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ABBREVIATIONS AND ACRONYMS

CRISPR Clustered Regularly Interspaced Short Palindromic Repeats

DNA Deoxyribonucleic acid

GM Genetically Modified

GMO Genetically Modified Organism

HDR Homology Directed Repair

ISAAA International Service for the Acquisition of Agri-biotech Applications

NHEJ Non Homologous End Joining

PCR Polymerase Chain Reaction

RNA Ribonucleic Acid

LGS1 Low germination stimulant 1

1.0 INTRODUCTION

Genome editing (also referred to as gene editing) comprises a group of technologies that give scientists the ability to change an organism's DNA. These technologies allow addition, removal or alteration of genetic material at particular locations in the genome. The technologies make use of site-directed nucleases that create breaks in the DNA strands and thereafter use the cell DNA repair mechanisms to introduce desired changes.

In 2012, Jennifer Doudna, Emmanuelle Charpentier, and their teams elucidated the biochemical mechanism of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) technology. By making precise targeted cuts in DNA, CRISPR ushered in endless potential in areas of medicine, agriculture, biomaterials and so on. In nature, CRISPR-Cas9 is a bacterial adaptive immune system, whereby pieces of DNA from invading viruses are cut by a bacterial nucleases, CRISPR associated proteins. The DNA fragment that is cut off is saved as memory for fighting future infections. The CRISPR-Cas9 system can be engineered to edit eukaryotic DNA by designing guide RNA complementary to the target sequence.

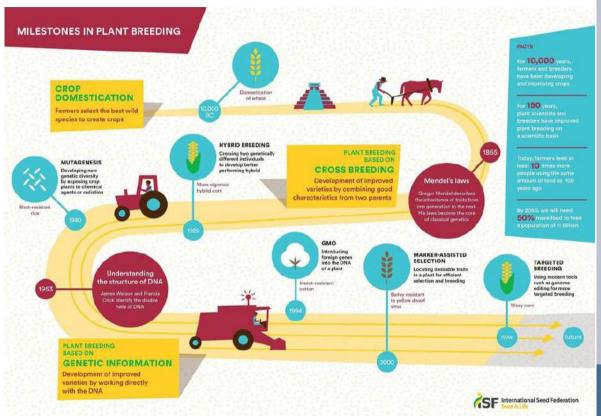
The guide RNA has a 20 base pair protospacer motif with flanking homology to the cut site of interest. Cas9 binds to this protospacer motif in the guide RNA, which in turn binds to the site of interest. Cas9 then binds to a protospacer adjacent motif (PAM) in the genomic DNA, and catalyzes a double strand break (DSB) in the DNA at a position three base pairs upstream of the PAM. If a homology

arm is provided with the CRISPR-Cas9 cassette, homology-directed repair (HDR) will occur, otherwise the cell will employ non-homologous end joining (NHEJ) to create small indels at the cut site of interest.

To date, CRISPR genome editing technology has been applied in studying gene function, human disease research including pathogenesis of hereditary diseases, gene therapy, livestock and crop genetic improvement. Genome editing differs from genetic modification in that the latter generates modifications in the genome via stable integration of DNA elements which do not occur naturally. The resulting organisms and (most) products thereof can be identified with event-specific polymerase chain reaction (PCR)-based methods targeting the insertion site. New breeding techniques such as genome editing have diversified the breeder's toolbox for generating useful genetic variability in both plants and animals. Several of these techniques can introduce single nucleotide changes without integrating foreign DNA while generating organisms with the intended phenotypes.

Since the discovery of CRISPR technology, scientists in many parts of the world have sought to use it to achieve different objectives in their research involving plants or animals. The purpose of this booklet is to highlight genome editing projects and experts in Africa making use of this technology in their respective fields.

