Phenotypic Characterization of Transgene-Free SDN-1 Edited Rice for Enhanced Nitrogen remobilization.

Credit: E. Arcillas, IRRI

CGIAR project on

Genome Editing

Annual Technical Report 2024

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This publication has been prepared as an output of the CGIAR Research Initiative on Genome Editing. Any views and opinions expressed in this publication are those of the author(s) and are not necessarily representative of or endorsed by the CGIAR System Organization.

Boundaries used in the maps do not imply the expression of any opinion whatsoever on the part of CGIAR concerning the legal status of any country, territory, city, or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Borders are approximate and cover some areas for which there may not yet be full agreement.

The Artificial Intelligence (AI) software ChatGPT was used to support the editing of parts of this report, specifically to improve clarity, grammar, and style. ChatGPT was not used to generate the content of the report. All edits made with AI assistance were reviewed and validated by the authors to ensure accuracy, coherence, and alignment with the original intent.

Acknowledgements

We would like to thank all funders who supported this research through their contributions to the CGIAR Trust Fund.

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CGIAR Technical Reporting 2024

CGIAR Technical Reporting has been developed in alignment with CGIAR's Technical Reporting Arrangement. This annual report ("Type 1" Report) constitutes part of the broader CGIAR Technical Report. Each CGIAR Research Initiative/Impact Platform/Science Group Project (SGP) submits an annual "Type 1" Report, which provides assurance on progress towards end of Initiative/Impact Platform/SGP outcomes.

As 2024 marks the final year of this CGIAR Portfolio and the 2022-24 business cycle, this Type 1 Report takes a dual approach to its analysis and reporting. Alongside highlighting key achievements for 2024, the report also provides a cumulative overview of the 2022-24 business cycle, where relevant. This perspective captures the evolution of efforts over the three-year period. By presenting both annual and multi-year insights, the report underscores the cumulative impact of CGIAR's work and sets the stage for the transition to the 2025-30 Portfolio.

The 2024 CGIAR Technical Report comprises:

- **Type 1 Initiative, Impact Platform, and SGP Reports:** These annual reports present progress towards end of Initiative/Impact Platform/SGP outcomes and provide quality-assured results accessible via the CGIAR Results Dashboard.
- **Type 3 CGIAR Portfolio Practice Change Report:** This report provides insights into CGIAR's progress in Performance Management and Project Coordination.
- **Portfolio Narrative:** Drawing on the Type 1 and Type 3 reports, as well as data from the CGIAR Results Dashboard, the Portfolio Narrative synthesizes insights to provide an overall view of Portfolio coherence. It highlights synergies, partnerships, country and regional engagement, and collective progress.
- **Type 2 CGIAR Contributions to Impact in Agrifood Systems: evidence and learnings from 2022 to 2024:** This report offers a high-level summary of CGIAR's contributions to its impact targets and Science Group outcomes, aligned with the Sustainable Development Goals (SDGs), for the three-year business cycle.

The Portfolio Narrative informs the 2024 CGIAR Annual Report – a comprehensive summary of the organization's collective achievements, impacts, and strategic outlook.

Elements of the Type 2 report are integrated into the CGIAR Flagship Report, released in April 2025 at CGIAR Science Week. The Flagship Report synthesizes CGIAR research in an accessible format designed specifically to provide policy- and decision-makers at national, regional, and global levels with the evidence they require to formulate, develop, and negotiate evidence-based policies and investments.

The diagram below illustrates these relationships.

