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Chapter

Genetic Modification and Application in Cassava, Sweetpotato and Yams

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Abstract

Cassava (*Manihot esculenta* Crantz), sweetpotato (*Ipomoea batatas*) and yams (Dioscorea spp.) are important root and tuber crops grown for food, feed and various industrial applications. However, their genetic gain potentials are limited by breeding and genetic bottlenecks for improvement of many desired traits. This book chapter covers the applications and potential benefits of genetic modification in breeding selected outcrossing root and tuber crops. It assesses how improvement of selected root and tuber crops through genetic modification overcomes both the high heterozygosity and serious trait separation that occurs in conventional breeding, and contributes to timely achievement of improved target traits. It also assesses the ways genetic modification improves genetic gain in the root and tuber breeding programs, conclusions and perspectives. Conscious use of complementary techniques such as genetic modification in the root and tuber breeding programs can increase the selection gain by reducing the long breeding cycle and cost, as well as reliable exploitation of the heritable variation in the desired direction.

Keywords: application, genetic modification, genetic gain, transgenic plants, roots and tubers

1. Introduction

Root and tuber crops including cassava (*Manihot esculenta* Crantz), sweetpotato (*Ipomoea batatas*) and yams (Dioscorea spp.) are important crops with increasing food, feed and industrial applications in Sub-Saharan Africa and many other regions of the world [1–3]. These crops possess great potential to contribute to food, nutrition and income security of many livelihoods worldwide, but this potential is to be fully exploited. Variety development through breeding is among the activities targeted at unlocking the potential of these crops for food, feed and industrial applications [3].

Despite their importance, conventional breeding of root and tuber crops is limited by many challenges and heavily depends on the traditional techniques for exploitation of the existing variation. These challenges include high degree of genetic heterozygosity, genetic overloading, serious separation of progeny, few flowers, Irregularity in flowering time and flowering intensity, low pollen fertility, self-incompatibility, cross incompatibility, polyploidy, and low fruit set rate [3–6].

Genetic modification technologies are among many advances made to traditional breeding practices in plants, animals, and microbes to increase productivity and enhance food quality. Plant genetic modification is the oldest technique utilized in simple selection, where plants that exhibit desired characteristics are selected for continued propagation [7]. The advent of modern technology and various molecular analytical tools has improved upon simple selection for detection of elite plants expressing desired traits. Genetic modification is an important alternative and complementary technique for the genetic improvement of crops including roots and tubers. It is a powerful tool that can be used to introduce a number of genes with important agronomic traits, such as disease resistance, insect resistance, and high yield and quality. Genetic modification shows great potential for the genetic improvement of crops including roots and tubers and can compensate for the limitations of conventional breeding. The application of transgenic methods to cassava, sweetpotato and yam improvement programs is particularly important due to the difficulties associated with conventional breeding of these crops. However, an efficient plant regeneration system is imperative to achieve successful transformation [8].

Since the advent of genetic modification, rapid progress has been noted for cassava, sweetpotato and yam breeding programs through various of international non-profit organizations and scientists from developed countries. For instance, HarvestPlus and BioCassava Plus, have made remarkable achievements by transforming conventional breeding into molecular breeding [9, 10]. This book chapter focuses on genetic modification in selected root and tuber crops, applications, potential and future prospects for the genetic improvement of these economically important crops.

1.1 Concepts of genetic and nongenetic modifications

Genetic modification or transformation is the directed desirable transfer of gene or insertion of DNA from one organism to another along with the subsequent stable integration and expression of a foreign gene in the genome [7]. It also refers to the targeted manipulation of genetic material, and nontargeted, nontransgenic methods such as chemical mutagenesis and breeding applied to alter the genetic composition of plants, animals, and microorganisms. Genetic engineering refers to recombinant deoxyribonucleic acid (rDNA) methods that permit a gene from any species to be inserted and subsequently expressed in a food crop or other food product [7]. Although the process involving rDNA technology is not inherently hazardous, the products of rDNA technology may only be hazardous if inserted genes result in the production of hazardous substances.

Nongenetic engineering techniques of genetic modification such as embryo rescue involves placing of plant or animal embryos produced from interspecies gene transfer, or crossing, in a tissue culture environment to complete development [7]. Somatic hybridization of nongenetic engineering technique involves removal of the cell walls of a plant, forceful hybridization of cells and induction of mutagenesis. Irradiation or chemical mutagenesis is useful for the induction of random mutations in DNA [7]. The development of genetic modification approaches has enhanced an array of techniques that could be exploited to advance food production.

2. Genetic modification technology

The acquisition of new genes that confer selective merits is a relevant factor in genome evolution. Significant proportions of prokaryotic and eukaryotic genomes

originated from the exchange of genetic material among related or unrelated species through horizontal gene transfer (HGT). The HGT technique has been noted as one of the key sources of molecular variability and driver of evolution [11, 12]. This HGT often results in the occurrence of crown galls and the mechanism of HGT has been well understood and reported [12–14]. Moreover, Kyndt et al. [12] found that all the 291 tested accessions of cultivated sweetpotato contain one or more transfer DNA (T-DNA) sequences similar to the cultivated sweetpotato clone "Huachano", suggesting that an Agrobacterium infection occurred in evolutionary times. This finding also depicts the importance of plant–microbe interactions, and given that this crop has been eaten for millennia, it might change the negative mindset and paradigm governing the "unnatural" status of transgenic crops. The plant regeneration system of a typical root crop such as cassava was fully developed in the 1990s using somatic embryogenesis, shoot organogenesis from cotyledons of somatic embryos (somatic cotyledons), and friable embryogenic calli (FEC) (**Figure 1**). The key media components used for the establishment and optimization of the plant regeneration system have been well noted by Liu et al. [15]. The common transgenic techniques utilized for the genetic transformation of root and tuber crops are Agrobacterium-mediated gene delivery and particle bombardment. The explants used for transformation include somatic cotyledons and FEC.

