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REVIEW

Potential applications of the CRISPR/Cas technology for genetic improvement of yam (*Dioscorea* spp.)

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Abstract

Yam (*Dioscorea* spp.) is an economically important crop grown in the tropical and subtropical regions, producing tuberous roots that serve as a staple food, an income source, and an excellent source of various pharmaceutical precursors. Yam production is constrained by disease and pest infestations and a range of abiotic stresses. Genetic improvement can significantly mitigate these challenges, improve productivity, expand the yam markets, and increase economic gains. However, several intrinsic attributes of the crop have curtailed progress in yam breeding. Advanced genetic engineering such as genome editing by sequence-specific nucleases has emerged as complementary approaches to conventional breeding techniques. Mainly, the clustered regularly interspaced short palindromic repeats/CRISPR-associated protein (CRISPR/Cas) system for genome editing has provided robust platforms for gene function analysis and crop improvement in the post-genomic era. Despite its significance, research towards improving the yam species remains under-represented compared to other staple tuber crops such as cassava and sweet potato. Thus, it is critical to explore avenues for increasing the genetic gains from this under-exploited crop. The present review focuses on the progress and prospects for applying the CRISPR/Cas technology for yam improvement. The study elaborates on the currently available CRISPR/Cas tool for yam genome engineering and explores the potential applications of this toolkit in mitigating the various challenges encountered in yam production and consumption. Furthermore, we have delved into the challenges associated with this technology and the improvements made to minimize these challenges. The insights presented herein provide a guide for yam improvement to increase genetic gains from this under-researched and under-utilized resource.

KEYWORDS

CRISPR/Cas, *Dioscorea* spp., genome editing, yam improvement

1 | INTRODUCTION

Yam (*Dioscorea* spp.) is a multi-species, tuberous food crop with tremendous economic, sociocultural, and pharmaceutical importance. In terms of global production of tuber crops, yam ranks fourth after potato, cassava, and sweet potato (Chandrasekara & Kumar, 2016). Yam is grown by smallholder farmers on 8.7 million hectares of land with global production of 72.6 million tons, to which Africa contributes over 96% (Bhattacharjee et al., 2018; FAOSTAT, 2018). West Africa accounts for 66.7 million tons of yam, and over 99% of yam production lies in a five-country "yam belt" that includes Nigeria, Benin, Togo, Ghana, and Côte d'Ivoire (66.44 million tons). Nigeria is the world's largest grower of yam, with an annual production of 47.5 million tons, accounting for over 65% of the global yam production (FAOSTAT, 2018).

Yam is a staple food and an income source for approximately 300 million people worldwide, particularly in sub-Saharan Africa. Several attributes of the yam plant, such as diversity of maturity periods and the potential for long-term storage, make these tubers vital for food security in developing countries (Mignouna et al., 2008). The tubers are a source of food security to food deficient in low-income countries, providing approximately 200 kilocalories daily. Yam tubers are rich in vitamin C, essential minerals, dietary fibre, and starch but significantly low beta carotene, riboflavin, and thiamine (Chandrasekara & Kumar, 2016). Besides, the starch content of some *Dioscorea* species is higher than cassava and most cereal crops and therefore has a high potential for the production of industrial starch (Ezeocha et al., 2012). It also produces various secondary metabolites, including alkaloids, diterpenoids, and steroidal saponins, which serve as essential precursors of pharmaceutical excipients. Despite the enormous economic importance, the crop has not shown progressive productivity gain over the last decades due to various production constraints, including the high cost of planting materials, high labour costs, poor soil fertility, low yield potential of local varieties, pests such as nematodes, diseases like anthracnose and viral infections, and shortage of quality seed yam of popular landraces and released varieties (Bhattacharjee et al., 2018; Darkwa et al., 2020).

Previous efforts towards yam improvement by empirical breeding have generated varieties with improved traits, including good organoleptic attributes, wide adaptability, and resistance to multiple pests and diseases. However, classical yam breeding is constrained by the crop heterozygous, dioecious, and polyploid nature, vegetative propagation, poor seed set, non-synchronous flowering, and long breeding cycles (Mignouna et al., 2008). Therefore, improvement of the yam germplasm necessitates applying modern biotechnological tools such as genetic transformation and genome editing that can allow direct manipulation of the genome.

Transgenesis has been applied in the last decade to complement classical breeding efforts in improving most crops, including vegetatively propagated crops. However, transgenic research in yam has only been limited to proof-of-concept efforts with the introgression of reporter genes. This slow progress can be attributed to the limitation of resources for yam research, lack of good genomic information, and genetic resources for yam (Nyaboga et al., 2014). The recent sequencing of various yam genomes (Saski et al., 2015; Siadjeu et al., 2020; Tamiru et al., 2017) coupled with modern breeding tools offers unprecedented opportunities to accelerate yam biology research and genetic improvement.

Unlike traditional breeding systems, modern crop breeding with engineered nucleases enables the precise and efficient alteration of the plant genome. Sequence-directed nucleases (SDNs) have been extensively used in numerous plant species to improve agricultural traits and speed up gene function analysis (Bortesi & Fischer, 2015). The most recent and widely used SDNs include zinc-finger nucleases (ZFNs) (Ramirez et al., 2008), transcription activator-like effector nucleases (TALENs) (Weeks et al., 2016), and CRISPR/Cas systems. While engineering ZFNs and TALENs are time-consuming and involve complicated design protocols, the CRISPR/Cas technique is robust, cost-effective, and easier to implement (Jinek et al., 2012).

The CRISPR/Cas-based genome editing can synergize conventional breeding by making precise changes in the yam genome to develop new traits such as disease resistance and integrate such traits into elite cultivars for wide distribution. Several comprehensive reviews discussed the application of CRISPR/Cas tools for the improvement of tropical crops (Haque et al., 2018; Islam, 2019; Molla et al., 2020). Harnessing precise gene modification could mitigate the major yam production constraints, notably disease vulnerability, post-harvest deterioration, and low yield potential. The genome editing technologies also offer possibilities for improving the yam nutritional composition, enhancing tolerance to abiotic stresses, and metabolic engineering of products applicable to pharmaceutical, biofuel, and agricultural industries. This review article intends to provide an overview of the challenges facing yam production and consumption as well as the recent progress and perspectives to explore the application of CRISPR/Cas-based genome editing to improve yam, an essential but neglected tuber crop.

2 | CONSTRAINTS FOR YAM PRODUCTION

The demand for yams by consumers in sub-Saharan Africa is increasing, but their production is declining due to biotic and abiotic stresses. These constraints include susceptibility to pests (e.g. insects and nematodes) and diseases (e.g. anthracnose, viruses, tuber rots), high cost of seed material, declining soil fertility, and low yield potential (Adegbite et al., 2008). It has been estimated that more than a quarter of yam produce is annually lost to pests and diseases. Yam pests reduce both the quantities of yam produced and their quality, making them unattractive to consumers. Yam is susceptible to disease infestation at the seedling stage, during and after harvesting (Adegbite et al., 2008).