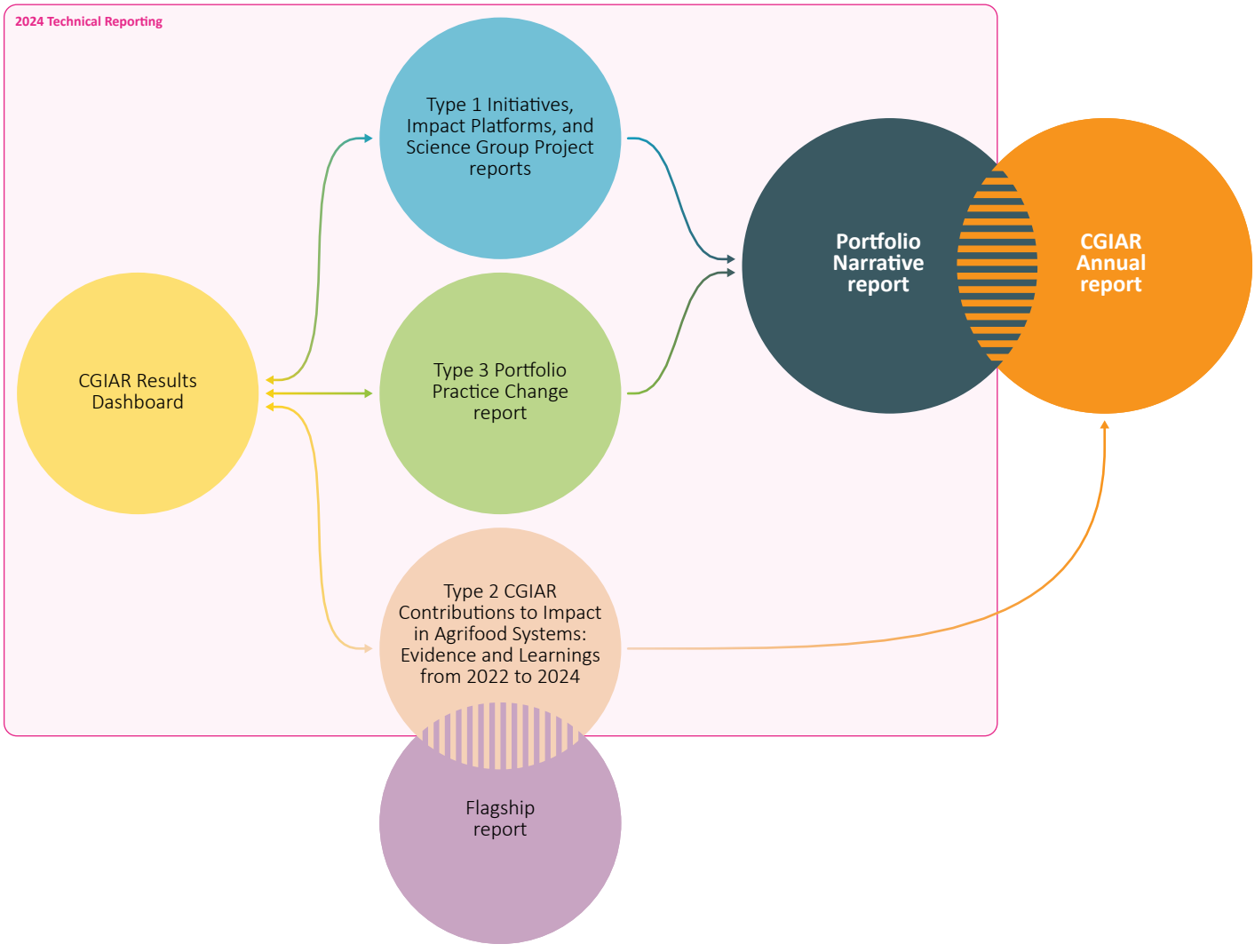


Figure 1. CGIAR's 2024 Technical Reporting components and their integration with other CGIAR reporting products.