2.1 *Agrobacterium*-mediated genetic modification method

Agrobacterium-mediated genetic modification has been the method of choice for the development of genetically modified crops. The major merits of Agrobacterium-mediated genetic modification are its high frequencies of single-copy integration, high reproducibility, transformation efficiency, stable expression of transgenes, utilization of simple equipment, ease of accessibility, ability to transfer low copies of DNA fragments carrying the desirable genes at higher efficiencies with minimal cost and the transfer of very large DNA fragments with low rearrangement [17].

The first attempt to transform cotyledonous embryos of cassava MPer183 with *Agrobacterium tumefaciens* CIAT1182 started in 1993–1994, but the transgenic

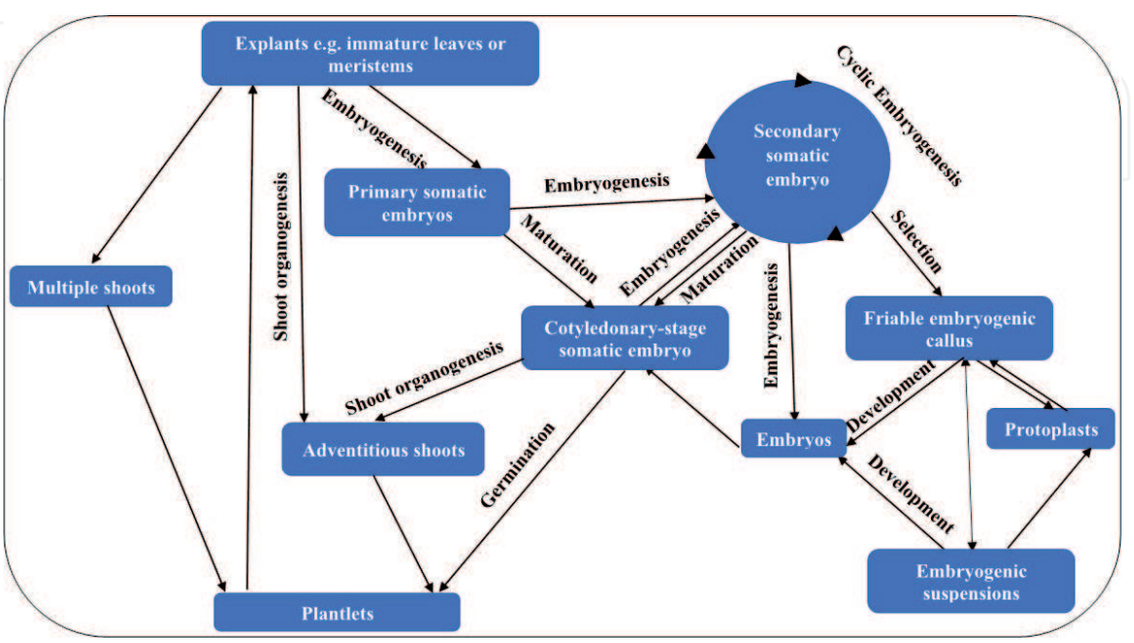

Figure 1.
Illustration of an in vitro plant regeneration system of a typical root crop. Redrawn from Zhang [16].

nature of regenerated plants could not be verified by Southern analysis [18]. The first successful Agrobacterium mediated cassava genetic transformation was done in Potrykus laboratory at ETH Zurich in 1996 [19]. Several Agrobacterium strains sheltering different binary vectors such as LBA4404 (pTOK233), LBA4404 (pBin9GusInt), C58C1 (pIG121Hm), and EHA105 (pBin9GusInt) were used for the transformation of cassava somatic cotyledons. Gonzalez et al. [20] successfully transformed TMS60444 with the *A. tumefaciens* strain ABI using Agrobacterium-mediated FEC transformation. The transgenic nature of the two plant lines resistant to paromomycin was confirmed by glucuronidation glycosidase (GUS) assay and Southern analysis. The embryogenic suspensions of TMS60444 were transformed with *A. tumefaciens* LBA4404 using negative- and positive-selection agents [21]. A total of 12 morphologically-normal transgenic lines have been developed, of which, are five are mannose selected and seven hygromycin resistant. Moreover, polymerase chain reaction (PCR) and Southern analysis confirmed the successful integration of the transgene into the genome. The confirmation of expression of the transgene in the regenerated plants was done using reverse transcription (RT)–PCR and Northern analyses. In 2000, Sarria et al. [22] successfully transformed a herbicide (phosphinotricin, ppt)-resistance gene into the cotyledons of cassava MPer183 by an Agrobacterium-mediated method and found stable transgenic plants resistant to Basta spray (at concentrations of 200 mg/L or more). An efficiently robust and reproducible transformation protocol for cassava embryogenic suspension culture with *A. tumefaciens* has also been reported [23]. Of the 31 GUS-active plants identified, 14 were found with 100% GUS activity, whilst the remaining lines had 72% GUS activity. The transgenic nature of these plants was detected using the southern blot analysis. Zhang et al. [24] successfully introduced a synthetic artificial storage protein 1 (ASP1) gene encoding a storage protein rich in essential amino acids (80%) into embryogenic suspensions of cassava using Agrobacterium-mediated FEC transformation. The ASP1 tetramer was detected in the leaves and primary roots of transgenic cassava plants by Western analysis. Another achievement in the cassava genetic modification is the development of transgenic cassava with a lower cyanide content using MCol2215 cotyledon explants [25]. Jørgensen et al. [26] constructed several RNAi and antisense vectors to interfere with the expression of CYP79D1 and AYP79D2, and transformed the somatic cotyledons of cassava MCol22 using an Agrobacterium-mediated technique. In 2009, a multi-auto-transformation (MAT) vector system of isopentenyl transferase (ipt) type was utilized for the production of marker-free transgenic cassava plants with conversion efficiency up to 19–21% via shoot organogenesis of KU50 somatic cotyledons [27]. Zhang et al. [28] developed transgenic cassava with senescence-inducible expression of the ipt using Agrobacterium-inoculated TMS60444 somatic cotyledons via shoot organogenesis. These achievements contributed to cassava transformation for the verification of a tissue-specific promoter [29, 30], the resistance to African cassava mosaic virus (ACMV) [31], increased protein content [32], and improved cassava brown streak virus resistance [33].