Anthracnose disease, caused by the fungus *Colletotrichum gloeosporioides*, is considered the most economically significant field disease of yams (Bhattacharjee et al., 2018). The fungal pathogen causes leaf necrosis and die-back of the stem of the infected yam plant, which impairs the plant photosynthetic competence and subsequently leads to over 90% production losses (Egesi et al., 2007).

Plant-parasitic nematodes such as *Scutellonema bradys* have an enormous economic impact on crop productivity because they reduce the yield and quality of tubers and facilitate fungal and bacterial attacks, both in the field and during storage (Coyne et al., 2016).

Yams are afflicted by viruses from the genera of *Aureusvirus*, *Badnavirus*, *Carlavirus*, *Comovirus*, *Cucumovirus*, *Fabavirus*, *Macluravirus*, *Potexvirus*, and *Potyvirus* (Bömer et al., 2019). Among these viruses, *Yam mosaic virus* (YMV, genus *Potyvirus*), *Yam mild mosaic virus* (YMMV, genus *Potyvirus*), and *Dioscorea bacilliform* viruses (DBVs, genus *Badnavirus*) are widespread in West Africa. YMV is considered the most devastating viral pathogen of yam (Seal et al., 2014). The spread of yam viruses in West Africa has been attributed to the use of diseased propagules and unrestricted introduction of infected germplasm through porous land borders (Bömer et al., 2019).

3 | GENETIC IMPROVEMENT OF YAM

The constraints mentioned earlier to yam production and consumption require the development of multidimensional strategies that ensure food security while maintaining environmental integrity without negatively impacting agricultural sustainability. The subsequent sections highlight the progress made to improve the scientific understanding and technological capabilities for enhancing the yam crop. It also highlights some potential strategies that could be implemented to achieve sustainable crop production and provide maximum nutritional and economic gain to yam farmers and consumers.

3.1 | Conventional breeding of yam

Most research efforts in yam have been limited to understanding the crop genetics and generating its genomic information (Saski et al., 2015). Yam breeding programs have focused on resolving the primary challenges in yam production and consumption, including pest and disease resistance, increased tuber yield potential, and unique combinations of multiple desired attributes (Mignouna et al., 2008). Over the last five decades, breeding programs have identified several trait progenitors and released many improved yam accessions. For instance, resistance to YMV has been identified in some breeding lines of *D. rotundata*, and attempts have been made to incorporate this resistance into agronomically valuable varieties (Mignouna et al., 2001a).

The advancement of yam breeding programs to contemporary levels for designing new genotypes with resistance or tolerance to biotic and abiotic stresses has been significantly constrained by the lack of systematic knowledge and understanding of the genetics and genomics of the crop (Tamiru et al., 2017). Thus, the availability of many genomic resources, including genome-wide molecular markers, will accelerate the breeding efforts and application of genomic selection in yams. Previously AFLP (amplified fragment length polymorphism) markers associated with anthracnose resistance in *D. alata* have been identified (Mignouna, Njukeng, et al., 2001a; Petro et al., 2011). In a recent study, Bhattacharjee et al. (2018) developed an EST (Expression Sequence Tags)-SSR (simple sequence repeat)-based genetic linkage map and identified quantitative trait loci (QTLs) for anthracnose resistance in *D. alata*. Notably, the linkage map and QTLs could fast-track breeding for anthracnose resistance in yam. At present, these traditional efforts for trait identification have been augmented by novel techniques such as genome sequencing, functional genomics research, and genome editing (Tostain et al., 2006). Besides, the recent sequencing of the genomes of *D. rotundata* (Tamiru et al., 2017), *D. alata* (Saski et al., 2015), and *D. dumetorum* (Siadjeu et al., 2020) are expected to expedite the identification and breeding of novel traits for improvement of yam.

3.2 | Genetic engineering of yam

Genetic engineering can complement conventional breeding towards yam improvement. Several studies have

developed systems for transient and stable transgene expression in yam, including particle bombardment (Tör et al., 1993), polyethylene glycol (PEG)-mediated transfection (Tör et al., 1998), and *Agrobacterium*-mediated transformation (Nyaboga et al., 2014; Quain et al., 2011). Among these protocols, *Agrobacterium*-mediated transformation is the most preferred because it is readily available, facilitates the integration of a low copy number of transgene segments into the genome, and is relatively inexpensive. However, the regeneration of transgenic plantlets was not feasible in all these studies, except Nyaboga et al. (2014), who reported stable *Agrobacterium*-mediated transformation and subsequent recovery of transgenic events. We have further optimized the transformation protocol based on several factors such as preculture of the explants, micro-wounding of the explants through sonication, *Agrobacterium* cell density, vacuum infiltration during co-cultivation, and including the antioxidants in the regeneration medium. The optimized protocol is currently used for the transformation of *D. rotundata* and *D. alata*. Figure 1 shows the various steps of stable genetic transformation of yam using nodal explants. The transformation efficiency using nodal explants is relatively low. Therefore, we further developed an efficient and reproducible system for plant regeneration via somatic embryogenesis (Manoharan et al., 2016). This system was further refined for the generation of friable embryogenic calli (FECs) (Syombua et al., 2021, unpublished). The FECs are highly proliferative and provide excellent target explants for genetic transformation. In cassava, for instance, FECs are considered the most suitable target tissues for regeneration and transformation (Nyaboga et al., 2013).

Genetic engineering has been applied to mitigate crop production and consumption challenges by enhancing crop yield, nutrient levels, and pest and disease resistances (Ahmad & Mukhtar, 2017). For instance, RNA interference (RNAi), one of the most widely applied strategies for crop engineering, has emerged as a valuable tool for gene silencing. Suppression of pathogen effectors or virulence factors by RNAi has been used to generate resistance against cassava mosaic virus (Ntui et al., 2015), *Fusarium graminearum* in barley (Schöneberg et al., 2018), potato late blight (Jahan et al., 2015), and rice sheath blight (Tiwari et al., 2017) among others. Thus, these strategies could potentially engineer resistance to nematodes, anthracnose, and viral diseases in yam.

Despite the availability of a system for stable gene integration in yam, there is currently no report on the integration of agronomically important traits in this crop. The primary impediments to yam improvement by the transgenic approach include lack of efficient regeneration protocols and scarcity of knowledge on appropriate target genes in yam (Tamiru et al., 2017). All crops rely on the availability of efficient and robust transformation and regeneration protocols to recover the transgenic events (Nyaboga et al., 2014). However, most of the studies showed a low transformation and regeneration efficiency. This challenge is further compounded by the high genotype dependence of most tissue culture protocols and regeneration recalcitrance of some farmer preferred varieties (Paul et al., 2020). One possible way for mitigating this challenge in yam is to improve the transformation and regeneration efficiency using morphological regulator genes such as *WUSCHEL2* (*Wus2*), *Baby boom* (Bbm), and *SHOOT MERISTEMLESS* (*Stm*). The morphological regulators have been used to improve the plant transformation efficiency of other recalcitrant crops such as cereal crops (Gordon-Kamm et al., 2019; Masters et al., 2020). Employing these morphogenic transcription factors in plant genetic transformation and editing systems offers avenues for genotype-independent improvement of yam (Kausch et al., 2019). As yam is a monocot, its transformation can be improved by overexpressing *Bbm* and/or *Wus2* well-characterized genes from maize or upregulating the yam orthologs of the morphogenic regulators (Figure 1b).

