROS known as an oxidative burst [25]. In cassava roots, an oxidative burst occurs within 15 min of harvest [21], resulting therefore in an early PPD. Other early events that result in rapid PPD include, increased activity of enzymes that modulate ROS levels, such as catalase, peroxidase, and superoxide dismutase [22]. Further evidence in support of a role of oxidative stress in PPD comes from the observation that cassava cultivars that have high levels of b-carotene (which quenches ROS) are less susceptible to PPD [26]. Reduction of ROS and PPD was also shown to be induced by cyanogenesis, suggesting that a possible solution to cassava PPD is to reduce the cyanide-induced accumulation of ROS.

2.5. Cassava pathogens

Cassava viruses constitute a major challenge to cassava production; of particular importance are cassava mosaic geminiviruses (CMGs) (Family, *Geminiviridae*: Genus, *Begomovirus*), which cause the cassava mosaic disease (CMD) in all cassava growing regions of Africa and the Indian subcontinent. CMGs are transmitted by the whitefly vector, *Bemisia tabaci* (Gennadius) and through cuttings used routinely for vegetative propagation. Tuberous root losses due to CMD range from 20 to 100% [27]. With the emergence of new molecular and sequencing capabilities, CMGs have been shown to exhibit considerable sequence and biological differences and so far, 11 species have been described in the cassava growing regions of African and the Indian subcontinent [28] and some of these viruses co-infect the same plant resulting in a synergistic interaction, characterized by severe symptoms (**Figure 3**). Interestingly, cassava was introduced in Africa from South America [29], yet CMGs are not found in South America and therefore these viruses are likely recent descendants of geminiviruses adapted to indigenous uncultivated African plant species [30]. The problem of CMGs has been compounded by the emergence, in eastern Africa, of cassava brown streak disease (CBSD), which is caused by cassava brown streak viruses (CBSVs (Family, *Potyviridae*: Genus, *Ipomovirus*). Like CMGs, CBSVs are transmitted by the whitefly vector [31] and through infected stem propagules. For a long time, CBSD was considered to be limited to lowland coastal regions of Tanzania, and to a limited extent in lowland areas of Uganda [32], northwestern Tanzania, southern Uganda [32–34]. Since 2004, however, the CBSD epidemic has spread around the Great Lakes Region to affect eastern Uganda, western Kenya, the Lake Zone of Tanzania, Rwanda, Burundi and the DRC [35, 36]. The most damaging symptoms of CBSD are found in tuberous roots, including brown, corky necrosis of the starchy tissue, occasional radial constrictions and a reduction in the content of starch and cyanide [32, 34]. Yield losses are estimated to be up to 70% in highly susceptible cultivars [37]. In additional to viral pathogens, is cassava bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. *manihotis (Xam)*. CBB is considered to be one of the most relevant plant pathogenic bacteria because of the yield losses, estimated to be 70%, it causes in cassava [38, 39].

Use of resistant varieties has been the most effective in controlling CMD in Africa thanks to the discovery in the 1930s that some of the cassava varieties being grown were less affected by CMD than others. Thus, resistance breeding began in Ghana, Madagascar, Tanzania and



Figure 3. Cassava plant mixed infected by two cassava mosaic geminiviruses displaying severe mosaic and leaf distortion and size reduction, resulting in plant stunting.

elsewhere in Africa [40, 41]. In the last 2 decades, use of genetic engineering to produce virus resistant cassava has gained considerable attention, especially with the discovery of RNA interference pathways [42].

2.6. Tolerance to drought

In most cassava growing regions of the world, the cassava growth cycle is typically interrupted by months of drought, influencing various plant physiological processes and resulting in depressed growth, development and yield [43, 44]. Although cassava is a drought tolerant crop, there is a range of drought-tolerance levels in available germplasm. Thus, growth and productivity of genotypes with a low threshold of drought tolerance in marginal areas are constrained by severe drought stress, especially during the earlier stages of growth [45]. Indeed, experimental data suggest that root production is positively correlated with the life span of individual leaves [46] and increased leaf retention was found to increase root yield under irrigated and stressed conditions [47]. With continuous advances in genome science, there will be opportunities to enhance drought tolerance in the cassava crop. For example, Zhang et al. [46] have shown that transgenic cassava expressing *isopentenyltransferase (IPT) gene* under the control of senescence-activated promoter (SAG12), delayed leaf senescence under both greenhouse and field conditions, leading an increase in drought resistance. Also, identification of miRNA gene targets involved in post-transcriptional abiotic stress regulation could prove useful in engineering cassava for drought resistance [48].

