



## Overview of CRISPR-Cas9 Technologies and its Application in Crop Improvement

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### Abstract

Genome editing offers a range of solutions for more efficient development of crops that are productive, adapted to stresses, climate-resilient, and less dependent on agro-inputs. Clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR-associated protein (Cas9) technology is the current dominant tool used for genome editing. Originally, a Cas9 nuclease was employed to induce a double-strand break in its target site, causing the deletion of a few base pairs, inversion and gene integration to deliver desired changes in organisms. Aside from the primary nuclease activity (knock-in/out), a Base editor system, Gene priming and Cargo chauffeuring activities have been reported to deliver functionalities to specific regions in the DNA such as regulating transcription and fluorescence DNA for visualizing and understanding biological systems. Limitations of the scope of Cas9 activity were also eliminated by the recent development of more Cas9 orthologues (Cpf1-RR and Cpf1-RVR). Cas9 together with the advent of novel base editing tools that enable precise genome modifications and DNA-free genome editing via ribonucleoproteins demonstrate significant promise in the development of future crop improvement strategies. However, large-scale adoption of CRISPR/CAS will require optimizing strategies while accounting for costs, ease of implementation, and potential impacts on production gains. This review focuses on CRISPR application in plants, advances in CRISPR technology, regulations that may disadvantage scientists, resources for the smooth application of CRISPR and the preparedness of Africa to benefit from CRISPR technology.

**Keywords:** Genome editing; CRISPR Cas9; Applications; Crop improvement

### Introduction

Mutagenesis conferring genome changes in organisms may either turn off (Gene knockouts) or on (Gene knock-in) the function of genes in target regions. This results from a disruption in the synthesis of essential amino acids relevant in diverse metabolic pathways. Genetic mutants are essential for elucidating the genetic and molecular controls of many important biological mechanisms. The use of mutant-induced genetic variations in plant breeding commenced in the 1960s when radiation and chemical mutagenesis were developed to select favorable genetic combinations. Alterations in the sequence of the Deoxyribonucleic acid (DNA) (single or double-stranded) of organisms are critical for gene disruption. At present, gene transfer and elimination can be achieved through conventional crosses, mutagenesis or through biotechnological approaches like genome editing. Mutant libraries of several model plants have been generated by physical, chemical, or insertion (T-DNA/transposon insertion) mutagenesis and, more recently, by genome editing techniques [1].

Traditional mutagenesis approaches often generate random mutations in the host genome, which could often cause deleterious effects that may not be intended and have many drawbacks including large screening populations to identify desired variants. Since the late 2000s four families of engineered and programmable nucleases including Mega-Nucleases, Zinc Finger Nucleases (ZFNs), Transcription Activator-Like Effector nucleases (TALEN) and the Clustered Regularly Inter-Spaced Short Palindromic Repeats (CRISPR) Cas9 changed mutagenesis from random events to targeted modification of genomes. These developments have limited mutations to specified regions of the genome, which, have helped decipher the function of genes more accurately.

Interspaced Short Palindromic Repeats (CRISPR)–Cas9-associated protein technology is the current dominant genome editing tool used to manipulate and change the DNA of organisms. The CRISPR

technology mimics the surveillance system performed by immune memory in bacteria that enables it to recognize and degrade foreign nucleic acids. The engineered CRISPR-Cas9 system relies on single guide RNA (sgRNA), a small non-coding RNA composed of a crRNA homologous to a genome target region and a tracrRNA that binds to CAS9. Target sites are generally located in the exon regions of an open reading frame in order to induce effective frameshift mutations. The target region of CRISPR sgRNA is usually 3 bp upstream of the NGG Proto-spacer Adjacent Motif (PAM) (Meng et al., 2017). The Cas9 has a bi-lobed architecture, with a large globular recognition lobe (REC) and a small nuclease lobe (NUC) with two nuclease domains, RuvC and HNH used to edit the DNA at the right position [2]. The CRISPR/Cas9 system can be delivered to targeted cells either indirectly by biological transfer (*Agrobacterium tumefaciens*) or directly by microprojectile bombardment of cultured tissues, electroporation or chemical treatment (Polyethylene glycol-PEG) of protoplast suspensions. Alternatively, it can be delivered as ribonucleoprotein complexes in bombarded tissues or protoplasts. However, CRISPR applications in plants have largely depended on *Agrobacterium*-mediated T-DNA transformation, which is limited to a narrow range of genotypes within a species. The traditional CRISPR genome editing process relies on the double-strand break (DSB) repair capacity of recipient cell types, the promoter under which the Cas9/gRNA is expressed and the regeneration efficiency of the crop (Ding et al., 2016). The DNA breaks