Regulatory legislation for genetically modified (GM) crops also limits the application of transgenesis for crop improvement. Most countries have enacted stringent restrictions on the research, production, and marketing of GM products, which slow or impede the realization of the technology's benefits (Komen et al., 2020).

3.3 | CRISPR/Cas-based genome editing for crop improvement

Innovation in crop improvement is critically needed to enhance the production for food and nutrition security. The advances in CRISPR/Cas-based genome editing have facilitated efficient and targeted manipulation in several crops, showing its potential for fast-tracking crop improvement (Chen et al., 2019). The CRISPR/Cas system, developed from the adaptive immune system of bacteria and archaea, is the most advanced and preferred genome editing system. The system is based on the induction of double-stranded breaks (DSBs) at a target site and subsequent repair of DSB either through non-homologous end joining (NHEJ) or homology-directed repair (HDR) (Kim & Kim, 2019). Based on the repair, the editing can be SDN1, SDN2, SDN3. The SDN1 is a very efficient, error-prone repair of a targeted DSB through NHEJ, leading to a mutation causing gene knockout, gene silencing, or a change in the function of a gene. Whereas SDN2 is less efficient and high fidelity through HDR, it allows introducing the mutation(s) at the target site. SDN2 is through a template-guided repair of a targeted DSB using a repair template

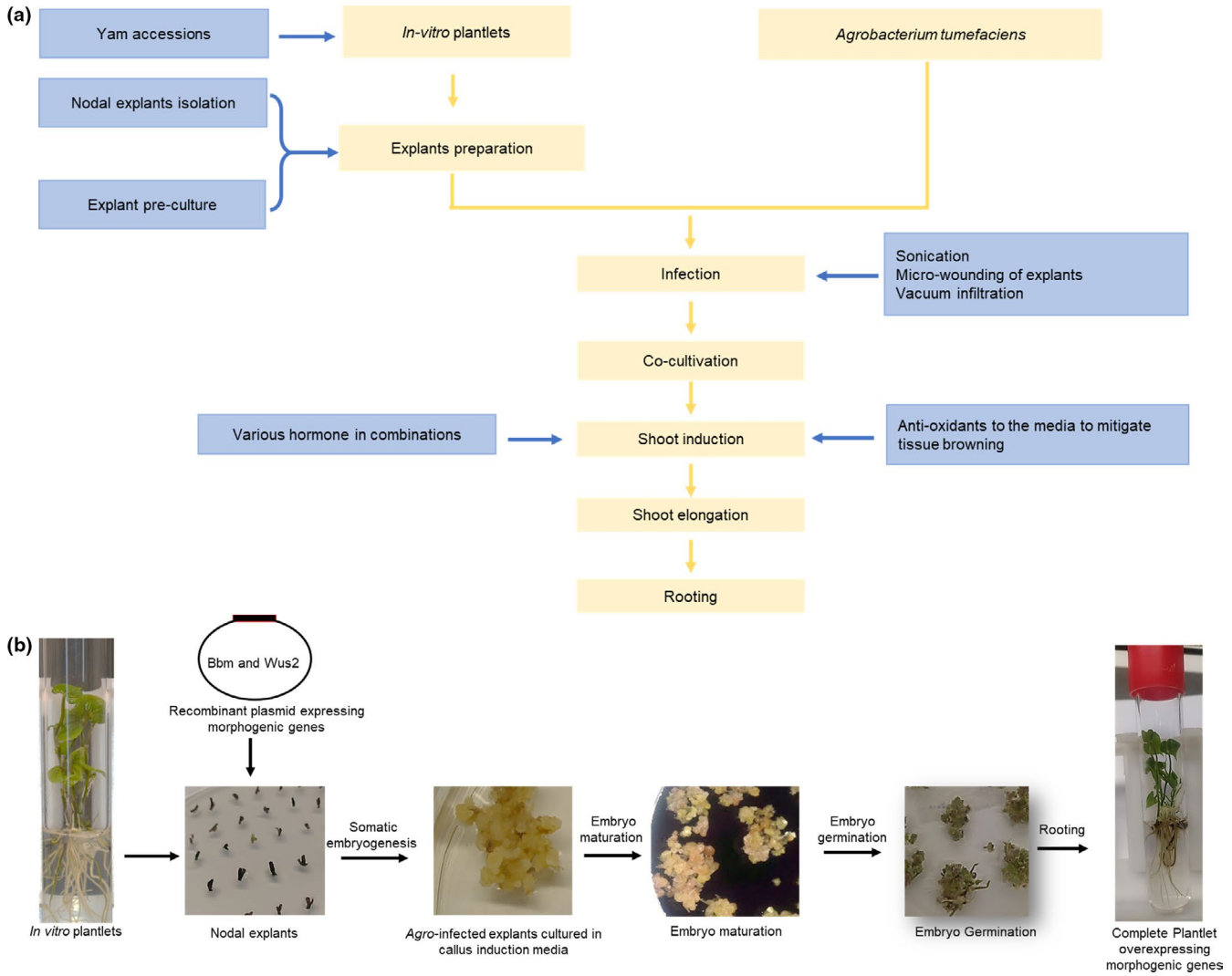

FIGURE 1 Possible strategies for developing robust and genotype-independent transformation system for yam. (a) Transformation protocol currently used in IITA transformation laboratory. (b) Strategy to improve yam transformation using the morphogenic genes such as *Bbm* and *Wus2*

with one or several small mutations. SDN3 is also less efficient and high fidelity generated using a donor sequence through a template-guided HDR repair of a targeted DSB. SDN3 leads to the insertion of the entire gene at the target site. The SDN1 and SDN2 are indistinguishable from mutations obtained through spontaneous natural mutations and, therefore, not subjected to regulation as GM crops in several countries (Tripathi et al., 2020).

The widely used Cas9 from *Streptococcus pyogenes* and its variants (SaCas9 or StCas9) have been shown to recognize PAM (protospacer adjacent motif) sequences in the canonical NGG sequence and non-canonical NGA, NAG, or NGCG (Kamburova et al., 2017). Recently, numerous studies have demonstrated the potential for using CRISPR/Cas12a as an alternative tool for genome editing in various organisms, including plants. Cas12a utilizes a thymidine-rich PAM site, 5′-TTTN-3′, guided by a single CRISPR RNA (crRNA). This system, previously known as Cpf1, is an endonuclease of the class 2 CRISPR family from *Prevotella* and *Francisella*1 (Alok et al., 2020). The CRISPR toolbox also contains the type VI CRISPR/Cas13, which targets RNA instead of DNA (Abudayyeh et al., 2017). Base editing and prime editing are the further evolution of CRISPR/Cas-based tools for precise genome modification (Abdulla et al., 2020). They can directly create point mutations in genomic DNA without inducing a DSB and do not require a DNA donor template.