1.1 MILESTONES IN PLANT BREEDING





Emmanuelle Charpentier

Max Planck Unit for the Science of Pathogens, Berlin, Germany

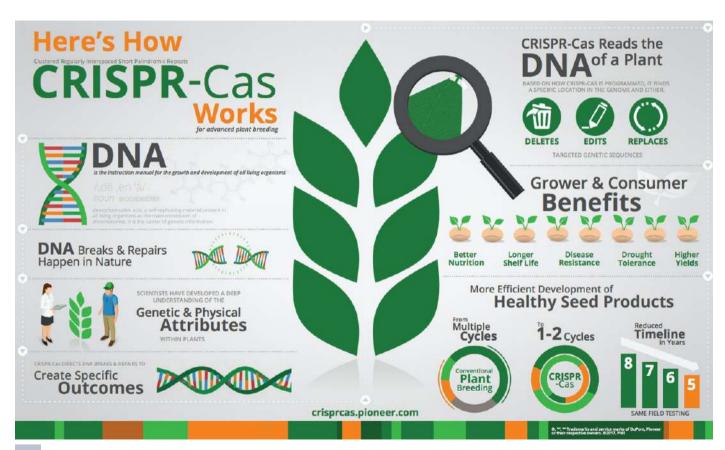


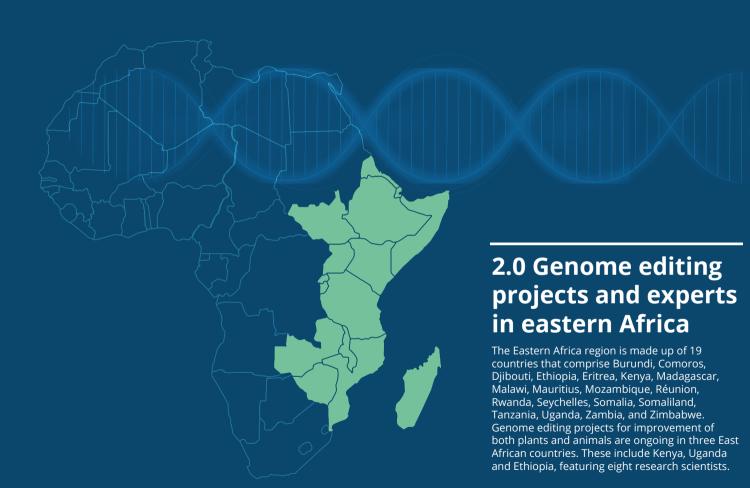
Jennifer Doudna

University of California, Berkeley, USA

The pair won the Nobel Prize in Chemistry 2020 for development of CRISPR/Cas9, a method for genome editing.

1.2 HOW CRISPR GENOME EDITING WORKS IN AGRICULTURE





Evaluation of Striga resistance in Low Germination Stimulant 1 (LGS1) mutant sorghum



Prof. Steven RunoProfessor of Molecular Biology

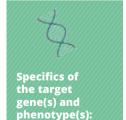
Affiliation: Kenyatta University



Parasitic weed Striga is a huge constraint to production of sorghum and other cereal crops. Most cultivated cereals, including maize, millet, sorghum, and rice, are parasitized by at least one Striga species, leading to enormous economic losses. The Striga genus has over thirty species distributed over 50 countries in sub-Saharan Africa (SSA), causing an estimated 7 billion dollars' worth of crop losses every year.



Evaluate LGS1 gene knock-out in conferring Striga resistance in sorghum.



LGS1

Mutant alleles at the LGS1 locus drastically reduce Striga germination stimulant activity.



Application of reproductive biotechnologies to develop a transgenic goat as a model for genetic control of animal diseases



Wilkister Nakami Nabulindo

PhD Graduate Fellow

Affiliation: International Livestock Research Institution



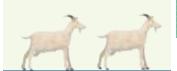
Animal trypanosomiasis is one of the diseases that cause huge losses to livestock-dependent communities in sub-Saharan Africa and efforts for its control and eradication have not been successful for decades. Scientists have in the recent past discovered a gene (Apolipoprotein L1) in primates that encodes proteins that cause lysis of trypanosomes in the body hence making the primates resistant to trypanosomiasis. A group of scientists from New York State University (Jayne Raper and co-team) have developed a synthetic version of the ApoL 1 gene that is compatible with caprine genome. This gene could be transferred to livestock to develop genetically resistant animals through transgenesis



- To investigate the feasibility of introducing a synthetic Apol1 gene into the genome of a group of goats and confirm its expression pattern
- Generation of African indigenous goat carrying the APOL 1 transgene that confers resistance to trypanosomiasis



Specifics of the target gene(s) and phenotype(s): Genomic regions that have been validated in mice and encompassing the validated synthetic APOL1 sequence will be transferred into the 'protected' ROSA26 locus using a ROSA26 miniBAC. Establish cultures of donor spermatogonial stem cells from the Kenyan Galla goats' testis, after which the ApoL 1 clone will be introduced into the ROSA26 locus of the spermatogonial stem cells by homologous recombination (CRISPR-Cas9 system). Validate synthetic APOL1 in the goat ROSA26 sequence between intron 1 and exon 2, which will also carry the neor selection marker gene. Integrants will be selected with G418 and single copy integration events will be selected by quantitative PCR-based loss of allele assay. The antibiotic resistance genes that will be used to select transformed cells will be excised before creation of transgenic animals.