3. RNA-based functional genomics technologies in cassava improvement

3.1. Hairpin dsRNA, co-suppression and antisense RNA silencing

The hairpin dsRNA (hpRNA), anti-sense silencing and co-suppression strategies have been extensively employed in crop improvement [49–51]. In cassava improvement, hpRNA and antisense silencing procedures have been employed mostly in virus control [52–56]. An indirect approach where the hpRNA is used to knockdown the expression of V-ATPase A, an enzyme that provides force for many transport processes, has been used to control whitefly vectors of CMGs and CBSVs [57, 58].

The antisense strategy has also been used to inactivate allergens and toxins in cassava, especially in the inhibition of hydrogen cyanide (HCN), which is the product of linamarase-mediated hydrolysis of linamarin. The presence of residual linamarin and its breakdown product (acetone cyanohydrin) in cassava-based food products has been a cause for concern because of their possible effects on human health. As discussed above, the first committed steps in linamarin biosynthesis is catalyzed by cytochrome P450 genes (CYP79D1 and CYP79D2) and therefore efforts have been made to knockdown CYP79D1 and CYP79D2 using hpRNA-mediated silencing so as to reduce HCN toxicity in cassava. Thus, transgenic cassava lines containing antisense copies of both genes exhibited almost complete absence of linamarin in tuberous roots [10]. Unfortunately, this approach could not be applied extensively as transgenic cassava lines exhibited poor tuberous root development.

In spite of the encouraging early results obtained from the use of hpRNA, co-suppression and antisense RNA silencing in crop improvement, this approaches have been tempered by several disadvantages associated with these approaches, these include poor stability of the transgene in transformed plants, dependence on the expression levels of the antisense strand, and limited penetration of the silencing signal to the appropriate target cells due to target-sequence folding (reviewed in Fondong et al. [59].

3.2. Small RNA (sRNA)-mediated silencing

The limitations of hpRNA, co-suppression and antisense RNA silencing strategies are, to a large extent overcome in sRNA strategies, including especially artificial microRNAs (amiRNAs) and trans-acting siRNA (tasiRNA). microRNAs constitute a well-studied class of sRNAs; their biogenesis starts with the transcription of long primary RNAs (pri-miRNAs) [60, 61]. miRNAs function in a homology-dependent manner against target mRNAs to typically either directly cleave at highly specific sites or to suppress translation. The amiRNA silencing technique exploits the biogenesis and function of endogenous miRNAs to silence genes in plants. In this approach, the endogenous miRNA-miRNA duplex in a native miRNA precursor is replaced with a customized sequence designed from the target gene. Upon processing, the amiRNA redirects the miRNA-induced silencing complex to silence the targeted mRNA, thereby generating a loss-of-function phenotype for the gene of interest [62–66]. The amiRNA strategy has especially been used in targeting plant viruses (reviewed in Fondong et al. [59]. However, there has been little application in cassava improvement. Indeed, to our

knowledge, the only report of use of amiRNA in cassava improvement is the replacement of miR159 precursor with amiRNAs from cassava brown streak viruses in miR159 precursor; transgenic *Nicotiana benthamiana* lines thus produced were virus resistant [67].

It is important to note that the amiRNA platform has several advantages over the hpRNA strategy, including the fact that amiRNAs are small and thus have a reduced likelihood of off-targeting and the approach can easily be multiplexed via use of polycistronic miRNA backbone. In addition, processing of miRNA is not affected by changes in temperature compared with hpRNA-derived siRNAs whose levels decrease at low temperatures [68]. Thus, it is likely that this platform will prove useful in studying cassava gene function. A major limitation of the amiRNA strategy is that the small size of the amiRNA (21nt) increases opportunities for loss of complementarity between the amiRNA and the target gene, and genes from the same family with variations may not be silenced using a single amiRNA. To reduce these risks, a multimeric amiRNA approach in which multiple amiRNAs targeting different conserved regions of the gene can be adopted as has been reported in plant virus control [69, 70].