3.4 | CRISPR/Cas9-based genome editing tool for yam

The availability of a well-annotated reference genome of *Dioscorea* spp., genetic transformation protocols, and

advancement in bioinformatics tools makes yam a suitable candidate for developing improved varieties via genome editing. Recently, a CRISPR/Cas9-based genome editing tool was developed for yam (*Dioscorea rotunda*) targeting the *Phytoene Desaturase* (*PDS*) gene, a gene involved in carotenoid biosynthesis as a visual marker (Syombua et al., 2020, Figure 2a). Mutation in the *PDS* gene results in albino and dwarf phenotypes due to disruption of the photosynthetic machinery, gibberellin, and carotenoid biosynthesis. Previously, PDS has been used as a visual marker gene for establishing genome-editing protocols in several plant species such as Arabidopsis (Wang et al., 2005), cassava (Odipio et al., 2017), grapevine (Wang et al., 2018), petunia (Zhang et al., 2016), maize (Liang et al., 2014), apple (Nishitani et al., 2016), soybean (Du et al., 2016), rice (Banakar et al., 2019), and banana (Ntui et al., 2020) among others.

Syombua et al. (2020) demonstrated that the CRISPR/Cas9 could induce targeted mutations in the *PDS* gene, disrupting its function and produced stable phenotypical changes in yam. The established CRISPR/Cas9-based genome editing system for yam showed an efficiency of 83% in the accession Amola. The system needs to be tested further with more target genes and more yam accessions. The

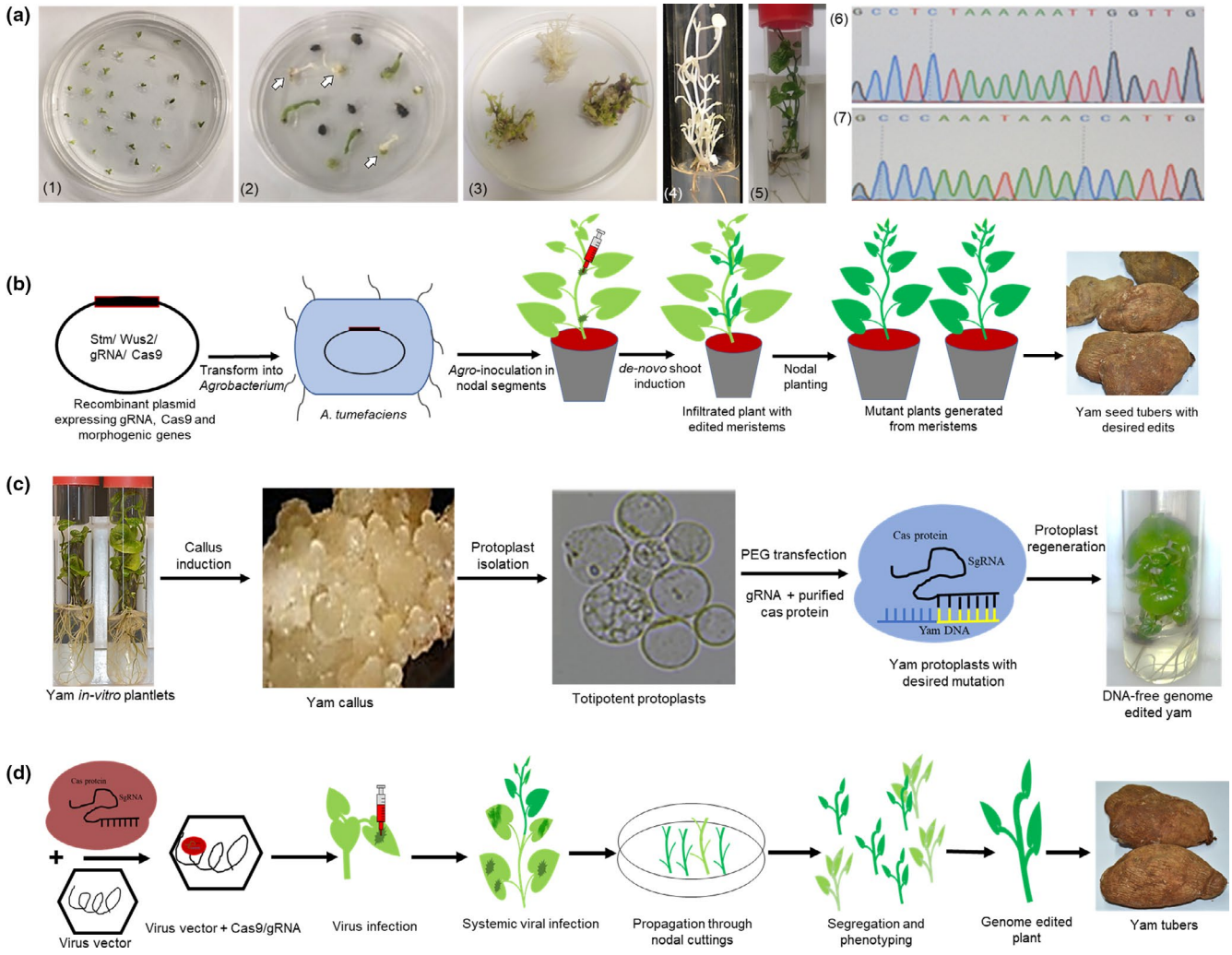

FIGURE 2 Schematic illustration of strategies for delivery of CRISPR/Cas reagents to yam tissues. (a) Plasmid-based delivery of CRISPR/Cas9 reagents through *Agrobacterium*-mediated transformation of nodal explants and regeneration of edited plants of yam. 1: Transfection of CRISPR/Cas9 reagents (plasmid containing *Cas9* gene and gRNAs targeting the *PDS* gene) onto yam nodal segments via *Agrobacterium*-mediated transformation, 2–3: Regeneration of mutants; arrows show putative albino shoots, 4: Edited yam plantlets showing complete albino phenotype due to disruption of function of *PDS* gene, 5: Green control wild-type plantlet, 6: Sanger sequencing chromatogram of a wild-type yam plantlet, 7: Sanger sequencing chromatogram of an edited yam plantlet showing targeted mutation. (b) Delivery of CRISPR/Cas reagents along with morphogenic regulators (*Wus* 2 or *Stem*) through inoculation in the nodes of the young plantlet to induce *de novo* meristems, which subsequently generate the edited plantlets. (c) Direct delivery of ribonucleoprotein (RNP) complexes to yam protoplasts by PEG transformation and recovering the DNA-free genome edited plantlets. (d) Delivery of CRISPR/Cas reagents using viral vector *in planta* and generating the edited plantlets

established CRISPR/Cas9 system allows the precise modification of yam genes for functional genomics and trait improvement in yam. In another study, Feng et al. (2018) reported targeted mutation in the *farnesyl pyrophosphate synthase* (*Dzfps*) gene of *Dioscorea zingiberensis*, a perennial vine exclusively cultivated to produce pharmaceutical diosgenin. Even though these studies showed high editing efficiency, the genetic transformation of yam still needs improvement.