For sweetpotato, Kyndt et al. [12] reported that the Agrobacterium-mediated gene delivery system was utilized for T-DNA integration, the interruption of an *F-box* gene, and the subsequent insertion of foreign T-DNA into the sweetpotato genome. This is believed to have occurred during the evolution and domestication of this crop. White et al. [34] suggested that the identification of gene sequences in IbT-DNA1 and IbT-DNA2 imply that the transforming Agrobacterium was probably Agrobacterium rhizogenes, an ancestral form of A. rhizogenes or a closely related species (perhaps extinct) of A. rhizogenes. The *Ib*T-DNA1 corresponds to TR-DNA (typically containing the auxin biosynthesis genes *iaa*M and *iaa*H), and *Ib*T-DNA2 corresponds to TL-DNA (harboring the *Rol* genes). The gene organization and DNA

sequences of the T-DNAs are similar to, but distinctly different from, the ORFs of the Ri and Ti-plasmids in well characterized *Agrobacterium* strains. The identified *RolB*/*RolC* region represents a new member of the *RolB* family indicating that, unlike the T-DNA found in *Nicotiana* spp. [35], the *Agrobacterium* strain (or species) that transferred its T-DNA into the sweetpotato genome is uncommon.

For yams, the Agrobacterium-mediated gene delivery system is the most preferred technique utilized for the genetic modification of the crop [14, 36]. Initial development of a transient genetic modification of *Dioscorea rotundata* using the Agrobacterium-mediated produced no transgenic plants [37]. However, the first fast, efficient and reproducible protocol for Agrobacterium-mediated transformation of *D. rotundata* resulted in the generation of stable transformations and the regeneration of complete transgenic yam plants [8]. This achievement laid the foundation for the full implementation of genetic engineering and gene editing in yam. Based on the review, it is clear that Agrobacterium-mediated transformation system is the most prominent genetic modification technique due to the availability of a large number of transgenic plants. The transformation efficiency of this technique can be improved using a protocol based on somatic cotyledons as explants for the transformation of cassava, sweetpotato and yams. Thus, it is the most widely-used method for genetic engineering in the studied root and tuber crops.

2.2 Biolistic-mediated genetic modification method

Biolistic transformation or gene gun or particle bombardment technique is often utilized for plant transformation studies. The technique involves FEC induction, subculturing, somatic embryogenesis, and plant germination. The biolistic transformation technique involves series of protocols and stages [15]. These include the subculturing in SH liquid medium, followed by supplementation with 50 μM picloram, without selection for 2 weeks. The samples are further subjected to SH liquid medium with 25 μM paromomycin for 4–5 weeks, solid SH medium with 25 μM paromomycin for 4 weeks, and Murashige and Skoog (MS) medium with 5 μM picloram for embryogenesis. The next stage involves the development of transformed cell clusters into somatic embryoids in maturation media (MS medium supplemented with 0.5% activated charcoal). The final stage is the regeneration and verification of transgenic plants exhibiting GUS-positive and paromomycin resistance using Southern analysis [15]. This technique was successfully conducted on somatic cotyledons of cassava genotypes CMC40, MPer183, MCol22, and TMS60444 suspensions [21, 38]. Twenty transgenic plants of TMS60444 and 11 transgenic lines of MCol22 were obtained. The GUS, Southern blot, and RT-PCR assays indicated the successful integration of the transgene into the plant genome. Transgenic cassava has also been produced from the plasmid constructs pHB1 and pJIT100 using FEC of TMS60444 and particle bombardment [39]. Of the dozens of transgenic plants produced using the pHB1 and pJIT100 constructs, some have been analyzed at the molecular level. Zhang and Puonti-Kaerlas [40] used particle bombardment to transfer the plasmid pHMG into TMS60444 embryogenic suspensions. Selection from the dozens of transgenic cassava plants produced in less than 15 weeks was based on either negative hygromycin or positive mannose. Zhang et al. [41] also utilized FEC and a particle bombardment technique to investigate ACMV resistance in cassava.

The success of this method depends on the high efficiency of the particle bombardment and shoot organogenesis. The long time utilized for the FEC induction, subculturing, somatic embryogenesis, and plant germination causes a low efficiency of plant regeneration and a high rate of somaclonal variation. This indicates low probability of success due to its complicated operation and its susceptibility to

many factors. Thus, the technique is infrequently used for genetic transformation in roots and tubers. This necessitates the establishment of a robust standard protocol for FEC-based transformation for each cultivar.

2.3 The friable embryogenic callus genetic modification method

The embryogenic callus tissue utilized in this genetic modification technique are friable as they tear, slough and bleed more easily when touched. For the cassava embryogenic callus genetic modification, the induction of primary somatic embryos on the embryogenesis induction medium of the immature young leaves and apical or axillary meristems are useful for the establishment of cyclic secondary somatic embryogenesis using the subculture of the induction medium [15]. However, continual subculturing of the secondary somatic embryos on Gresshoff and Doy (GD) medium, supplementation with 12 mg/L picloram results into production of FEC, formation of non-embryogenic calli and secondary somatic embryos [15]. Establishment of an embryogenic suspension for rapid multiplication in liquid Schenk and Hildebrandt (SH) medium containing 10–12 mg/L picloram requires appropriate selection of FEC and subculturing on the GD solid medium in the long-term. Culturing of embryogenic suspension cells on MSN solid media with 1 mg/L naphthaleneacetic acid, produce somatic embryos and subsequently cotyledon-stage somatic embryos that germinate to plantlets [42, 43]. The FEC and embryogenic suspension cultures are susceptible to Agrobacterium infection, and favorable to particle bombardment, making the delivery of foreign genes easy. Cassava transformation is usually done using FEC and/or embryogenic suspensions by *A. tumefaciens* or particle bombardment.