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**Received:** 13-Dec-2022, Manuscript No: acst-22-83315, **Editor assigned:** 28-Dec-2022, PreQC No: acst-22-83315 (PQ), **Reviewed:** 13-Jan-2023, QC No: acst-22-83315, **Revised:** 27-Jan-2023, Manuscript No: acst-22-83315 (R), **Published:** 31-Jan-2023, DOI: 10.4172/2329-8863.1000549

**Citation:** Bissah MN, Kotey (2023) Overview of CRISPR-Cas9 Technologies and its Application in Crop Improvement. Adv Crop Sci Tech 11: 549.

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caused by the nuclease are generally repaired by non-homologous end joining (NHEJ) eventually causing deletion and/or insertion of nucleotide(s) or, when a DNA repair template with homology regions with the target site is provided, homology-directed repair (HDR). In the NHEJ approach, the cell repair mechanism attempts to rejoin the cut ends but loses or induces a few bases in the process: a situation that can result in gene alterations due to frameshift mutations. In the HDR situation, donor repair templates are supplied and the DSB repair may result in the integration of the donor sequence to allow targeted modification of genes by small to large nucleotide replacement or insertions but its efficiency remains low in flowering plants [3]

### Advances in CRISPR-CAS 9 technology development and applications

The original CRISPR-Cas9 protein uses a combination of an enzyme that cuts DNA (Cas9, a nuclease) and a guiding piece of genetic material (guide RNA) that specifies the location in the genome to be targeted. The application scope of CRISPR – Cas9 was expanded with the use of new CAS9 variants or CAS proteins with different specificities, notably the PAM, base editing, prime editing and simultaneously targeted mutations (multiplexing). Recently [4], Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas12a nuclease (Cpf1), dCas9 transcriptional regulators, base editors, PRIME editors and RNA editing tools are widely used in basic research.

### Recognition of guide RNA

The original CRISPR-Cas9 technology is based on the commonly used *Streptococcus pyogenes* Cas9 system which recognizes only Proto-spacer adjacent motif (PAM) = NGG. Alternative genome editing technologies including CRISPR *Prevotella and Francisella* 1 (Cpf1) or Cas12a have opened up CRISPR editing to additional areas of the genome have been developed [5]. The Cas9 and Cpf1 differ in the structural organization as the Cpf1 protein is smaller, use of a shorter guide as the maturation of crRNA by Cpf1 does not require the assistance of trans-activating crRNA (tracrRNA). Whereas Cas9 cleaves the DNA close to the PAM site with blunt ends, Cpf1 cleaves distal to the PAM site and produces staggered ends, which offer the opportunity for further modification at the same would also facilitate HDR. The Cpf1 recognizes TTTV as well as TTTT PAM sites increasing the target range for genome editing for the Cas9 tool. The Cas9 and Cas12a both cut DNA, while the Cas13 family has been shown to target RNA.

### Nuclease Activity

Recently, new and more powerful techniques to edit genes have been developed based on the disabled activity of the Cas9 nuclease. When the nuclease activity of the Cas9 protein is disabled it is referred to as dead (dCas9). The dead Cas9 can be fused to transcription activators, epigenetic modifiers and Fluorescence-labeled Epitope-tagged to modulate transcription reporter gene function [6]. New technologies have been developed for gene activation, replacement and insertion to satisfy various demands of genome editing for functional genomic studies which eliminate the necessary injury due to the double-strand break (DSB) associated with traditional Cas9 technology.