Gene editing to control maize lethal necrosis in Africa for improved maize productivity and grain harvests





Senior Research Scientist, Head of Maize Lethal Necrosis (MLN) Section

Affiliation: Kenya Agriculture and Livestock Research Organization (KALRO) National Agricultural Research Laboratories (NARL), Kabete

Partnership with other institutions & Roles:

- CIMMYT Initial mapping, germplasm, breeding, phenotyping
- Corteva Genotyping, fine mapping, cloning, editing, phenotyping
- USDA-ARS Phenotyping support (validation of edits)
- KALRO Field support, advocacy, consulting, deployment



Maize lethal necrosis (MLN) disease causes severe losses to maize in Kenya and neighbouring countries. Traditional breeding approaches are time consuming and disrupt the favorable characteristics of elite varieties, whereas gene editing can achieve MLN resistance without altering desirable traits and performance of the target susceptible elite lines and varieties.



- Introduce resistance against MLN disease directly into parent inbred lines of popular commercial maize varieties, which are currently susceptible to the disease, and reintroduce them into the farmers' fields in Kenya with possible scaling out to other countries in East Africa
- Build expertise of Kenyan scientists and stakeholders through seminars, workshops, scientific visits, support and mentor one Kenyan student to conduct PhD research within the project.



 A strong quantitative trait locus (QTL) on maize chromosome 6 confers a high-level of resistance against MLN disease.



Genetic improvement of banana for control of bacterial wilt disease



Dr. Leena Tripathi

Director for Eastern Africa Hub

Affiliation: International Institute of Tropical Agriculture (IITA)



Control of Xanthomonas wilt disease of banana in East Africa.



To develop genome-edited banana resistant to bacterial wilt disease.



Phenotype:

Target genes are disease susceptibility 'S' genes and phenotype is disease resistance.



Modulation of energy homeostasis in maize to develop lines tolerant to drought, genotoxic and oxidative stresses



Dr. Elizabeth Njuguna

Former doctoral fellow

Affiliation:

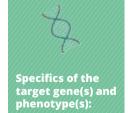
- VIB-UGENT Center for Plant Systems Biology, Ghent University, Belgium
- Plant Transformation Laboratory, Kenyatta University, Kenya



Maize - drought susceptibility



- Overall objective: Metabolic engineering of Poly(ADP-ribosyl)ation pathway (a stress response pathway) to broaden stress tolerance in plants by maintaining energy homeostasis during stress conditions.
- One approach: Knock-down of the maize PARP gene expression using CRISPR/CAS9 gene editing as a strategy for abiotic and genotoxic stress tolerance



Genes: Poly(ADP-ribose) polymerase (PARP1 and PARP2)

Expected Phenotype: Maize tolerant to drought, DNA damage and oxidative stresses.









- 1. Accelerating African Swine Fever Virus (ASFV) vaccine development via CRISPR-Cas9 and synthetic biology technologies
- 2. CRISPR/Cas9 gene editing of Theileria parva for the development of vaccine against East Coast fever (ECF)



Dr. Hussein Abkallo

Post-Doctoral Fellow

Affiliation: Vaccine Biosciences/

Animal and Human Health (AHH)/

International Livestock Research Institute (ILRI)







Animals: pigs (African Swine Fever Virus) and cattle (Theileria parva)



Generation of live-attenuated vaccines



Targets viral (ASFV) and parasite (Theileria parva) virulence genes to weaken (reduce the virulence of) the pathogen to elicit strong a strong and effective immune response when animals are vaccinated.