2.4 Shoot organogenesis-based genetic modification method

This technique involves the induction of somatic embryogenesis from immature leaves and apical or axillary meristems using green cotyledons as explants and plant regeneration via shoot organogenesis to overcome genotype restrictions [44]. However, an efficient shoot organogenesis using mature green cotyledons has been developed from secondary somatic embryos in AgCOM medium supplemented with N6-benzylaminopurine and $AgNO_3$ (an ethylene action inhibitor) [45]. The shoot organogenesis-based genetic modification method is most applicable and suitable for the biolistic or Agrobacterium-mediated genetic transformation since these techniques accommodate the initiation of shoot primordia from cut ends and epidermal cells as well as the regeneration of transgenic plants in a short cycle of 3–4 months. Zhang et al. [21] established that subjection of transgenic shoots to rooting sensitivity tests could quickly eliminates false-positive transgenic plants, thereby preventing difficult and expensive molecular analyses. This is also a reliable method for screening transgenic cassava plants.

3. Novel genetic transformation technologies

3.1 Genetic transformation

Advances in transgenic technology has led to the development of different schemes for the delivery of target genes into plant cells [15]. Selectable-marker genes including herbicide- and antibiotic-resistance genes, and the reporter genes such as GUS, luc, chloramphenicol acetyltransferase, and green fluorescent protein. The selectable-marker and reporter genes are used for screening and monitoring

of putative transgenic plants. However, there are lots of public concern about the biosafety of using these marker genes are widely used for genetic transformation. This necessitates use of suitable alternatives as selectable markers. Roots and tubers are conventionally vegetatively propagated crops with the merit of reduced risk of horizontal gene transfer to relevant organisms and pathogens through pollen. This indicates the necessity of developing safe marker genes or marker-free technology in root and tuber crops.

3.2 Marker-free technology

Development of marker-free transgenic plants involves co-transformation, homologous recombination, site-specific recombination, and transposition systems [15]. These techniques have potential for application in cassava, sweetpotato and yams once the genetic transformation systems of the crops are further improved and optimized.

3.3 Co-transformation system

The co-transformation system involves the co-transformation of plant cells with a pair of plasmid vectors, each carrying a selectable-marker gene or a target gene. The selectable marker and target gene integrate into different loci on the chromosome at the same period. Since the two genes integrate at different loci, the selectable-marker gene segregates to produce marker-free transgenic plants. This procedure works for sexually producing crops [46]. For asexually reproducing crops, conventional breeding is utilized for development of new varieties. Thus, co-transformation via sexual hybridization technique is a useful means of producing marker-free cassava, sweetpotato and yam transgenic plants as an intermediate putative parental genotype. The major demerits of this method include its labor intensiveness and the long cycle for the occurrence of genetic transformation [15].

3.4 The recombination system

The site-specific recombination systems reported include Cre/loxP (Cre: causes recombination; loxP: locus of crossing X2 over in P1), R/RS (R: recombinase; RS: recognition site), Gin/gix (Gin: inversion of the G loop; gix: Gin-inversion complex sites), and FLP/FRT (FLP: flipping DNA; FRT: FLP recombination target), consist of a recombinase enzyme and corresponding specific recognition sequences [15]. The functions of the recombinase enzyme are to identify and mediate the recombination of two specific recognition sequences in the same direction leading to the simultaneous and independent formation of the cyclic DNA and the chromosome [15]. This facilitates the elimination of the selective marker gene. Of the three recombination systems, Cre/loxP is the most widely used and studied system. Dale and Ow [47] first utilized the Cre/loxP system to develop transgenic tobacco. The application of site-specific recombination system is still in its infancy stage in root and tuber crops due to its lower transformation efficiency. Saelim et al. [27] reported marker-free transgenic KU50 cassava produced using the MAT vector system (containing the yeast site-specific recombination Rint/RS system mediated excision of DNA fragments and the ipt phenotypic marker gene from recombination sites) developed by Ebinuma et al. [48]. The development of excessive and overgrowth buds in transgenic plants is caused by isopentenyl transferase gene. Genetic recombination during subculture eliminates the expression of this gene to produce marker-free transgenic plants. The conversion efficiency and the proportion of normal growing plants are 19–21% and 32–38%, respectively, indicating

feasibility of its use. Zuo et al. [49] developed the Cre-loxP-XVE system and could be worth testing in root and tuber transgenic program.

3.5 The transposon system

Transposable elements (TEs) or jumping genes or transposons are sequences of DNA that move from one site in the genome to another. The transposons take advantage of their characteristic conservative cut-paste mechanism to transfer genes from one site to another in the chromosome [15]. This attribute of transposons reduces the probability of genetic linkage at the new site even after occurrence of transposition to the new site. This peculiar attribute of TEs is useful for the removal of unwanted marker gene from the transgenic plants. The removal of the unwanted marker gene is accomplished in two ways: (i) the marker gene could be placed between the TEs and the repeat sequence Dissociator (Ds) element; or (ii) the target gene could be placed between the Ds sequences. During transposition, the marker gene maybe lost or separates from the target gene. The occurrence of the transposon is achieved by progeny segregation; therefore, its application in root and tuber crops is very difficult.