### Base Editing

Base editing is the conversion of one base or base pair into another (e.g. A:T to G:C, C:G to T:A) or the introduction of point mutations at specific sites which permits re-code the DNA sequences. The Adenine (Adenine - Guanine) and cytidine (cytosine-uracil-thiamine) base editors introduce mutations that largely do not disrupt the function

of genes. The cytidine deaminase enzyme removes an amino group from cytosine converting it to uracil, resulting in a U-G mismatch which gets resolved via DNA repair pathways to form U-A base pairs. Subsequently, a T gets incorporated in the newly synthesized strand forming T-A base pairs [7]. This results in C-G to T-A conversion in a programmable manner. Unlike cytidine deaminases, adenine DNA deaminases do not occur in nature. In 2017, David Liu and the group developed ABEs by using *Escherichia coli* TadA (*E. coli* TadA) through extensive protein engineering and directed evolution. *Escherichia coli* TadA is a tRNA adenine deaminase that converts adenine to inosine in the single-stranded anticodon loop of tRNA Arg. It shares homology with the APOBEC enzyme. The procedure involves the conversion of Cas9 nuclease to nickase followed by the fusion of the nickase with the deaminase enzyme (either cytidine deaminase or engineered adenine deaminase for C-G to A-T and A-T to C-G conversions respectively). The base editing technique permits the conversion of alleles by base change, the creation of premature stop codon for knock-out and the creation of new alleles of genes that do not exist in nature and has the advantage of overcoming the long procedure of introgressing new alleles. For instance, the acetolactate synthase (ALS) gene in tomatoes has been edited using *Agrobacterium*-mediated transformation with a Cytidine base editor changing amino acids essential for the protein to be targeted by the chlorsulfuron herbicide. The authors reported the production of chlorsulfuron-tolerant plants with up to 71% efficiency. This development allows the use of selective herbicides to control weeds in tomato plantations [8].

**Prime editing** is a genome editing technology by which new genetic information is written directly into a targeted DNA site in the genome of living organisms. This CRISPR-based strategy occurs without double-strand breaks (DSBs) or donor templates and permits various base-to-base conversions, including both transitions (C→T, G→A, A→G, and T→C) and transversion mutations (C→A, C→G, G→C, G→T, A→C, A→T, T→A, and T→G) (Hassan et al., 2020). The technology uses a fusion protein, consisting of Cas9 nickase fused to an engineered reverse transcriptase enzyme together with an extended guide RNA (prime editing guide RNA: pegRNA), which possesses a reverse transcription (RT) template that encodes the desired edit and a primer binding site (PBS). The prime editing technology has potential applications in plant biology research. However, high editing efficiency in diploid crops (eg rice 21%) has been reported compared to polyploid crops (eg Wheat 1.4%) [9]. In rice, the OsCDC48 gene controls chlorophyll synthesis. A single base substitution in OsCDC48 is responsible for premature senescence and death phenotype in rice 21.8% in OsCDC48-T1), lower editing efficiency (0.05-0.4%) was reported in other tested gene targets in prime editing can be used to generate crop plants for tolerance to biotic (disease tolerance, pest tolerance) as well as abiotic stresses (salinity, drought, and heat stress). Prime editing could be applied to improving plant resistance to pathogens and in the field of plant-microbe interactions. Improve the resistance spectrum of crops to diseases through functional screening to identify gene variants conferring resistance phenotypes.

### Multiplex Genome Editing

Another important development in the CRISPR-Cas9 technology is the ability to effectively express multiple sgRNAs for simultaneous editing of two or more genes. Multiplexing thus addresses the extremely time-consuming and laborious bottleneck of conventional cross-breeding methods for pyramiding multiple QTLs. In China, a CRISPR/Cas9-mediated multiplex genome editing system was used to simultaneously mutate three major genes in rice which negatively

regulated grain weight including Grain Width 2 (GW2), Grain Width 5 (GW5) and Thousand-Grain Weight 6 (TGW6). All three genes generated mutants (gw5tgw6 and gw2gw5tgw6) which showed notably larger grain sizes than that of the wild-type. In hexaploid bread wheat, CRISPR-Cas9 has been used to modify multiple alleles to confer heritable resistance to powdery mildew [10].