Feed the Future Striga Smart Sorghum for Africa (SSSfA)



Prof. Steven Runo

Professor of Molecular Biology

Affiliation: Kenyatta University



Dr. Margaret Karembu, MBS

Project PI

Affiliation:Director, ISAAA
AfriCenter



SSSfA Project Launch





Parasitic weed Striga (witchweed) adversely affects food and nutritional security for over 300 million people in sub-Saharan Africa. The weed is hard to manage because of its well-adopted parasitic lifestyle. Current control methods are either ineffective or too expensive for smallholder farmers in Africa. The SSSfA project aims to impart Striga resistance in sorghum by using genome editing (GEd).

In addition, the project endeavors to develop a sustainable model for developing home-grown genome edited Striga resistant sorghum. This involves integrating capacity development to ensure routine GEd in self-sustaining plant biotechnology laboratories dubbed Africa Biosciences Hubs (AfriBIOHubs) in Kenya and Ethiopia. These hubs will support future GEd, innovation and bio-entrepreneurship and demonstrate local capacity to commercialize agri-biotech products.



- i. Develop and commercialize GEd Striga resistant sorghum.
- Develop capacity for research and commercialization of GEd products.
- iii. Nurture an enabling environment for genome editing.



Pilot findings demonstrated that Striga resistance can be achieved using GEd through successfully mimicking a natural mutation called low germination loci 1 (lgs1) that makes some sorghum varieties resistant to the parasite through ineffectiveness in stimulating seeds of Striga to germinate. The SSSfA project is building on these findings to develop a technology platform for production and commercialization of Striga resistant sorghum for improved livelihoods of African smallholder farmers.

Improving oil qualities of Ethiopian mustard (Brassica carinata) through application of CRISPR/CAS 9-based genome editing



Prof. Teklehaimanot Haileselassie Teklu

Associate Professor

Affiliation: Institute of Biotechnology, Addis Ababa University



Misteru Tesfaye

PhD Student /Senior Oilseeds Researcher

Affiliation: Addis Ababa University, Institute of Biotechnology (IoB)



Tileye Feyissa

Associate Professor

Affiliation: Institute of Biotechnology, Addis Ababa University













Studies show that the level of erucic acid in Ethiopian germplasm materials as well as in Brassica carinata varieties released earlier is in the range of 31-51% of total fatty acid much beyond the nutritionally acceptable level (<5%). The emergence of novel gene editing tools like CRIPR/Cas9 has opened a good opportunity for improving the quality of B. carinata through editing targeted genes so that the crop can be applicable for both food/feed and oleochemical industries.



Objectives of the project and/ or proposed intervention(s):

- To explore the distribution of metabolites among 144 B. carinata genotypes for its bio-industrial applications
- To develop B. carinata genotype with low erucic and glucosinolate for food and feed application
- iii. To develop B. carinata genotypes with wax ester for industrial application
- iv. To enhance the level of erucic acid for industrial applications.



Specifics of the target gene(s) and phenotype(s):

Target gene

- For food- FAE1 and FAD2 genes,
- For feed GTR1 and GTR2 genes
- For industry- FAR and WS genes

Application of targeted gene editing for development of high yielding, stress resistant and nutritious crops



Dr. John Odipio

Scientist (Biotechnologist)

Affiliation: National Agricultural Research Organization (NARO)

National Crops Resources Research Institute (NaCRRI)-Namulonge Campus



Cassava:

- 1) Limited knowledge of molecular basis of flowering
- 2) Lack of double haploid lines and efficient methods for double haploid induction in cassava

Rice: No sources of resistance to rice yellow mottle virus

Maize: No sources of resistance to maize lethal necrosis



Objectives of the

project:

Completed:

- Efficient proof of concept developed for gene editing in cassava (https://www. ncbi.nlm.nih.gov/pmc/ articles/PMC5651273/)
- Production of fertile flowers and seeds by CRISPR/Cas9 mediated editing of endogenous anti-flowering genes in cassava (under peer publication)

On-going project:

Demonstration of proof of concept for gene editing by targeting marker gene PDS under NARs tissue culture system

Scheduled

- . Generation of knowledge and methods for haploid induction for rapid cassava breeding and faster delivery of stress resistant, high yielding and nutritious farmer preferred varieties
- Development of novel sources of resistance to devastating rice yellow mottle virus through gene editing
- Development of novel sources of resistance to maize lethal necrosis through gene editing





Genes:

Completed

- 1. Phytoene desaturase
- 2. Terminal flower 1

Scheduled

- 1. Centromere localized genes
- 2. Host susceptibility genes

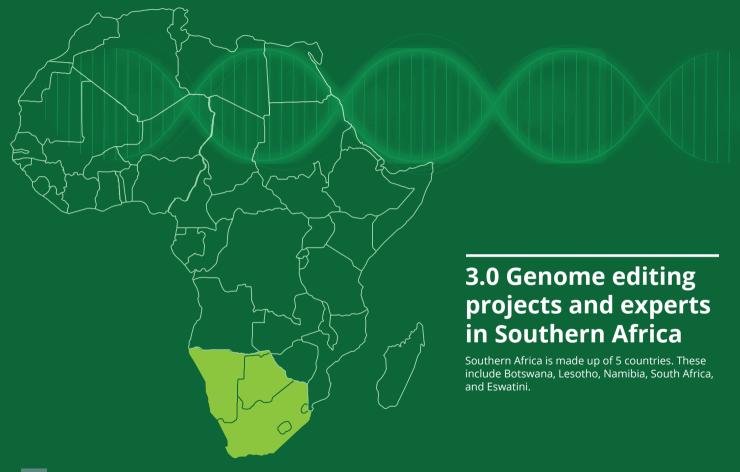
Phenotypes:

Completed

- 1. Photo bleaching
- 2. Early flowering

Scheduled

- 1. Short homozygous plants
- 2. Virus resistant edited plants



High-throughput screening of genes associated with the response of cassava to geminivirus South African cassava mosaic virus (SACMV).



Chrissie Rey
Professor
and Principal
Investigator



Patience Chatukuta Postdoctoral Research Fellow

Affiliation: School of Molecular and Cell Biology

University of the Witwatersrand





Cassava is recalcitrant to transformation, thus making reverse genetics approaches of studying the plant's response to cassava mosaic disease (CMD) time-consuming, taking at least 8 months. We exploit the use of protoplasts to study genes putatively associated with cassava's tolerance to CMD. The use of protoplasts in combination with gene editing techniques drastically reduces the time in which key genes involved with the response to CMD can be identified to 6 weeks. These key genes can then be targeted for biotechnological improvement of African cassava varieties for improved tolerance/resistance to CMD and improvement of yields thereby.



- To silence genes putatively associated with the response to SACMV infection in susceptible and tolerant cassava landrace protoplasts using CRISPR gene editing
- To measure target gene expression and viral load in wild and mutant (gene-edited) SACMV-infected cassava protoplasts
- To identify the hub or key genes associated with SACMV tolerance in cassava protoplasts



Target host genes are those which are known to be targeted by or respond to geminiviruses, such as the ubiquitin proteasome system genes (e.g. E3 ligases), transcription factor genes (e.g. WRKYs), and resistance genes (e.g. NLRs).

Genome editing of potato



Prof. James Lloyd

Professor of Plant Biotechnology

Affiliation: Stellenbosch University





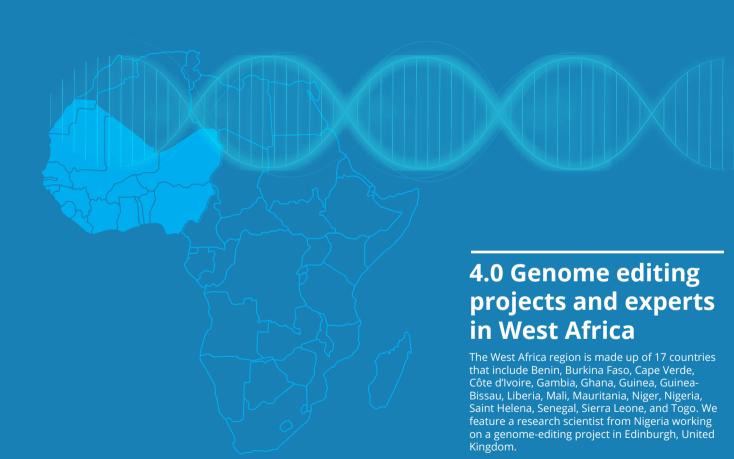
Viral infection in potatoes.



Production of potato virus-resistant potatoes.