3.6 Non-antibiotic selection

In the non-antibiotic selection system, growth of transformed cells is favored by the development, promotion or additional metabolic activity, without affecting non-transformed cell growth by hormonal stimulation or inhibition by starvation from nonmetabolizable sugars, or death by antibiotics [15]. Unlike the conventional antibiotic-selection systems, the non-antibiotic techniques exhibit better acceptability by the public as they are considered to be safer than the antibiotic-selection systems. Selectable-marker genes from hormonal action Isopentenyl transferase (ipt) and indole-3-acetamide hydrolase (iaaH) are reportedly the most widely used hormone metabolism genes [15]. The Isopentenyl transferase gene was cloned from *A. tumefaciens* T-DNA and is related to cytokinin biosynthesis. The overexpression of ipt in transgenic plants has been observed to cause phenotypic variations in them [15]. This necessitates use of an inducible promoter to regulate its expression or the ipt gene should be applied in a site-specific recombination enzyme system or transposon system to build a highly-efficient marker-removal system. Saelim et al. [27] opined the usefulness of the Rint/RS system for excision of the ipt gene from transgenic cassava KU50. This technology is useful in the genetic transformation of crops with long cycle of breeding. The iaaH is also a safe selective-marker gene detected in the regulation of hormone metabolism. The overexpression of iaaH results in abnormal production of transgenic plants. However, the expressivity of iaaH can be modulated, inactivated or removed [15].

3.7 Selectable-marker genes based on sugar metabolism

The selectable genes consist of selectable markers utilized to facilitate the isolation of plasmid-containing transformants [15]. Selectable markers are useful for genetic transformation as they permit plant cells to grow under conditions that prevent the growth of untransformed tissue. Selectable-marker genes based on sugar metabolism comprise of the mannose phosphate isomerase (pmi) gene and the xylose isomerase (xylA) gene [50]. The pmi system is a positive selection system that uses D-mannose as a selection agent. The mechanism of the system is well reviewed by Liu et al. [15]. The pmi gene has been widely applied in the transformation systems of rice, corn, wheat, and sugar beet [51]. The pmi-mannose system has

also been used in cassava transformation system [40]. For validation of this technique, Zhang [16] constructed a pHMG binary vector that uses the visual marker GUS gene, independent expression cassettes of the pmi and hygromycin phosphotransferase genes in its T-DNA region. They observed 82.6% Agrobacterium-mediated transformation of embryogenic suspensions by the mannose selection system compared to 100% hygromycin selection system. Transgenic plants were also generated from application of the biolistic transformation of somatic cotyledons as explants, and mannose as the selective agent [40].

Another selectable marker xylA gene, encodes xylA and catalyzes the conversion of D-xylose through D-xylulose tautomerism. The growth of transformed cells is promoted in the medium containing a carbon source material such as D-xylose, whereas the growth of non-transformed cells is inhibited by the lack of a suitable carbon source [15]. Haldrup et al. [52] noted the production of transgenic plants using D-xylose as the carbon source. Application of this technique is yet to be fully exploited in root and tuber crops.

3.8 Tissue-specific promoter

Liu et al. [15] reported three types of promoters based on spatial and temporal expression patterns including inducible promoters, constitutive promoters, and tissue- or organ-specific promoters. For cassava genetic transformation, the CaMV 35S promoter and methyl jasmonate and salicylic acid-induced nopaline synthase gene promoter are the widely used promoters. These promoters regulate genes of interest and selective marker gene or reporter gene. The gene expression level of CaMV 35S promoter in transgenic cassava leaves was noted to be higher relative to the storage roots indicating possible influence on the function of certain genes in cassava storage roots [29].

Further studies to increase the specific expression of a target gene in cassava organs and tissues led to the discovery of specific promoters in leaves or storage roots. Zhang et al. [29] detected two cassava promoters such as p15/1.5 of a cytochrome P450 protein, and p54/1.0 of the cassava glutamic acid-rich protein, Pt2L4, from a cassava storage root cDNA library. The gene expression patterns of these promoters show close association with cassava vascular tissues and storage root development, especially in the starch-rich parenchyma cells of the storage root. Their activities are also stronger than those of CaMV 35S promoter. The function of the glutamic acid-rich protein promoter has been well articulated [30, 53–55]. Application of p54/1.0 promoter regulated the dsRNA expression for interference with the granule-bound starch synthase (GBSS)I expression producing amylose-free transgenic cassava [56].

Tuber-specific class I patatin promoter was noted to regulate several gene expression. Ihemere et al. [57] reported its gene regulation activity in the expression of the *Escherichia coli* gene, glucose-1-phosphate adenylyltransferase (glgC) with insensitivity to the substrate, by site-directed mutation in cassava for increased starch content. Siritunga and Sayre [25] noted its regulation activity in CYP79D1 and CYP79D2 antisense gene expression in cassava for decreased toxicity of cyanide. Abhary et al. [32] reported the role of the promoter in the expression of a zeolin fusion protein to increase protein content. A promoter regulating protein family AAI_LTSS of unknown function in cassava was found strongly expressed in the secondary xylem of the carrot [58].

Leaf senescence inducible promoter SAG12 was found to regulate the ipt gene that moderate extended leaf longevity and improved the drought resistance in transgenic [28]. Leaf specific cab1 promoter applied in transgenic cassava regulated transgene expression Siritunga and Sayre [25]. Based on existing information on

tissue-specific promoters, greater application of this technology is envisaged in future root and tuber breeding programs.

4. Applications of genetic modification technology in cassava, sweetpotato and yam improvement programs

Application of functional genomics and genetic engineering has contributed to resolve the problems associated with the germplasm enhancement of root and tuber crops. For cassava, improved agronomic traits achieved by transgenic technology include virus resistance, improved nutritional quality, reduced cyanide content, improved biomass, and delayed post-harvest physiology deterioration in storage roots.

4.1 Resistance to pests and viral diseases

Pests and viral diseases are transmitted via infected stems, vines, tubers from generation to generation, subsequently causing yield losses. For instance, the cassava mosaic disease (CMD) accounts for about 20–95% yield reductions [59]. It is the major cassava disease in Africa and the Indian peninsula. The CMD is caused by several cassava geminiviruses and their satellite components, including the ACMV, Eastern ACMV, and Indian cassava mosaic virus [59, 60].