### Transgene Free Edited Products

An important feature of the agricultural application of CRISPR-Cas9 technology is the delivery of non-transgenic traits in crops. In seed-propagated crops, the CRISPR/Cas9 transgene can be easily eliminated through Mendelian segregation in progenies. This process alone distinguishes the technique from the class of GMOs and hence absorbs CRISPR edits from rigorous testing regimes. The transformation of protoplasts followed by plant regeneration allows either the delivery of a ribonucleoprotein complex or the transient expression of delivered plasmid DNA without DNA integration into the host genome. Such methods are particularly useful for vegetatively propagated crops. have optimized CRISPR/Cas9 ribonucleoproteins (RNPs) to completely avoid transgene integration reported 12.9% and 10% edited and transgene-free plants in the first generation of tomatoes and potatoes. Transgene-Free Genome CRISPR editing technologies have also been used to engineer bacterial blight, caused by *Xanthomonas oryzae*, herbicide tolerance and better organoleptic and nutritional value.

### Applications of CRISPR Technologies in Crop Improvement

3.1 CRISPR-Cas9 genome editing methods have been exploited in several spheres of research including breeding and development of agricultural crops, animals and human health genetic applications. In agriculture, CRISPR-Cas9 has been applied to modify and understand the functions of genes controlling major agronomic, nutritional and economic traits in a vast array of crops to achieve improved yield performance, enhanced nutritional quality (bio-fortification) as well as biotic and abiotic stress tolerance. There are several efforts of gene function analysis in important crops including Banana, cassava, maize, millet rice, sorghum, soybean, tobacco and tomatoes. show some of the current applications of CRISPR/Cas9 systems in crops are for studying gene function, improving agricultural traits, creating male-sterility mutants, and inducing haploids.

The role of genomics in plant research and sustainable crop production is imminent and it will become even more crucial in the future. Two key applications of CRISPR that will revolutionize agriculture more than the green revolution are the fixing of hybrid and enhancing diversity to improve adaptation.

### Promoting Apomixes for Hybrid Vigor Preservation through the CRISPR/Cas9 editing

Hybrid vigor is a phenomenon in which progenies display greater performance than parents for a specific trait. This phenomenon has been established in many crops; however, the phenotype is usually lost in the following generations.  $F_2$  progeny seeds from hybrid often experience trait segregation, which lowers global plant performance. Hence farmers are compelled to renew hybrid seeds which sour the cost of production. Presently, hybrid seed production in rice relies on the implementation of male sterility systems whereas hybrid maize production relies on manual emasculation. Thus, year in and year out, huge resources are committed to a labor-intensive practice in maize and the development of male sterile lines in order to produce hybrid seeds which translates into a high seed cost for hybrids. To eternalize

the benefits of useful hybrids, hybrids must be propagated by seeds so that the benefit of hybrids can reach a large number of farmers. Alternative male sterility systems can be generated by CRISPR/Cas9. In rice, through the use of CRISPR/Cas9, sterile male lines have been obtained by mutating the OsGELP34 gene which encodes a putative GDSL lipase that plays a vital role in rice male reproduction (Yuan et al. 2020). An elite rice restorer line using the nptII gene as a plant selection marker Agrobacterium-mediated genetic transformation.