A second class of sRNAs used in crop improvement is tasiRNAs, which are produced from noncoding *TAS* genes, which have been identified in all examined land plants. *TAS* genes differ from most other genes in that they do not code for a protein, but rather produce long noncoding RNA transcripts, which are subsequently processed into 21nt tasiRNAs. Synthesis of tasiRNA is initiated by miRNA-directed and Argonaute (AGO) protein-mediated cleavage of *TAS* transcripts, of which four (TAS1, 2, 3, 4) have been extensively studied in Arabidopsis (see reviews Allen and Howell [71] and Yoshikawa [72]. Two models of tasiRNA biogenesis, referred to as "one-hit" and "two-hit", have been described in Arabidopsis [73]. The tasiRNA strategy is very efficient, highly predictable in processing siRNAs and can easily be multiplexed to target multiple genes, especially genes from the same family; yet it remains an underutilized strategy in plant improvement. It has been used to successfully engineer resistance to plant viruses [74, 75]. In cassava virus control, transgenic *N. benthamiana* containing Arabidopsis *TAS1a* gene modified with tasiRNA from the cassava geminivirus, *East African cassava mosaic Cameroon virus* (EACMCV) exhibits strong resistance to the virus (Fondong et al., unpublished). Fifty-four tasiRNAs and fifteen possible *cis*-nat- siRNAs were identified in cassava infected with cassava bacterial blight, and many of these loci were induced or repressed in response to *Xam* infection [76]. A similar transgenic strategy using a *TAS* gene modified with tasiRNAs from *Xam* could be promising. This finding emphasizes the potential potency of this strategy in plant virus control.

4. Role of reverse genetics and gene editing techniques in cassava improvement

4.1. TILLING and EcoTILLING

TILLING is a non-recombinant reverse genetics approach used to identify novel sequence variation in genomes, with the aims of investigating gene function and/or developing useful

alleles for breeding. TILLING involves induction of mutations in the plant genome using classical mutagenesis approaches followed by traditional or high throughput deep sequencing to identify the mutations in the gene of interest [77–79]. This technique has been used in allele discovery in different plant species [80–83]. EcoTILLING, which is an adaptation of the TILLING, is used in detecting rare single nucleotide polymorphism (SNPs) or small INDELs in target genes in natural populations [84]. In EcoTILLING, mismatches formed by hybridization of different genotypes in a test panel are cleaved with CEL I, which is a mismatch-specific endonuclease from celery. A valuable application of EcoTILLING in plants is in the search for variation in disease resistance genes. There are only a few reports of the use of TILLING or EcoTILLING in cassava improvement. Of these, is the recent report of irradiation of seeds of elite cassava lines and wild *Manihot* species in an effort to broaden the genetic base of the germplasm pool so as to expand the industrial uses of cassava [85]. The study led to the discovery of small granules, which are abnormal amylose starch molecules resulting from a mutation. These small granules are ideal for industrial ethanol production due to the fact that they facilitate the activity of starch-degrading enzymes [86]. Because of the promise of the technique in cassava improvement, the International Institute of Tropical Agriculture (IITA) in Ibadan, Nigeria, is developing a TILLING protocol for discovery of important cassava traits [87].

TILLING has several advantages over other crop improvement techniques: (1) it produces a spectrum of allelic mutations that are useful for genetic analysis, (2) it is applicable to any organism, (3) mutations that are difficult to be detected by forward genetics can be revealed via TILLING since it can focus at on the gene of interest, and (4) it is a non-transgenic method, hence there are no biosafety or environmental concerns [88]. The main disadvantages of TILLING are the requirement of locus-specific polymerase chain reaction (PCR) products (difficult for gene families with very similar sequences and in polyploids) and the inability to detect mutations near simple sequence repeats (SSRs) (because of the flare caused by polymerase slippage-induced deletions) [89].