Transgenic technology has played an important role in obtaining virus resistant cultivars [61]. Chellappan et al. [62] utilized pILTAB9001 and pILTAB9002 harboring the wild-type and mutant AC1 genes of ACMV-Kenya. These wild-type and mutant AC1 genes regulate the cassava vein mosaic virus promoter and the pea Rubisco terminator, which enables the production of transgenic TMS60444 plants with increased resistance to mosaic disease [62]. The initial inoculation assay detected transgenic plants resistant to several cassava geminivirus diseases from Africa. However, these transgenic plants were later susceptible to CMD infection in a closed-field trial in Kenya. With the aid of improved antisense RNA technology, Zhang et al. [63] developed transgenic cassava plants with increased ACMV resistance targeting the viral mRNAs of Rep (AC1), TrAP (AC2), and REn (AC3). Several transgenic clones remained symptomless after biolistic inoculation of ACMV at infection pressure of 100 ng viral DNA plant^{-1}. Decreased and attenuated symptom development were also detected even at higher viral DNA doses. Significant reduction in viral DNA accumulation was observed in the leaves of transgenic ACMV-resistant plants. Application of RNAi-mediated gene-silencing approaches, siRNAs, homologous to either the common region or AC1 in transgenic cassava plants suppressed the replication of African mosaic virus, leading to recovery after infection with ACMV [64] or immunity to infection by the virus [31]. Bi et al. [65] screened cassava germplasms from using Agrobacterium-mediated inoculation in combination with CMD-resistant molecular markers RME1, SSRY28, and NS158. Findings showed that the cassava germplasms lacked CMD-resistant genes, suggesting the necessity of introducing and integrating disease-resistant cassava genotypes from Africa into the current breeding program, while developing CMD-resistant cassava using different transgenic approaches.

Cassava brown streak disease (CBSD) is another important viral disease of cassava in Africa. A sequence analysis of CBSD showed that the causative virus belongs to potato virus-Y of the ipomovirus family [66]. Two subspecies of CBSD are cassava brown streak virus (CBSV) and cassava brown streak Mozambique virus (CBSMV) [67]. Transgenic approach is noted to be more promising for development of CBSD clones relative to the traditional cassava-breeding method. Resistant

cassava lines have been developed by transferring the virus coat protein gene or through RNA (siRNA) interference [33, 68]. Cassava bacterial blight (CBB) disease (gum disease), caused by *Xanthomonas manihotis*, mainly affects cassava leaves. Transcriptomic studies have shown a rapid change in cassava genes after infection by this disease [69]. The main insects that infest cassava are whiteflies, cassava mealybugs, cassava green mites, and stemborers, while root-knot nematodes are the most widely-reported parasitic nematodes on cassava. The cultivation of insect resistant cassava genotypes increases the yield and quality of the crop. Insecticide proteins including Bt Cry proteins, protease inhibitors, α-amylase inhibitor, and plant lectins, could aid insecticides, as a high expression of these products in transgenic cassava might facilitate increased insect resistance.

Targeted genome alteration technique is a promising tool for yam breeding. Successful application of the CRISPR/Cas9 technology resulted into inactivation of the endogenous banana streak virus by editing the virus sequences to develop resistant plantain [70]. Yam viruses have also been found to be integrated into the genome of yam and for the development of yam genotypes resistant to yam mosaic virus using CRISPR/Cas9 approach [71, 72]. The major challenge of the CRISPR/Cas9 technology is that it may recognize sequences with up to five mismatched bases suggesting high rates of off-target effects [73]. However, techniques such as DNA-RNA chimeric guides, Cpf1, a single RNA endonuclease that employs a T-rich PAM on the 5′ side of the guide, and specific point mutations have been developed to mitigate this challenge [74, 75].

The recently established gene-editing technique, the clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) system, resulting from the adaptive immune system of *Streptococcus pyogenes*, is a notably potent tool for targeted genome editing in many species [76]. Gene editing and genetic engineering technologies have been reported to facilitate improvement of the productivity and nutritional quality of yam. This was achieved through the Genome-Enabled Platforms for Yam Project launched in 2016 in collaboration between scientists at the International Institute of Tropical Agriculture (IITA) and Iowa State University (https://www.nsf.gov/awardsearch/showAward?AWD_ID=1543888). Moreover, a genome-editing tool for yam using phytoene desaturase (a key enzyme in the β-carotene biosynthesis pathway, which converts the colorless phytoene to colored carotenoids) as a marker is being developed [77]. Targeted traits such as resistance to yam mosaic virus and anthracnose diseases, herbicide tolerance and nematode resistance are being investigated using yam gene editing and genetic engineering technique.

Feng et al. [76] successfully applied the CRISPR/Cas9-mediated targeted mutagenesis in *D. zingiberensis* using an *A. tumefaciens*-mediated transformation method. Their study aimed at an essential gene involved in the synthesis of secondary metabolites, known as the farnesyl pyrophosphate synthase gene (Dzfps). They detected five types of mutations among the transformed plants at the predicted double-stranded break site. Feng et al. [76] also found that the transcript levels of Dzfps and the content of squalene in isolated mutants were drastically decreased relative to those in wild-type plants and concluded that CRISPR/Cas9 is a rapid and efficient method for targeted genome modification in *D. zingiberensis*.