Section 1: **Fact sheet, executive summary and budget**

Science Group Project name	Accelerating Crop Improvement through Genome Edit
SGP name	Genome Editing
Science Group	Genetic Innovation
Science Group Project Lead	Inez Slamet-Loedin (i.slamet-loedin@cgiar.org)
Science Group Project Co-lead	Judy Chambers (j.chambers@cgiar.org)
Science Group	Genetic Innovation
Start – end date	01 October 2023 – 31 September 2025
Geographic scope	**Regions:** Global
Allocated budget	**2023 (Q4 only):** $0.48M · **2024:** $2.08M

EXECUTIVE SUMMARY

Over the past year, the CGIAR Science Group Project on Accelerating Crop Improvement through Genome Editing (Genome Editing), involving six CGIAR Centers, made significant progress in addressing social, policy, technology licensing, and regulatory challenges related to the release and public acceptance of genome-editing (GEd) products. Following a product life cycle approach, the Initiative ensures smooth market deployment.

Work Package 1 (WP1) focuses on fostering an enabling environment through capacity development and regulatory frameworks. The Research Enabling and Oversight (REO) Unit, alongside its advisory team, will provide strategic leadership on GEd issues. A baseline analysis and capacity gap assessment helped Centers share expertise and optimize resources.

Training workshops by IITA, IRRI (in Africa), and Alliance of Bioversity International and CIAT covered genome editing tools, regulatory requirements, and licensing. IRRI also supported IITA's stewardship audit, ensuring compliance with private-sector standards. Additionally, a joint internal communications strategy was developed.

To secure Freedom to Operate (FTO) for essential editing tools, the Initiative in collaboration with the CGIAR System Office established a basic licensing template between a technology provider and Centers. However, Centers must sign individually, with one Center already on board. These efforts align with WP1's goal of strengthening capacity and establishing a unified GEd research and deployment platform.

Work Package 2 (WP2) uses genome editing technologies to accelerate demand-driven traits. GEd offers faster and more precise breeding, addressing challenges like food security, disease resistance, and reduced fertilizer dependency. Consultations with CGIAR breeders, Market Intelligence Targeted Product Profile surveys and partners from national agricultural research systems (NARS) confirmed the current traits-crop combination targets with new priorities like drought, salinity tolerance, improved nutrition, and hybridization enhancement also suggested.

Several crops have shown progress:

1. **Cassava:** The CRISPR-Cas9 gene-editing technology was used to edit the MeSWEET gene promoters for cassava bacterial blight (CBB) resistance. Four edited lines were selected for infection assays and field validation was planned.
2. **Potato:** Genome editing targeted the eIF4E gene for Potyvirus Y (PVY) resistance. Protoplast isolation and mini-calli induction were established and prime editing constructs were evaluated. Challenges were observed in plant regeneration from mini-calli.
3. **Banana:** Transgene-free genome editing: the Cas-CLOVER approach combined with PiggyBac transposase was explored. Over 100 edited banana events were generated. Further refinement is needed to enhance transgene elimination.
4. **Rice:** Efforts focused on improved nitrogen remobilization with third generation (T3) preliminary data showing enhanced biomass under low nitrogen and stress conditions. A dossier for non-genetically-modified certification is under development to allow reliable field testing. Prime editing protocols were established for African and Asian cultivars.
5. **Wheat:** CRISPR/Cas9 edits targeted Lr67 (resistance to rust and powdery mildew) and MLO (powdery mildew susceptibility). Resistance screening and prime editing optimization are ongoing.

A workshop in Nairobi fostered collaboration between African scientists and regulators on genome editing, intellectual property (IP), and biosafety. A standardized protocol for non-transgenic status applications was developed for one crop with possible adaptation to other crops. A joint research project was initiated to test an alternative open-source system in one crop. A joint research project was initiated to test an alternative open-source system in one crop. Overall progress in both Work Packages (WPs) aligns with the End-of-Initiative outcomes (EOIO).

Vietnamese asian farmers spray fertilizers working in rice fields.
Credit: Attasit saentep/Shutterstock

Section 2: **Progress towards End of Initiative outcomes**

Science Group Project-level theory of change diagram

This is a simple, linear, and static representation of a complex, non-linear, and dynamic reality. Feedback loops and connections between this Initiative and other Initiatives' theories of change are excluded for clarity.