To generate the genome-edited crops, the CRISPR reagents, including Cas9 and single-guide RNAs, are delivered to the explants, and then the edited cells or tissues are cultured in tissue culture to develop complete plantlets. The development of edited plants through tissue culture is found to be inefficient, genotype-dependent, and time-consuming for several crops such as yam. Therefore, the *de novo* meristem induction system through expression of the developmental regulators can be used to develop genome-edited events more efficiently, within a shorter time, and overcome the genotype barrier (Maher et al., 2020). The CRISPR reagents and the developmental regulators such as *Wus2* and *Stm* can be delivered to nodal explants, which then induce the formation of meristems to produce shoots with targeted gene edits (Figure 2b). These edits can be stable and transmitted to the next generation. This approach can make genome editing of yam more efficient and alleviate the tissue culture bottleneck in crop improvement, enabling CRISPR/Cas-mediated gene editing for important traits in yam.

The current genome-editing system for yam relies on *Agrobacterium*-mediated transformation using a plasmid expressing the sgRNA and Cas9 gene. The plasmid-based delivery of CRISPR reagents through *Agrobacterium* or microprojectile bombardment into the plant cells is most common. This approach results in transgenic plants as the foreign gene(s) from the plasmid construct integrates into the plant genome, which can be removed by backcrossing and selecting transgene(s) free events. Generally, the delivery of CRISPR/Cas reagents by transgenic methods has significant drawbacks, including regulatory restrictions governing transgenesis (Voytas & Gao, 2014), prolonged breeding cycles for segregation of foreign DNA, and unanticipated genome damage or changes (Jupe et al., 2019). Numerous attempts have been made to deliver DNA-free reagents as preassembled Cas9 protein-gRNA ribonucleoproteins (RNPs) directly into plant cells (Liang et al., 2017; Malnoy et al., 2016; Svitashev et al., 2016). These RNPs directly edit the target cells immediately after delivery and are rapidly degraded, leaving no traces of foreign DNA elements. The DNA-free delivery system mainly utilizes protoplasts as the recipient explant. Protoplasts offer excellent targets for DNA-free genome editing as the RNPs can be easily delivered by PEG-mediated fusion (Figure 2c). Our laboratory is currently exploring the possibility of DNA-free genome editing of yam. Now, we are optimizing the regeneration of complete plantlets from protoplasts. Even though the editing efficiency is high using protoplasts, the regeneration of whole plants from edited protoplasts remains a challenge in several crops (Ghogare et al., 2021). The other common approach for DNA-free genome editing involves the biolistic bombarded of CRISPR reagents into callus or immature embryos (Zhang et al., 2021). But generally, this system is less efficient.

RNPs rapidly mutate the target sites soon after transfection and are immediately degraded by endogenous cell proteases, reducing the possibility of off-target mutations and ensures no traces of foreign DNA (Tripathi et al., 2019a; Woo et al., 2015). Yam is vegetatively propagated, and backcrossing for T-DNA segregation is challenging because the crop has a poor seed set and a lengthy breeding cycle (Mignouna et al., 2008). Thus, the ability to generate mutants without integrated foreign DNA is an attractive approach for developing yam plants with desirable traits.

Another approach for DNA-free genome editing of the crop is through the delivery of CRISPR/Cas reagents using viral vectors (Ma et al., 2020). The RNA virus-based vector can be used for DNA-free delivery of the CRISPR/Cas reagents *in planta* to develop the edited plants (Figure 2d). Ma et al. (2020) demonstrated that over 90% of plants regenerated from virus-infected tissues contained targeted mutations. Although the viral vector remains stable even after mechanical transmission, it can easily be eliminated from the edited plants during regeneration or later.

3.5 | Potential application of CRISPR/Cas genome editing in yam

3.5.1 | CRISPR/Cas for functional genomics research in yam

Targeted mutagenesis has wide applications in the functional annotation of plant genomes, significantly augmenting traditional gene identification and characterization strategies. Compared to previous approaches of chemical and physical mutagenesis, TILLING (targeting induced local lesion in genomes), and RNAi, CRISPR/Cas is precise, faster, efficient, and reproducible (Liu et al., 2019). Besides, some genes are controlled by quantitative traits, and the conventional QTL mapping and genome-wide association studies (GWAS) proves laborious. Therefore, the CRISPR/Cas technology coupled with the recent sequencing of the yam genome (Saski et al., 2015; Siadjeu et al., 2020; Tamiru et al., 2017) and pedigree analysis could facilitate rapid and efficient genes characterization in yam. This system is quick and accurate, hence beneficial for

crops such as yam, in which gene functional characterization lags behind. By creating mutants and then evaluating the subsequent loss-of-gene-function phenotype (Huang et al., 2018), it will be possible to quickly elucidate the functions of various genes in the yam genome.

3.5.2 | Strategies for improving yam for disease resistance using the CRISPR/Cas technology

Biotic stresses resulting from pathogens and pest infestations cause up to 25% yam yield losses annually (Anukwuorji et al., 2016). Thus, developing yam accessions resistant to economically significant pests (insects and nematodes) and diseases (e.g. viruses, tuber rot, and anthracnose) will improve the yield and economic value of this tuber crop. The yam pathogens can be controlled by manipulating the host plant genes through CRISPR/Cas technology (Table 1).

CRISPR/Cas for virus resistance

Yams are vegetatively propagated from seed tubers, and most farmers obtain planting material from their farms or surplus material from their neighbours. This practice facilitates pathogen accumulation and perpetuation from the infected low-quality material, particularly viruses. Subsequently, farmers suffer substantial yield losses and a reduction in the yam crop quality (Mantell & Haque, 1978). Yam viruses also impede the international exchange of germplasm. CRISPR/Cas technology can be applied to control yam viruses by targeting viral genomes or host susceptibility genes (Table 1).

Eukaryotic translation initiation factors, including *eIF4E*, *eIF(iso)4E*, and *eIF4G*, are host factors with redundant functions in plants and aid in replicating plant RNA viruses (Sanfaçon, 2015). Thus, editing of the yam *eIF* locus could generate resistance against yam viruses as has been demonstrated in other crops such as cucumber against *cucumber vein yellowing virus*, *zucchini yellow mosaic virus*, and *papaya ringspot virus*-type W, and rice against *rice tungro spherical virus* (Chandrasekaran et al., 2016; Macovei et al., 2018). Similarly, CRISPR/Cas9 was applied to generate resistance against the *cassava brown streak virus* (CBSV) in cassava plants by modifying two *eIF4E* isoforms (Gomez et al., 2019). The mutations delayed and weakened CBSV symptoms in cassava shoots, attenuated disease severity and incidence in the storage roots, and reduced tuber necrosis. This success in modifying the *eIF4* gene to generate virus resistance in various crops demonstrates the feasibility of its application to obtain virus resistance in crops whose genomes are less characterized, such as yam. This approach could specifically generate resistance to yam RNA viruses, including YMV, YMMV, yam asymptomatic virus 1 (YaV1), and *Dioscorea mosaic-associated virus* (DMaV).