4.2 Improved stress resistance

Cassava is susceptible to cold stress, resulting into decrease root yield and cumbersome maintenance of cassava stems. In southern China, winter, rain, snow, and freezing temperatures cause severe frostbite to cassava stems [15]. In 2008, damage of cassava stems caused by cold stress in Guangxi amounts to several billion

RMB. Thus, development of low temperature resistant cassava clones aids easier stem storage and increasing cultivation in temperate countries, thereby meeting the demand for raw cassava materials for the industries. High expression of the C-repeat/dehydration-responsive element-binding factor 3 gene regulated by a low temperature inducible or CaMV 35S promoter has been noted to significantly improve cold resistance in transgenic cassava [15]. The growth and yield of cassava are influenced by intermittent drought in tropical and subtropical regions. Application of leaf senescence induced promoter, SAG12, for expression of the ipt gene, transgenic cassava revealed prolonged leaf life, and improved resistance to drought stress [28]. This study demonstrates a useful strategy for the improvement of drought resistant and high yield cassava clones. Several authors have also noted a large number of genes associated with stress resistance using high-throughput genomic and transcriptomic analyses of cassava [78–80]. Functional verification of these genes is needed for better understanding of the molecular mechanisms of cassava resistance to different stresses and establishment of the theoretical basis for cassava genetic improvements.

4.3 Enhanced nutritional quality

Nutritional quality enhancement in roots and tubers can improve the dietary and nutritional balance of people who utilize them, particularly in central and western Africa. Zhang et al. [24] noted the production of transgenic cassava exhibiting an artificial storage protein enriched with essential amino acids, under the control of the CaMV 35S promoter. The transgenic plants were detected expressing ASP1 at both the RNA and protein levels. The leaves of transgenic plants had increased proline and serine contents, while the amounts of aspartic acid, alanine, and methionine were decreased compared to non-transgenic plants. Altered composition of amino acids and reduced cyanide content were also observed in transgenic plants [32]. Thus, cassava protein enhancement using transgenic approach is practically feasible and is a useful means of reducing protein deficiency in poverty-stricken regions [81].

The BioCassava Plus project team has developed transgenic cassava clones with value-added traits including virus resistance, improved protein content, and increased vitamin A, iron, and zinc contents [10]. The HarvestPlus project team has also developed β-carotene-rich cassava clones [82].

4.4 Reduced cyanide content

Siritunga and Sayra [25] utilized an Arabidopsis leaf-specific promoter to drive the antisense expression of cytochrome P450 genes (CYP79D1 and CYP79D2). In vitro tests of the linamarin content of the transgenic leaves revealed a decrease of 60–94% compared to the control, while a 99% decrease was observed in the storage roots of cassava, suggesting the transport of linamarins from leaves to storage roots. White et al. [83] noted that at transcript level, the hydroxynitrile lyase content in cassava roots is only 6% of that in the leaves. The overexpression of hydroxynitrile lyase reduces the acetone cyanohydrin content of roots, thereby accelerating the detoxification process. The overexpression of hydroxynitrile lyase was achieved through the cloning of the cDNA of the gene encoding hydroxynitrile lyase between the CaMV 35S promoter and the pea ribulose bisphosphate carboxylase terminal sequence, and transformed into MCol2215 [84]. The authors found a 40–135% increase in the hydroxynitrile lyase activity in transgenic plants, compared to 800–1300% found in the storage roots of cassava. However, no changes were detected in the total amount of linamarin and lotaustralin detected in the whole

plant [84]. After harvesting, the detoxification capacity of the root was greatly enhanced. Jørgensen et al. [26] conducted similar trial by RNAi and found a 92% decrease in the cyanogenic glucoside contents of cassava storage roots.

4.5 Improved starch content and quality

Starch quality is one of the key agronomic traits for selection of elite cassava genotypes. The ratio of amylose to amylopectin determines the property of starch granules, and influences the quality of various starch products utilized in the pharmaceutical, chemical, and paper-making industries. Starch synthesis is regulated by AGPase, starch synthase (SS), and starch-branching enzyme (SBE). These three main enzymes have been successfully cloned from cassava [85–87]. The inhibition of AGPase activity results in partial or complete termination of starch synthesis. Thus, improvement of the AGPase activity contributes to the conversion of sugar to starch, which subsequently increases the starch quantity. The reduction of allosteric feedback regulation by fructose-1,6-bisphosphate was detected by genetic modification of the *E. coli* glgC gene (encoding AGPase) by site directed mutagenesis (G336D) [57]. Transgenic cassava plants expressing the mutant glgC gene showed a 70% enhancement in AGPase activity, and up to a 2.6-fold increase in biomass. The quality of cassava starch depends on amylose and amylopectin content. Antisense RNA technology has been used to reduce GBSS expression in the potato resulting into decreased amylose content in potato tubers [88]. Using antisense GBSSI RNA under the control of the CaMV 35S promoter, waxy transgenic cassava plants were generated [89]. Waxy transgenic cassava clones have also been developed using the cassava vascular-specific p54/1.0 and CaMV 35S promoters to drive the expression of hairpin dsRNA homologous to cassava GBSSI. Starches from waxy transgenic plants revealed altered biological and physico-chemical properties [56]. Thus, the control of GBSS activity is an effective way to regulate amylose synthesis.

4.6 Delayed post-harvest physiological deterioration

Postharvest physiological deterioration (PPD) limits the storability and utilization of cassava. The PPD is a physiological and biochemical decay process caused by an oxidative burst in storage root cells of cassava [90]. The PPD phenomenon has a close relationship with reactive oxygen species (ROS) [90]. Analysis of the proteins and enzymes influencing PPD using the cDNA-AFLP technique showed that most of the proteins and enzymes are involved in signal transduction, ROS, cell wall repair, programmed cell death, metabolite transport, signal transduction, and a series of biological processes [91]. The upregulation or downregulation of key enzymes or factors in the PPD pathway by the overexpression or RNAi might effectively slow or decrease the PPD activity [91]. Study of the temporal and spatial expressions of genes related to ROS production and scavenging in cassava PPD, and the functional verification of key genes, indicates the possibility of interference of the PPD process by the regulation of ROS-scavenging activities [15]. These genes are involved in the regulation of glutathione-peroxidase cycle, the ascorbate-glutathione cycle, and the peroxidase-oxidoreductase cycle [15]. The genes also combine with superoxide dismutase to generate univalent, bivalent, and trivalent overexpression or RNAi vectors to transform cassava. Secondary metabolites of PPD such as scopoletin and diterpenoids, are synthesized in deteriorated roots [92]. The interference with the biosynthesis of the secondary metabolites might also influence PPD. Morante et al. [93] noted the discovery of germplasms of radiation mutants that suppress the occurrence of PPD. These results provide gene resources for the amelioration of PPD through traditional and molecular breeding techniques.