4.2. Clustered regularly interspaced short palindromic repeat (CRISPR)

As indicated above, cassava transformation and crossing are challenging and thus gene editing is potentially a method that can be used to improve the crop. The clustered regularly interspaced short palindromic repeats (CRISPRs) and associated protein (Cas) approach has recently gained wide application in gene editing. In bacteria and especially archaea, CRISPRs/Cas is a nucleic acid-based adaptive immune system, which confers molecular immunity to foreign nucleic acids, including plasmids and viruses (see review Barrangou [90]. CRISPR genomic loci consist of repeat sequences, typically 20–50 bp in length, separated by variable spacer sequences (or protospacers) of similar length that match a segment of invading nucleic acids. These protospacers serve as a molecular memory of prior infections and together with repeat sequences, constitute CRISPR RNAs in the CRISPR locus [90, 91]. CRISPR RNAs are used as guides by Cas proteins for base-pairing with and degradation of complementary sequences in invading DNAs [90, 91]. The CRISPR/Cas system is functional in eukaryotic systems, for which the *Streptococcus pyogenes* endonuclease Cas9 (Cas9) has been harnessed for

efficient eukaryotic genome editing and gene regulation [92, 93]. The ease of deployment of the CRISPR/Cas9 system is due to its dependence on RNA as the moiety that directs the Cas9 nuclease to a desired DNA sequence [94, 95].

The functionality of CRISPR/Cas9 system in eukaryotes has revolutionized genome editing and in a very short time since its discovery, has become a very useful tool in crop improvement. Successful examples have been reported for several crops with complex genomes (reviewed in Paul and Qi [96]). However, only a few reports of use of CRISPR/Cas9 system in cassava improvement exist and are still in the preliminary stage, these include CRISPR/Cas9-mediated modification of cassava flowering genes to induce flowering in this predominantly clonally propagated crop [97]. Because of the successful development of a modified geminivirus vector based on *Cabbage leaf curl virus* for a virus-guided delivery of CRISPR/Cas9 [98], it is likely that a similar vector system can be developed for cassava using the cassava geminivirus, *African cassava mosaic virus*.

There are drawbacks of the CRISPR/Cas system, including: (1) imbalance in stoichiometry between Cas9 and sgRNA ratio that may lead to off-target cleavage [99, 100]. (2) Many protospacer adjacent motif (PAM) sites may lead to undesired cleavage of DNA regions [101]; to resolve this problem, bioinformatics tools are being developed at whole genome sequence level to improve specificity [102]. (3) Codon usage varies across species and may affect Cas9 translation; several codon-optimized versions of Cas9 genes have therefore been harnessed for several individual crops [102] and there may be need for a cassava codon optimized Cas9. (4) CRISPR/Cas9 systems use exogenous promoters for Cas9 and sgRNA expression; for cassava, *Cassava vein mosaic virus* promoter [103] has been shown to be very efficient. (5) Homology between gene family members may complicate sequence targeting and directing sgRNAs to the 5′ region of the targeted gene has been proposed improve target specificity [102].

5. Natural host resistance to pathogen in cassava

5.1. Natural pathogen resistance and cassava virus control

It is now clear that the cassava geminiviruses and cassava brown streak viruses are the most important constraints to cassava production in the African [104]. Correspondingly, in Latin America, a diverse set of virus species that cause the cassava frog skin disease syndrome has a serious impact on cassava production [105]. Thus, considerable effort will be required to expand sources of resistance to cassava viral diseases and advances in genomics have provided new opportunities to explore sources of natural resistance. An important source of resistance that may be useful in cassava is non-host resistance. Mechanistically, non-host resistance is likely due to an intrinsic lack of susceptibility, which is a multigenic trait. It is now known that natural compounds, such as melatonin that modulate immune responses, such as ROS metabolism, calcium signaling and mitogen-activated protein kinase (MAPK) cascades, can be used to enhance natural resistance [106]. Notably, the recent identification and functional analysis of melatonin synthesis genes in cassava has provided a direct link

between melatonin and immune responses [107]. Furthermore, the importance of resistance targets that function as host susceptibility factors, such as translation initiation factors 4E and 4G in RNA viruses, have been studied in model systems and can potentially be exploited for CBSV resistance in cassava [108].