Apomixis is an asexual reproductive process that bypasses female meiosis and fertilization to produce embryos identical to the maternal parent. Apomictic reproduction generates clonal seeds and offers tremendous potential in permanently preserving hybrid vigor. This kind of reproductive process has been documented in many flowering plant species, although no major seed crops have been shown to be capable of apomixis. The ability to generate maternal clones and therefore rapidly fix desirable genotypes is an important breeding strategy to preserve superior allele combinations from heterosis. Apomixis breeding has followed three approaches that include (i) introgression of apomixis or its components from related species (ii) mutagenesis and (iii) genetic engineering approaches. Sexual reproduction is controlled by a cluster of genes; therefore Apomixis requires coordinated deregulation of several genes involved in reproduction. A number of genes play a critical role in apomixis, for instance, BBM and APOSTAT are candidate genes for the induction and maintenance of apomictic events. AtREC8 and AtSCC3 genes have been found to be essential to the monopolar orientation of the kinetochores during meiosis. The MTL gene leads to haploid induction whereas the DYAD gene is a regulator of meiotic chromosome organization and is required for progression through female meiosis. Recently, CRISPR/Cas9-mediated genome editing has been used to induce mutations to divert the natural sexual pathway toward apomixis. The dyad mutant of Arabidopsis is defective in female meiosis and produces diploid embryo sacs following disrupted meiosis. Centromeres are specified by a centromere-specific histone 3 (CENH3) proteins in plants. An apomictic effect is produced in Arabidopsis lines that contain a manipulated centromere-specific histone (CENH3). In rice partial apomixis was achieved almost two decades ago by triggering parthenogenesis in MiMe-generated unreduced female gametes by ectopic expression of a male-specific OsBBM. reported multiplex editing of three (key meiotic genes Omission of Second Division (OSD1), Absence of pairing and recombination (PAIR1), and Meiotic Recombination Protein (REC8) in hybrid rice to produce clonal diploid gametes. In three separate development, CRISPR/Cas9-mediated genome editing was used to synchronously knockout three genes in rice, PAIR1, OsREC8, and OsOSD1, OsSPO11-1, OsREC8, and OsOSD1 and OSD1, SPO11/PAIR1 and REC8. A common limitation of these reports are the efficiency of seed production and paucity of pathogenesis thus additional parthenogenesis or an inducer without fertilization is needed for MiMe phenotypes to achieve apomixis and resolve the issue of low fertility. Specific expression of BBM and MATL genes promotes the elimination of the paternal genome after fertilization whereas editing of BBM1 expression or disruption of MTL leads to clonal seed production and heritability for multiple generations. However, a mutation in the MTL gene leads to haploid induction at the expense of seed production. To resolve the issue of low fertility it has been suggested that Mutation in BBM and MATL can be used in combination with MiMe to obtain apomictic plants.

### Enhancing Fitness of Crops through Genetic Recombination

**Genetic recombination** is an important process for the maintenance of genetic diversity in organisms and provides the gene



combinations needed to survive in changing fitness optimum in changing environments. Genetic recombination facilitates natural selection in addressing bottlenecks for evolution in an ecological context. Meiotic recombination is needed for homologous pairing during meiosis. It also ensures that offsprings differ in their fitness and decrease the risk factor for the population. Several genes including RECQ4, FANCM and FIGL1 are anti-cross-over genes, the mutation of which allows an increase in Cross over number. explored the effects of mutating the orthologues of FANCM3, RECQ44 or FIGL15 on recombination in three distant crop species, rice (*Oryza sativa*), pea (*Pisum sativum*) and tomato (*Solanum lycopersicum*). The authors reported that the single recq4 mutation increased crossovers by about three-fold in these crops, suggesting that manipulating RECQ4 may be a universal tool for increasing recombination in plants.

### Regulation of Genome-Edited Products

The development of genome editing technologies within a safe and ethical framework requires global governance. Presently the Americas and Australians do not regulate genome-edited products without foreign gene integration but the European Union considers genome-edited products as GMOs, hence they are highly regulated. Africa and most parts of Asia are yet to come up with regulatory mechanisms. In the European Union (EU), organisms obtained through “recent methods” of mutagenesis must be classified as Genetically Modified Organisms (GMOs) and must follow stringent release procedures including prohibitive costs, lengthy assessments and approval timelines and labeling requirements. On the contrary, the United States Department of Agriculture (USDA) lifted regulations on genetically edited crops; including CRISPR materials that do not contain CRISPR/Cas9 anymore are considered non-GMO. For instance, genome-edited products like high oleic-modified soybeans and cold storable potatoes without transgenes have been field evaluated and commercialized as non-GMO products.