The knockout of integrated viral sequences from host plant genomes is a feasible approach for controlling dsDNA plant viruses, particularly badnaviruses. For instance, in the banana crop, resistance against the *banana streak virus* (BSV) was achieved by knocking out the integrated endogenous BSV sequences from the host genome, eliminating the chances of their activation to infectious viral particles (Tripathi et al., 2019b). This approach could be applied to control yam badnaviruses, which are prevalent in West Africa. They are pararetroviruses with the viral sequences integrated into the host yam genome (Seal et al., 2014).

CRISPR/Cas for resistance to fungal pathogens

Fungal pathogens causing the anthracnose disease represent the most economically significant field pathogen for *D. alata*, the most widely cultivated yam species globally. Anthracnose is a foliar disease caused by several related fungal pathogens of the *Colletotrichum* genus. It is the most widespread of all field diseases of yam, causing severe yield losses globally (Amusa et al., 2003). Other yam infecting fungi include *Botryodiplodia theobromae*, *Rosellinia bunodes*, *Aspergillus niger*, *Aspergillus tamari*, *Fusarium* spp. causing tuber dry rot and *Rhizopus* spp., causing tuber wet rot (Amusa et al., 2003).

Genome editing by CRISPR/Cas has demonstrated significant potential in generating fungal resistance in various crops, mainly by losing function of host susceptibility (S) genes. For instance, knocking out the mildew resistance locus proteins (MLO) has generated resistance to fungal pathogen in various crops, including tomato and wheat (Nekrasov et al., 2017; Wang et al., 2014). The *MLO* locus encodes plasma membrane protein and is evolutionarily conserved in monocots and dicots (Acevedo-Garcia et al., 2014).

The resistance against fungal genes was also demonstrated by targeting *Ethylene Response Factor 922* (*ERF922*) and *enhanced disease resistance 1* (*EDR1*), which are involved in ethylene signalling and pathogen resistance. For example, editing the *OsERF922* gene led to resistance against the fungal rice blast disease (Abdelrahman et al., 2018). Gene-edited wheat with a mutation in *TaEDR1* showed enhanced resistance to the powdery mildew disease (Zhang et al., 2017).

Another target gene for providing resistance to fungal diseases is *WRKY* transcription factors that regulate the plant's defence response. For instance, a genome-edited grapevine with the mutation in the *VvWRKY52* showed resistance against *Botrytis cinerea* (Wang et al., 2018). The *Non-Expressor of Pathogenesis-Related 3* (*NPR3*) gene

TABLE 1 Summary of potential target gene orthologs of yam that could be edited by CRISPR/Cas to improve traits

Target trait	Potential target gene(s)	Gene function	References
Virus resistance	*eIFs*	Involved in virus infection in plants, recessive resistance genes required for plant RNA virus–protein translation processes in several crop species	Gomez et al., 2019); Chandrasekaran et al. (2016)
Virus resistance	Dicer-Like (*DCL*) genes	DCLs play a key role in RNA-silencing mechanisms, acting in gene regulation via miRNAs and in antiviral protection in plants	Kwon et al. (2020)
Resistance to yam badnaviruses	Endogenous virus sequences	Viral genome integrated in the host plant genome	Tripathi et al. (2019b)
Fungal disease resistance	*MLO*	Susceptibility gene, inhibit resistance to fungal diseases	Nekrasov et al. (2017); Wang et al. (2014)
Fungal disease resistance	*NPR3*	Susceptibility gene, negative regulator of defense response	Backer et al. (2019)
Fungal disease resistance	*WRKY52*	Plant response to multiple biotic stress factors, negative role in defence signalling	Wang et al. (2018)
Fungal disease resistance	*PAL* and *LOX*	Involved in oxidative metabolism in plants	Bill et al. (2017)
Bacterial disease resistance	*SWEET14, SWEET13 and SWEET11*	Function as susceptibility genes to bacterial pathogens	Oliva et al. (2019); Xu et al. (2019)
Bacterial disease resistance	*DMR6*	Susceptibility factor to bacterial and fungal pathogens	Thomazella et al. (2021); Tripathi et al. (2021)
Bacterial disease resistance	*bZIP*	Confers disease resistance	Li et al. (2017)
Bacterial disease resistance	*DELLAs*	Participate in multiple physiological and developmental processes	Li, Liu, et al. (2018a)
Bacterial disease resistance	*RAV1 & RAV 2*	Regulates melatonin synthesis genes	Wei et al. (2018)
Herbicide tolerance	*ALS*	Encodes acetolactate synthase, which is involved in the biosynthesis of the branched amino acid	Tian et al. (2018)
Abiotic stress tolerance	*ERFs*	Contributes to plant survival during stress conditions	Debbarma et al. (2019)
Abiotic stress tolerance	*KUP*	KUP is responsible for potassium ion transport, which plays a vital role in the response of plants to abiotic stress	Ou et al. (2018)
Abiotic stress tolerance	*MAPKKK*	Plant response to abiotic stress	Ye et al. (2017)
Reduce post-harvest browning	*PPO*	Catalyzes the oxidation of phenolic compounds into highly reactive quinones	Nishitani et al. (2016); Waltz (2016)
Increase yield	*RBCS*	Negative regulator of photosynthesis	Donovan et al. (2020)
Increased Beta carotene content	*Lycopene epsilon-cyclase*	Participates in the carotenoid biosynthesis pathway	Kaur et al. (2020)
Enhanced starch accumulation in the roots	*Vacuolar invertase* (*VI*) & *cell wall invertase* (*CWI*)	Regulates sink strength and carbohydrate partitioning	Jin et al. (2009)
Improved starch quality	*Granule-bound starch synthase* (*GBSS*)	Elongation of amylose polymers during starch biosynthesis	Andersson et al. (2018)
Promote early flowering	*Flowering locus T*	Regulates flowering time	Odipio et al. (2017)

could be another target, as *NPR3* is a negative regulator of the defence pathway. Editing of *NPR3* in cacao conferred resistance against *Phytophthora tropicalis* (Fister et al., 2018).

The S genes are generally conserved in nature; therefore, their orthologs in yam can be targeted for developing resistance to fungal diseases. Yam genomes can be edited in a way similar to other crops in a targeted manner by editing the yam orthologs of known susceptibility genes (*MLO*, *EDR1*, *ERF922*, *NPR3*, and/or *WRKY52*) to produce new varieties with enhanced resistance to fungal diseases (Table 1).