Viruses that successfully infect the host induce changes in host cells by manipulating the host molecular pathways and host responses can provide clues for functional manipulation of resistance traits. It has been shown that CMGs [109] and CBSVs [110] induce global transcriptome reprogramming of cassava. In the case of CBSD, of the 700 overexpressed genes in a resistant cassava variety, none of the genes was identified as a resistance gene, instead most belonged to hormone signaling and metabolic pathway gene classes [110]. Interestingly, three functional genomic studies with *South African cassava mosaic virus* in three hosts, Arabidopsis [111], cassava [109] and *N. benthamiana* [112] revealed a small number of common differentially expressed genes at the early infection stage of full systemic symptoms. However, a common theme in all three hosts was virus-induced changes in hormone signaling, and primary and secondary metabolisms. Understanding the roles of host reprogramming and RNA silencing during cassava-virus interactions could be exploited to improve natural immunity in cassava.

5.2. Identification of cassava immunity-related or resistance (R) genes

Dominant and recessive genes have been associated with natural plant virus resistance [113]. Using a combination of genotype-by-sequencing (GBS)-based SNPs and physical mapping of scaffolds from cassava whole genome sequencing (WGS), 1061 cassava immunity-related genes were mapped [114]. Notably, from 105 putative *CMD2* genes identified from the CMD2 locus on chromosome 8 [115], 35 were identical to those identified in a RNA-seq study of SACMV-infected cassava genotype TME3 [109]. These genes could be strong candidates contributing to resistance in cassava. Proteins encoded by *R* gene usually occur as large families of proteins with nucleotide binding-leucine rich repeat (NLR) domains and function as indirect perception sensors of pathogen avirulence (avr) proteins. The determinants of apparent virus R gene-wide specificity lies in the leucine-rich repeat (LRR) domains and sequencing of wild cassava varieties may provide a source for discovery of new cassava virus resistance genes. Recently, 228 NLR and 99 partial NBS genes were mapped to the cassava reference genome (http://phytozome.jgi.doe.gov) and these genes show high sequence similarity to genes found in other plant species [116]. However, involvement of these genes in CMD or CBSD resistance is not known. Furthermore, microRNAs are master regulators that trigger processing of genes coding for NLR into phased small interfering RNAs (phasiRNAs) [117] and are therefore regulators of genes that are the first line of defense. Unveiling the role of miRNAs in cassava virus resistance would provide new tools in the combat against these viruses.

Based on a holistic approach, combining high-throughput transcriptome sequence data, public genomic data from cassava and Arabidopsis, Leal et al. [118] identified predicted immunity related gene (IRG) pathways, which showed that several cellular pathways are strongly related to immune response pathways. We will need to exploit these genomics data to identify evolutionarily diverse resistance or immunity genes in different cassava genotypes for development of durable resistance to cassava viruses.

5.3. Resistance against whitefly, *B. tabaci* (Gennadius)

Another approach to generate resistance to CMGs and CBSVs is through use of functional genomics to control the whitefly (*B. tabaci*), vector of both virus groups. Until recently, little was known about the molecular mechanisms of insect defense. Development of *B. tabaci* type B on Arabidopsis was shown to rely on the concomitant increase of salicylic acid and decline or unchanged levels of jasmonic acid and ethylene defense pathways [119]. Transgenic mediated overexpression or down-regulation of genes involved in lignin or other defenses against insect pests could be exploited to develop insect resistant cassava [120]. Application of functional genomics in insect resistance was recently elucidated by expressing an insecticidal fern protein in cotton, which exhibited resistance to whitefly [121]. Efforts in editing genes that play a role in whitefly resistance in cassava will thus play a role in developing cassava with resistance to the whitefly, vector of many cassava viruses.

6. Conclusion

The future challenge in cassava is the ability to combine desirable traits with different agronomic requirements using molecular breeding, gene editing and RNAi technologies. This is critically important, given that cassava is fundamental to food security in many parts of the world. In this chapter, we have discussed advances in the improvement of this crop, especially with regards to nutrient quality and biotic as well as abiotic constraints. We have also proposed novel genome editing technologies that will likely address some of the challenges faced by this crop. These include technologies such as amiRNA, tasiRNA, TILLING/EcoTILLING and CRISPR-Cas9, which provide enormous potentials in cassava improvement. Also, the increasing reduction in the cost of high-throughput sequencing and lessons from ongoing and past work will continue to provide new insights into additional new genome-editing and functional genomic approaches for the improvement of the crop.

Acknowledgements

The disease resistance work in Vincent N. Fondong's laboratory is supported by NSF-IOS-1212576.

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