Genome editing application in crop agriculture includes the enhancement of nutritional value, improving tolerance to stresses and domestication of crops. Some genome-edited products do not fall under the classification of GMOs. This necessitates that we develop guidelines to enable us to effectively regulate such without putting undue rigor on the products that do not fall under the category of GMO. In many countries, there is a lack of clarity as to the GMO or non-GMO status of genome-edited crop Germplasm. In Africa, no regulations are in place, probably because not much is being done in this field except for South Africa, Kenya, and Uganda. The safety application of genome-editing technology is a matter of discussion in the scientific community and many scientists have called for its regulation. However, the dominant view is that an international framework under which countries retain the right to make their own decisions is put in place.

### Importance of Genome Editing for Africa's Agriculture

In Sub-Saharan Africa, the majority of people depend on agriculture for food, nutrition and income security. Agriculture is constrained by the changing pattern of climatic variables and the increasing reduction of arable lands. Climate change is predicted to impact crop productivity through additional stress on crops. In addition pests and disease outbreaks that are devastating to farmers are becoming more common. Consequently, food crop production in many parts of Africa where agricultural activities are largely rain-fed will be affected. Many of the current crop varieties are less resilient to such conditions and may cause total crop failure, reduced income returns and consequently rising poverty, which, will hamper the attainment of Sustainable

Development Goals (SDGs) number 1 and 2. Hence the need to become both climate-proof and climate-smart through improved agricultural practices and resource-efficient varieties has become even more important. CRISPR-Cas 9 technology presents different strategies for crop improvement for greater resilience and better adaptation and its most important applications for food crops' improvement. Many of the production factors impeding agricultural productivity in Africa are affected by multi-gene controlled traits, which are difficult to improve and gene introgression is currently difficult to achieve. Multiplexed gene editing and transcriptional activation or repression of plant endogenous genes could be exploited to address complex traits while avoiding linkage drag during breeding and gene pyramiding. Major genes that negatively regulate important agronomic traits can be addressed through gene knockouts. The potential advantages of combining heterosis and apomixis in agricultural crops will have immediate benefits that are likely to dwarf the gains of the green revolution. In principle, CRISPR technology should facilitate the enhancement of traits and provide much-needed climate resilience for Africa.

Africa is endowed with a rich diversity of plant species that are used for food, feed and fuel. Most of these species are harvested from the wild, while others are underutilized and have not received enough scientific research recognition. These underutilized, semi-domesticated species together with their wild relatives are deficient in economically important domestication traits like germination inhibition, ripening, shattering and long processing time. CRISPR genome editing techniques could be applied to confer domestication traits on this germplasm to broaden crop genetic diversity, widen the food options and better adapt to climate variations. Selection for desirable agronomic traits could bring more diversity to the table to address food and nutritional security. The CRISPR technique can also help to address the setback in yield when current varieties are crossed with ancestral ones to introgress genes for resistance to stresses. On the African continent, CRISPR is being applied in the research of Banana Xanthomonas Wilt Disease (BXW), striga resistance, Maize Lethal Necrosis (MLN), Cassava Mosaic Disease (CMD) and salt tolerance. Banana *Xanthomonas* wilt indiscriminately affects banana cultivars with up to 100% yield loss. The technique can be extended to other crops which have consequences on the food security and incomes of farmers. CRISPR-Cas9 is currently being used in the diagnosis and management of viral diseases in bananas. The International Institute of Tropical Agriculture (IITA) in Kenya has demonstrated the local capacity to apply CRISPR-Cas9 genome editing to the development of disease tolerance to BXW disease. In Uganda, CRISPR-Cas9 is being used in research focused on overcoming the deadly Cassava Brown Streak Virus (CBSV) which is a damaging disease of cassava plants grown mainly in East Africa. These breeding efforts notwithstanding, African scientists currently have an unequal capacity, limited opportunities and incentives to realize the contributions that gene editing can make to address the intrinsic challenges plaguing African agriculture. The biggest drawback to fully exploiting the benefits of genome editing is the lack of sufficient resource allocation in the agricultural sector by various governments in Africa. Failing to take advantage of gene editing research could result in creating further inequalities in science, agricultural productivity and health in Africa. The number of people affected by hunger across Africa rose by 10 million people from 2018 to 2019 (FAO, 2020) with eight years remaining to the SDG 2 goal of 2030.