Eukaryotic initiation factor 4E (Eif4E)



Investigating the role of ANP32 proteins in the replication of Avian influenza Virus



Dr. Alewo Idoko-Akoh

Research Fellow

Affiliation: McGrew Group, Division of Functional Genetics & Development, The Roslin Institute & Royal (Dick) School of Veterinary Studies, The University of Edinburgh



Developing novel genetic anti-viral strategies to prevent avian influenza infections in poultry



Objectives of the

project:

- To identify the specific regions of ANP32 proteins needed for influenza virus protein interactions.
- ii. To use genome editing tools that we have developed to modify chicken cells to identify genetic variations in ANP32 genes that will have the most significant restrictive effect on avian influenza virus replication.
- Assess any global changes in the transcriptome of genomeedited chicken cells containing modified ANP32 proteins
- iv. Investigate in vivo replication of avian influenza virus in genomeedited chickens expressing modified ANP32 proteins

This information will inform control strategies for protection of poultry from avian influenza infection. It will also be of interest to researchers studying influenza virus in humans and livestock. See the following publication for some background information (Jason S. Long, Alewo Idoko-Akoh, Bhakti Mistry, Daniel Goldhill, Ecco Staller, Jocelyn Schreyer, Craig Ross et al. "Species specific differences in use of ANP32 proteins by influenza A virus." eLife 8 (2019): e45066)



ANP32 genes encode a family of nuclear proteins implicated in many molecular functions including transcriptional regulation, apoptosis, tumour suppression, protein phosphatase inhibition, messenger RNA export and regulation of intracellular transport. ANP32 genes include ANP32A, ANP32B, ANP32C, ANP32D and ANP32E. Differences between mammalian and avian ANP32A genes account for the poor replication of some avian influenza viruses in mammalian cells.



Genome Editing for improved resistance to Cassava Bacterial Blight (CBB) Disease



Dr. Ihuoma Chizaram Okwuonu

Biotechnology Research Scientist

Affiliation: National Root Crops Research Institute Umudike, Nigeria





Yield and harvest loss due to CBB disease



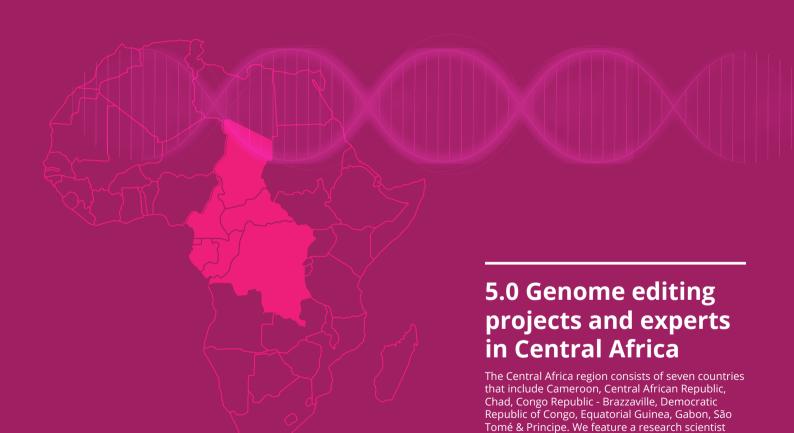
Objectives of the project:

To develop cassava resistant or with improved tolerance to CBB by disrupting gene(s) aiding disease establishment and spread.



Specifics of the target gene(s) and phenotype(s):

MeSWEET10a gene, a susceptibility gene for CBB targeted with CRISPR-Cas9



from Cameroon working on a genome-editing

project in Canada.

A combination of genome editing and BioID approaches to characterize a mitochondrial STAT3 function as a therapeutic strategy for multiple myeloma



Dr. Serges P. Tsofack

Scientific Research Associate

Affiliation: University Health Network (UNH) /University of Toronto



Genome editing in cancer cells, tissues and CRISPR/Cas9 approach to develop a new treatment regimen base on genomic instability



Objectives of the project:

CRISPR/Cas9 gene editing in genetic diseases (cancers). Understanding the mechanisms behind cancer drugs failure using different models and developing a next generation of drugs targets developing a new drug targets.