CRISPR/Cas for the control of bacterial diseases

The most significant bacterial pathogens in yam include *Erwinia carotovora* subsp. *carotovora*, the causative agent for bacterial wet rot in yam tubers (Amusa et al., 2003), and *Bacillus pumilus* (HSeu et al., 2010). CRISPR/Cas knockout of host susceptibility genes such as downy mildew resistance 6 (*DMR6*) and *Sugars Will Eventually Be Exported Transporters* (*SWEET*) has been proven to generate durable plant resistance against bacterial diseases. Notably, the expression level of *DMR6* is upregulated during pathogen infection and is a negative regulator of plant defence responses (Damme et al., 2008; Sun et al., 2016). Therefore, modulating the expression of *DMR6* gene homologs and/or its promoters in yam could generate resistance to yam bacterial pathogens. For example, tomato and banana with mutations in *DMR6* orthologs showed enhanced resistance against bacterial pathogens of the *Xanthomonas* species (Thomazella et al., 2021; Tripathi et al., 2021).

Susceptibility genes of the *SWEET* family constitute intercellular and intracellular sucrose transporters with key roles in bacterial pathogenesis. Therefore, crop cultivars with genetic variations in the *SWEET* genes are being developed for crop disease resistance (Gupta, 2020). In a previous study, precise modification of *SWEET* susceptibility genes (*OsSWEET14* and *OsSWEET11*) or their promoters enabled resistance to the bacterial pathogens *Xanthomonas oryzae* pathovar *oryzae* in rice (Oliva et al., 2019; Xu et al., 2019). These successes in generating crop resistance to various bacterial pathogens offer insights into yam gene homologs whose expression could be modulated by CRISPR/Cas to mitigate yam yield losses due to infestations by bacterial pathogens (Table 1).

Another approach to enhance the resistance to bacterial pathogens is to activate the endogenous genes involved in defence pathways such as *bZIP*, *DELLA*, *RAV1*, and *RAV2* (Li et al., 2018; Wei et al., 2018). These genes have conferred resistance to the bacterial blight disease of cassava. Thus, the yam endogenous defence genes can be upregulated through CRISPR activation (CRISPRa), which uses a modified version of Cas9 without endonuclease activity (dead Cas proteins; dCas) with added transcriptional activators to enhance the expression of the desired gene(s).

3.5.3 | Use of CRISPR/Cas for insect and pest management in yam

Yams are infested by various insects belonging to various genera and orders, including Coleoptera, Diptera, Hemiptera, Hymenoptera, Isoptera, Lepidoptera, and Thysanoptera (Morse & McNamara, 2015). As such, there is a need to adopt integrated approaches to manage the insect populations in the field and during storage. The yam crop is also susceptible to infection by nematodes of about ten species, including the *Meloidogyne* spp., *Scutellonema* spp., and *Pratylenchus* spp. (Adegbite et al., 2008; Imafidor & Mukoro, 2016). Yam nematodes destructively feed on the tuber tissues of growing yams in soil, causing quality deterioration and reducing the tuber size. Besides, nematode infestation of yams predisposes the tubers to attack by various pathogens, resulting in dry and wet rot diseases in stored tubers. Other economically significant yam pests include aphids (Odu et al., 2004), mealybugs (*Rastrococcus* spp.), and white Scale insects (*Aspidiella hartii*) (Kolombia et al., 2017; Kwoseh et al., 2005).

Various CRISPR/Cas-based techniques could be adopted for pest resistance in yam by either modifying the plant, the insect/pest, or both. These strategies could involve modifying yam pests to stall their infesting capacity or editing the plants to increase their competence to deter pests. There are six examples as described below. 1) Cadherin receptors in insect midguts could be knocked down by CRISPR/Cas. These receptors are involved in developing resistance against insecticidal proteins (Wang et al., 2016); hence, mutant insects can be easily targeted using insecticides. 2) Modifying the pest detoxification genes, such as the gossypol-inducing cytochrome P_{450} by CRISPR/Cas9, to increase their susceptibility to insecticides (Tyagi et al., 2020). 3) Targeting insect/pest genes, such as olfactory receptors that could interrupt the identification of mating partners or chemical communication between pests, could control pest populations (Wang et al., 2016). 4) Pest developmental genes, such as the Abdominal-A (*abd-A*) gene, could be mutated by CRISPR/Cas9 to compromise insect development (Wu et al., 2018). 5) The CRISPR/Cas9-mediated modification of volatile chemicals in the yam plant could aid pest management by deterring insects. For instance, aphid-infested plants release (E)-β-farnesene (Eβf), a volatile hydrocarbon that attracts the parasitic wasp *Diaeretiella rape*. The wasp subsequently feeds on the aphids, hence contributing to a reduced aphid population (Tyagi et al., 2020). 6) Editing the

pigmentation biosynthetic pathways in yam to alter the plant appearance; hence, pests cannot identify the host plant (Malone et al., 2009).

3.5.4 | Improving yam abiotic stress resistance by CRISPR/Cas

Various abiotic stress factors, including climate variability, drought conditions, and poor soils, negatively impact yam production and diversity, occasioning the abandonment of numerous cultivars for susceptibility reasons (Loko et al., 2015). Thus, yam breeders and technology developers will need to focus on providing accessions that can flourish in unsuitable weather conditions and soils with reduced nutrient profiles. Plant abiotic stress responses are characterized by overproduction of reactive oxygen species (ROS), which subsequently induces plant growth abnormalities, such as increased cell apoptosis, reduced photosynthetic rates, male sterility, and eventually reduced yield (Choudhury et al., 2017). Therefore, CRISPR/Cas-based modulation of genes involved in ROS redox balance such as *Respiratory Burst Oxidase Homologue* (*Rboh*) (Li et al., 2015) and *WRKY53* (Wang et al., 2017) could enhance abiotic stress tolerance in yam. The expression of genes coding for enzymes that quench ROS could also be overexpressed by CRISPR/Cas, including *SOD*, *CAT*, *APX*, and *GPX* (Huang et al., 2019).

Transcription factor gene families with primary roles in plant response to different stresses, such as *ethylene response factors* (*ERFs*), *heat shock factors* (*HSFs*), and *MYB*, could also be targeted to generate yam variants with resistance to abiotic stress (Debbarma et al., 2019). Other genes with critical roles in plant response to multiple abiotic stresses include *cis*-regulatory elements and structural and/or regulatory genes such as the dehydration-responsive element/C-repeat domain (DRE/CRT) (Zafar et al., 2020).

The feasibility of applying biotechnological tools to improve abiotic stress tolerance in root tubers has been demonstrated in sweet potatoes; integration of spermidine synthase genes derived from *Cucurbita ficifolia* (*FSPD1*) enhanced tolerance to drought and salinity stress (Kasukabe et al., 2006). In maize, drought-tolerant lines were generated by CRISPR-Cas-based modification of the *AUXIN REGULATED GENE INVOLVED IN ORGAN SIZE8* (Shi et al., 2017).