5. Future prospects of genetic modification for root and tuber improvement

Pests and diseases are among key factors affecting yield and quality of root and tuber crops. The progress made in genetic modification of these crops could open up many avenues to produce disease resistant varieties, through pathogen-derived resistance strategies, that would not be possible using conventional breeding approaches alone. In yams, host plant resistance to anthracnose has been suggested as a more viable alternative to control yam anthracnose disease (YAD) than use of chemical fungicides [94]. However, studies reveal lack of genotypes resistant to the disease [94]. Thus, the most efficient strategy for YAD control is possibly the development of disease resistant plants using the transgenic approach. These approaches could include the expression of genes encoding elicitors of defense response [95], genes encoding plant, fungal or bacterial hydrolytic enzymes [96] and antimicrobial peptides (AMPs) [97]. Most AMPs are non-toxic to plant and mammalian cells, with a broad-spectrum antimicrobial activity against fungi and bacteria.

Use of nematode resistant yam varieties can be an effective strategy in controlling the disease, however, there are no resistant varieties to nematodes. The application of transgenic approach could serve as a viable alternative for improvement of the nematode resistance of yam. Several transgenes have been noted to confer plant resistance to both tropical and temperate plant parasitic nematodes [98]. Cystatins inhibit nematode digestive cysteine proteinase activity, thereby suppressing the growth and multiplication of these pests [99]. Cystatin is one of the transgenes that has been successfully applied to control plant nematodes. The cystatins transgene confers improved resistance to a range of nematodes in different crops including potato, sweetpotato, rice, tomato, and plantain [100–105]. The transgene has displayed proven efficacy under field conditions [102]. Such an approach could be exploited for genetic enhancement of resistance of yam against nematodes in the near future.

In sweetpotato, genetic modification studies show that the suppression of βcarotene hydroxylase (CHY-β), which catalyzes the hydroxylation steps of both β-carotene into β-cryptoxanthin and β-cryptoxanthin into zeaxanthin, significantly increased the β-carotene and total carotenoid content in transgenic cultured cells [106]. Moreover, suppression of lycopene β-cyclase (LCY-β), which catalyzes the cyclization steps of lycopene to produce β-carotene, resulted in increased total carotenoid content [107]. Transgenic sweetpotato plants overexpressing an Or homolog, IbOr, showed increased carotenoid contents compared to non-transformed control plants [108]. These findings indicate that the CHY-β and LCY-β are key enzymes of carotenoid biosynthesis in sweetpotato that could be exploited as good targets for molecular breeding.

In many plants, transcription factors belonging to MYB-family are implicated in the control of a tissue-specific accumulation of anthocyanin. Mano et al. [109] investigated gene expression analysis using purple-fleshed clones and transformation using sweetpotato leaves and calli. Found that one of the MYB-type transcription factors in sweetpotato, IbMYB1, regulates anthocyanin accumulation in storage roots. The overexpression of this gene successfully induces anthocyanin accumulation in the storage roots of an orange-fleshed sweetpotato cultivar, resulting in higher radical scavenging activity [110]. Comparison of the structure of the IbMYB1 genes between high-anthocyanin content and non-anthocyanin cultivars revealed distinct IbMYB1 copies of IbMYB1-2a and IbMYB1-2b, shared only in the high-anthocyanin cultivars and their common ancestor 'Yamagawamurasaki' [111]. The PCR fragment amplified from IbMYB1-2a and IbMYB1-2b cosegregated with the pigmentation of the storage roots in the F1 progenies of high-anthocyanin cultivars, suggesting the usefulness of the PCR fragment as a selection marker for high-anthocyanin lines [111].

Transgenic sweetpotato plants overexpressing IbMYB1 were detected to exhibit an elevated total polyphenol level [110]. The gene expression of phenylalanine ammonia lyase (PAL), cinnamate 4-hydroxylase (C4H), and 4-hydroxycinnamoyl-CoA ligase (4CL), involved in the early steps of both anthocyanin and caffeoylquinic acids (CQA) biosynthesis of the transgenic plants, was upregulated. Tanaka et al. [111] also noted a suppressed expression of these genes in a white-fleshed mutant of 'Ayamurasaki'. Padda and Picha [112] detected variations in the CQA content in the storage roots of non-purple fleshed cultivars, leading to the speculation that, in addition to a co-regulation of the anthocyanin content, a specific regulation mechanism of CQA content exists.

6. Conclusion

Successful genetic modification and application in complementarity with conventional population improvement techniques and advanced genomics and phenomics tools can contribute and accelerate the genetic gain in cassava, sweetpotato and yams compared to using conventional technique only. Genetic modification technology in root and tuber crops is transitioning from development to application. This has great potential in promoting industrialization of these crops and their immense contribution to food security. Some of the successes of transgenic technology include virus resistance, improved nutritional quality, improved starch yield and quality, reduced cyanide content in cassava, improved biomass, and delayed post-harvest physiology deterioration in storage roots. Based on existing literature, genome editing should be incorporated into the root and tuber improvement programmes and targeted traits should be decided in consultation with breeders. The ethics and regulation of genetically modified and gene-edited crops should be seriously considered in the application of these technologies.

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Conflict of interest

The authors declare no conflict of interest.

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