Approach:

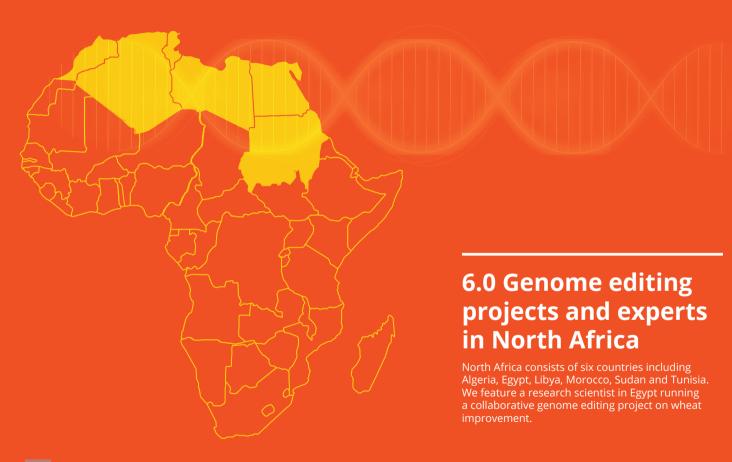
- 1. Understand a genomic instability in cancer patient particularly after a drug relapse;
- 2. Use a genetic variation to find new treatments. We strongly believe a functional genomics approach can lead us to molecular mechanism, which can be used for genetic disease treatment in human. These approaches can be easily translated to animals and plants disease special in agriculture areas. We employ in vitro and in vivo models, patient samples and bioinformatics tools.



Specifics of the target gene(s) and phenotype(s): STAT3



CAMEROON



Developing sal1 mutant drought tolerant wheat using CRISPR/Cas genome editing

Joint project between Faculty of Agriculture- Cairo University and USDA-ARS WRRC, Albany, CA



Prof. Naglaa Abdallah

Professor of Genetics

Affiliation: Department of Genetics, Faculty of Agriculture, Cairo University Egypt





Drought is one of primary stresses that limit crop productivity and cause economic losses. Development of abiotic stress tolerant crops like wheat is an important avenue to mitigate these problems and enable good agricultural yields, despite environmental challenges.



Objectives of the project:

- 1. Construction of the CRISPR/Cas9 transformation vectors
- 2. Generation of transgenic wheat plants
- 3. Screening of sal1 wheat mutants
- 4. Screening for stress tolerance in the sal1 mutant plants

Approach:

Use of genome editing techniques to generate drought stress tolerant wheat. Employing CRISPR-Cas9 to inactivate the Sal1 genes in wheat.



Specifics of the target gene(s) and phenotype(s):

Sal1

7.0 CONCLUSION

CRISPR genome editing technology offers a precise and efficient way of changing an organism's genetic material. This has presented the scientific community with an opportunity to address a myriad of challenges in health, agriculture, industry, environmental conservation and restoration. The inexpensive, simple and flexible technology comprises of an endonuclease protein whose DNA-targeting and cutting specificity can be programmed by a short guide RNA. Today, CRISPR technology has become an indispensable tool in biological research.

In agriculture, CRISPR genome editing is primarily being applied in improving crops with disease and pest resistance, abiotic stress tolerance and improved nutritional content. Due to its ability to generate genome-edited crops similar to those developed via conventional breeding, CRISPR technology is now regarded as one of the versatile tools for improving agricultural productivity to feed the rapidly growing population amidst climate change and dwindling arable land.

Modern biotechnologies are projected to play a critical role in building sustainable agricultural systems able to accommodate the rapidly growing demand for food. Globally, the first quarter of the 21st century has seen a major increase in undernourishment. Breeding of 'climate-change ready' and adaptable crop varieties is

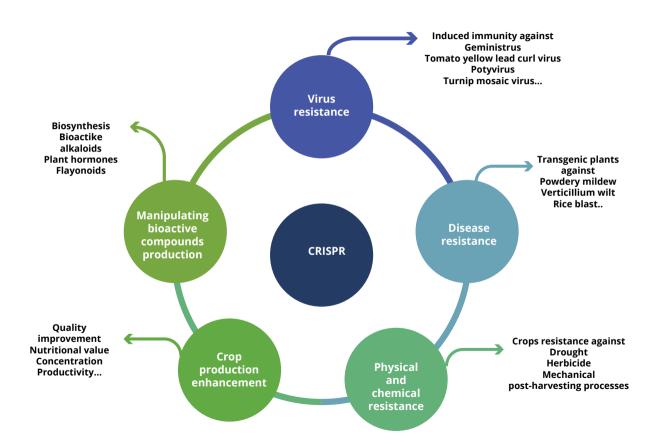
now more than ever critical in transforming agricultural productivity and ensuring global food and nutrition security.