3.5.5 | Improving yam nutritional quality by CRISPR/Cas

Many crops, including yam, experience browning due to the presence of polyphenol oxidase (PPO), especially during storage. In yam tubers, PPO changes the flavour, texture, and colour, thus reducing the commercial value (Jia et al., 2015). Notably, the CRISPR/Cas system can be applied to generate heritable and stable mutations on the yam *PPO* loci without affecting other crop attributes. The feasibility of applying this technology for nutrition improvement has been proven via the knockout of the *PPO* gene in potatoes, mushrooms, and apples (Halterman et al., 2016; Nishitani et al., 2016; Waltz, 2016) to create non-browning varieties.

According to Adepoju et al. (2018), raw yellow yam has significantly low levels of beta carotene and thiamine. Thus, the CRISPR/Cas approach could be applied to improve the nutritional potential of yam by redirecting the biosynthetic pathways to generate higher quantities of beneficial compounds and less anti-nutritional compounds (Sabzehzari et al., 2020). Lycopene cyclization during carotenoid biosynthesis involves two genes: *lycopene epsilon-cyclase* (*LCYE*) gene, which diverts the pathway towards biosynthesis of ε-carotenoids, and lycopene beta cyclase (LCYB), which catalyses the formation of β-rings (Richaud et al., 2018). Thus, mutations on the yam *LCYE* gene could accumulate the flux of biosynthetic precursors towards the β branch and hence increase the β-carotene contents. For instance, Kaur et al. (2020) manipulated the carotenoid biosynthetic pathway of banana by CRISPR/Cas9 to knock out the *LCYE* gene and obtained up to the sixfold increase in the β-carotene contents.

The thiamine content of yam could be enhanced by CRISPR/Cas-based overexpression of the genes involved in the biosynthetic pathway, primarily *thi1*, *thi4*, and *thiC*. In Arabidopsis, for instance, the simultaneous overexpression of *thi1*/*thi4* and *thiC* increased the seed and leaf thiamine contents by 2.6 and 3.4, respectively (Dong et al., 2015).

3.5.6 | Improving yam yield by CRISPR/Cas

The *Dioscorea* species is generally a low-yielding crop, and its cultivation is labour-intensive. For instance, the average yam yield is 8.8 t ha^{-1} (Frossard et al., 2017), while that of sweet potato and cassava are 12.2 t ha^{-1} and 12.8 ha^{-1} (Fermont et al., 2009), respectively. Besides, yams have a low multiplication ratio; hence, a significant fraction of each harvest must be preserved as subsequent planting material (Aighewi et al., 2015). The knockout of negative yield regulators can feasibly enhance crop yields (Sedeek et al., 2019). For instance, vacuolar invertase (VI) and cell wall invertase (CWI) regulate sink strength and carbohydrate partitioning in higher plants (Jin et al., 2009). Therefore, knocking down VI and CWI inhibitors could increase sucrose translocation to yam roots, hence increasing the tuber yield.

Another approach for engineering increased yield in yam could involve enhancing the photosynthetic efficiency to increase flux via the Calvin cycle, reduce photorespiration, increase carbon fixation rate, and increase the flag leaf area. It could be achieved by CRISPR-based knockout of negative regulators of photosynthesis. For instance, mutations on homologs of the ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) multigene family (*RBcs*), a rate-limiting enzyme that catalyses the first step in carbon fixation (Donovan et al., 2020), could be effected to increase the photosynthetic rate of yam and hence improve the yield. In a recent study, Chen et al. (2021) demonstrated that knocking out the *Negative Regulator of Photosynthesis 1 (NRP1)* increases the photosynthetic rate and crop biomass under field conditions.

3.6 | Application of CRISPR/Cas homology-directed repair for yam improvement

While the repair of Cas-generated DSB by NHEJ results in small random indels, HDR uses the genetic information from an artificial homologous repair template as blueprint to repair the break (Wada et al., 2020). Thus, genome modification through SSN-mediated HDR can be exploited for yam improvement by introducing novel gene functions, effecting gene replacement and knock-in, point mutations, or integrating foreign genes at desired sites in a predefined manner. The repair template can be customized to confer traits of interest, including disease resistance, enhanced nutrient contents, and abiotic stress resistance.

Allele replacement by HDR has huge prospects for accelerating crop breeding (Li et al., 2018); the many years of crossing and backcrossing involved in yam classical breeding can be reduced to 9 months of mutant generation. Among the eight predominant yam species in West and central Africa, *D. dumetorum* is the least labour-intensive (does not require staking), has the highest nutritional value [high protein content (9.6%), good balance of essential amino acids], and is high yielding (40 t/ha). However, the species is the least cultivated and consumed due to post-harvest hardening, a phenomenon in which the tubers harden within 24 hours after harvest, rendering them unpalatable (Adebowale et al., 2013). A recent gene functional analysis attributed this occurrence to the up-regulation of five genes, *MYB* transcription factor, *chlorophyll a/b binding protein1*, *2*, *3*, *4*, *xylan o-acetyltransferase*, and *cellulose synthase A* (Siadjeu et al., 2021). Thus, multiplex CRISPR-mediated HDR could be done to replace the genes with the corresponding elite alleles from other yam species, such as *D. alata*.

4 | CONCLUSION

Compared to other vegetatively propagated crops such as potato, cassava, and banana, research on the yam genome and efforts towards the application of biotechnological tools for yam improvement has substantially lagged. Efforts towards yam improvement by advanced biotechnological tools are beset by a dearth of information on the genetics of the crop and a lack of optimized regeneration and transformation protocols. Thus, the availability of technologies that allow for direct manipulation of the yam genome and involve less tissue culture steps is needed.

CRISPR/Cas-based gene targeting in yam could enable more precise and faster trait modification than the conventional transgenic approach. Traits that could be potentially targeted in yam include disease resistance, abiotic stress tolerance, increased tuber yield, and enhanced nutritional value (Table 1). Besides, CRISPR allows DNA-free genome modification and hence could mitigate the regulatory restrictions associated with transgenesis. For example, yam viral replicons could effectively deliver CRISPR reagents without the need for stable integration onto the yam genome.

More importantly, the capacity for multiplex genome editing should be explored for its ability to facilitate the simultaneous improvement of various traits in farmer preferred yam accessions. CRISPR/Cas could also offer insights into the molecular mechanism of pathogenesis of a virus or bacteria by specifically knocking down or knocking out different genes involved in pathogenesis. Considering the precision, simplicity, and versatility of the CRISPR/Cas technology, it is expected to fast-track studies on yam genes functions and mitigate the challenges encountered in yam breeding.

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AUTHOR CONTRIBUTIONS

LT is responsible for the original concept. ES, JT, and LT contributed to writing. ES and JT made figures. KW, BY, OG, and NE reviewed and edited the manuscript.

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