### Conclusion and Perspectives

CRISPR-Cas9 represents a significant change in how mutations

are made and presents a lot of possibilities hitherto not possible with conventional breeding for correcting abnormalities and improving the performance of current varieties. The future and the role of genome editing in plant research and sustainable crop production are eminent. Africa is at the inception -stages of using genome editing technology to improve crop production and utilization challenges. The predominant view in the literature is that CRISPR technology is simple and easy to apply mainly because the engineering advances have laid the groundwork for the creation, refinement and implementation of genome-modifying tools in advanced countries. However, a broad application of genome editing will rely on enhanced knowledge of genome organization and function, germplasm characterization, gene function characterization and identification of favorable variation in crops and their wild relatives. This should be complemented with phenotyping, omics and systems biology studies pursued across large-scale germplasm collections of important staples. There is also a need to commence the development of enabling technologies such as plant transformation protocols and tissue culture regeneration for important crops in order to take advantage of the opportunities in genome editing technologies. Comprehensive integration of these technologies is lacking in most African countries. However, there are regional centers, various CGIAR Centers, and universities that have equipped laboratories and institutions that support capacity-building in modern technological applications in Africa [11]. For instance, the Biosciences eastern and central Africa - International Livestock Research Institute (BeCA - ILRI) Hub in Nairobi, Kenya, is a shared agricultural research platform that exists to increase access to affordable, world-class research facilities. The Facility houses Genomics, Bioinformatics and Breeding platforms and offers research-related and capacity-building opportunities to African researchers. The African Orphan Crop Consortium (AOCC), Divseek Project, MoBreed and CultiVar are important initiatives that are helping to build the capacity of African scientists. The focus of the AOCC is to genetically sequence de novo and assemble and annotate 101 African Orphan Crops. The sequence information Generated from this project is openly accessible to all interested parties. DivSeek International Network aims to facilitate the generation, integration and sharing of data and information related to plant germplasm enhancement of useful exotic germplasm. The cultivar project is funded through ANR (the French National Research Agency) under the "Investissements d'avenir" programme and coordinated by Agropolis Fondation under the frame of I-SITE MUSE (ANR-16-IDEX-0006). Cultivar's main goals include the implementation of crop improvement as an impact pathway through leveraging new technologies and concepts. Educational institutions such as the West African Center for Crop Improvement (WACCI), African Center for Crop Improvement (ACCI), Makerere University Regional Centre of Excellence for Crop Improvement (MarCCI) and The UC Davis- African Plant breeding Academy are providing training to breeders in Africa to accelerate the use of modern plant breeding techniques by scientists in Africa. However, after acquiring skills in these modern techniques, scientists are unable to apply the skills to their local research because of a lack of supporting facilities. Different investments and policy actions from both national governments and development partners are needed to boost the current efforts at

using CRISPR technology to address the myriad of challenges facing agriculture in Africa.

### Acknowledgement

The Management of the CSIR-Plant Genetic Resources Research Institute (PGRRI) are acknowledged for their support.

### Compliance with ethical standards

N/A

### Competing interests:

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the publication of this article. All authors read and gave their consent for the manuscript to be published.

### Authors' contribution

Bissah drafted and prepared the manuscript. Asante, Ochar, Quain, Egbadzor, Ribeiro and Kotey wrote parts of the manuscript and provided a critical review.

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