The worsening impacts of climate change on food production, coupled with the increasing demand for food due to the burgeoning population has seen an increased prevalence of undernourishment. As of March 2023, 663 million people across 89 countries were undernourished (WFP Hunger Map 2023). Without fast and efficient interventions, the number of hungry people will reach 840 million by 2030.

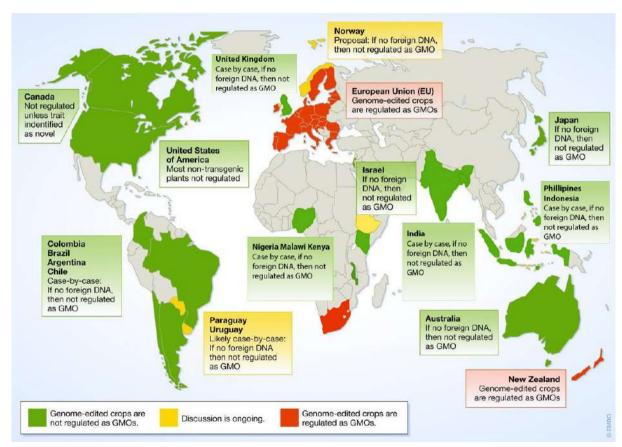
In Africa, over 250 million people (20 percent of the population) are undernourished. This situation has necessitated for rapid adoption of science, technology and innovations that improve the way food is produced. Genome editing is among the tools being employed in breeding crop varieties that are resilient and nutritionally superior.

As shown by the projects listed here, African scientists are moving fast to harness the potential of genome editing in developing crop varieties suited for the continent's modern agriculture. This spells a promising future where the inevitable impacts of climate change and the growing population are well mitigated through technology-supported, sustainable agricultural systems.

8.0 CRISPR GENOME EDITING: INSIDE A CROP BREEDER'S TOOLKIT



9.0 REGULATORY APPROACHES FOR GENOME EDITED PRODUCTS IN VARIOUS COUNTRIES



10 COMMUNICATING ABOUT GENOME EDITING IN AFRICA

Genome editing presents a unique and timely opportunity for improving agricultural productivity in Africa. It will improve the ease, speed, precision, cost, and generation time of higher-yielding, superior varieties, and breeds in crop and animal breeding. However, its deployment will rely heavily on developing and implementing policies and regulations that foster an enabling environment for research, development, and adoption. Importantly, communication approaches will either hamper or facilitate technology uptake.

In 2021, ISAAA AfriCenter launched the African Coalition for Communicating Genome Editing during the fourth edition of the Africa Biennial Biosciences Communication Symposium (ABBC). The Coalition provides stakeholders with a platform for open and transparent dialogue on genome editing in Africa. In addition, the Coalition will help in strengthening partnerships and synergizing the pertinent research, policy, and communication efforts in the region. The launch was presided over by Kenya's Cabinet Secretary for Industrialization and Trade Hon. Betty Maina.

The leadership of six African universities has expressed a strong commitment to supporting the Coalition in building scientists'

and policy makers' capacities in promoting informed decisions on genome editing. The universities include Nigeria's Ebonyi State University, Ethiopia's Addis Ababa University, Kenya's Kenyatta University, Masinde Muliro University of Science and Technology, University of Embu, and South Eastern Kenya University.

Dialogue on how products of genome editing should be regulated is ongoing. Kenya, Nigeria, and Malawi have published guidelines for determining the regulatory process for products derived from genome editing, with Ethiopia making progress in the drafting process. Public engagement is needed to keep pace with th rapid advancements, to avoid the inheritance of restrictive regulatory regimes. Key players in genome editing research, development, policies, and regulations must embrace constructive dialogue about the technology early.

We envisage that the Coalition will play a critical convening role as stakeholders dialogue on all aspects of genome editing and its application in Africa's agriculture.

To join the Coalition, contact Dr. Margaret Karembu, Director ISAAA AfriCenter at mkarembu@isaaa.org

Ngure G., Karembu M. (2023). Genome Editing in Africa's Agriculture: An Early Take-off. 3rd Edition. International Service for the Acquisition of Agri-biotech Applications (ISAAA AfriCenter), Nairobi Kenya.

To be featured in the Fourth Edition, contact Godfrey Ngure at gmngure@isaaa.org