CHALLENGE STATEMENT

- The growing demand for food will make production losses intolerable, a situation that will be further compounded by climate-induced declines in productivity. Conventional breeding alone will not be able to generate the increase in yields required to meet future demand for high-quality, nutritious food. A major technical drawback of conventional breeding is the inability to precisely select traits without including undesirable "co-segregating" traits from donor lines.
- Genome editing (GEd) is a cost- and time-effective solution that strategically complements conventional breeding. GEd enables precise and predictable refinement of crop genomes. Despite the significant change in impact achievable through GEd and recent positive regulatory trends in many countries, debate and regulatory developments remain ongoing. The discussion has shifted to the need for a sensible and pragmatic appreciation of benefits and risks, with a call to balance caution and innovation, recognizing that "governance must cope with a moving technical frontier." Within-species genetic changes using GEd are more readily accepted than transgenic innovations.
- The GEd Initiative takes a holistic and long-term view of future demand for GEd solutions as experience with technology and social acceptance grows. It streamlines the National Agricultural Research Service (NARS)–CGIAR gene-editing innovation pipeline and its supporting activities to reach the end user and create impacts. The Initiative will pursue activities with GEd innovations to support high standards of product stewardship as necessary for any breeding innovations, phase-gate the decision-making process, guide intellectual property management, facilitate licensing, encourage enabling societal and policy environments, and support the delivery of GEd products to market. There will be a focus on existing genome-editing innovations free of foreign DNA, as demanded by partners, along with enabling technologies or foundation tools to produce clonally propagated plants free of foreign DNA. Capacity development in collaboration with partners is designed to position CGIAR and its networks to use GEd more efficiently, effectively, and responsibly while building the local innovation climate.

SPHERE OF **CONTROL**

WORK PACKAGES

WORK PACKAGE 1

Research Enabling, and Oversight (REO) Unit.

WORK PACKAGE 2

Genome editing enabling technologies and Innovations for accelerated delivery of demand-driven traits.

SPHERE OF **INFLUENCE**

End-of-Project Outcomes

End-of-Project Outcome 1

Implementation of strategic approach for scaling GEd innovation.

End-of-Project Outcome 2

Policymakers and end-users are equipped and supported in using Genome Editing.

End-of-Project Outcome 3

Evaluated efficacy of market-driven genome editing products.

End-of-Project Outcome 4

Licensing Intellectual Property (IP) agreement on basic tools of genome editing technology.

Action Area Outcomes

Genetic Innovation

1 2 **1** · Research institutions, government analytical units, and scaling partners in the Global South have improved knowledge, skills, access to data, capacity to develop tools, innovations, and undertake research to support transformation of food, land and water systems contributing to livelihood, inclusion, nutrition, environmental and climate objectives.

3 **2** · CGIAR-NARS-SME networks use market segments, target product profiles to orient variety development and deployment towards those that provide larger scale benefits across the 5 Impact Areas.

1 2 **3** · National and private seed company breeding programs accelerate the development of varieties that provide larger scale benefits across the 5 Impact Areas.

2 3 4 **4** · Integrated seed systems increase the quantity of quality seed of improved varieties available to farmers for priority crops and in selected countries, geographies, and market segments.

4 **5** · Seed system actors promote the adoption of quality seed of improved varieties by women and men farmers in selected countries, geographies, and market segments.

3 4 **6** · GIAR partners develop and scale innovations that contribute to the empowerment of women and other social groups in food, land, and water systems.

SPHERE OF **INTEREST**

Impact Areas

Nutrition, Health & Food Security

1 3 4 · End hunger for all and enable affordable health diets for the 3 billion people who do not currently have access to safe and nutritious food.

Poverty Reduction, Livelihoods & Jobs

2 3 4 6 · Reduce by at least half the proportion of men, women and children of all ages living in poverty in all its dimensions according to national definitions.

Gender Equality, Youth & Social Inclusion

2 3 6 · Close the gender gap in rights to economic resources on, access to ownership of, and control over land and natural resources, for more than 500 million women who work in food, land, and water systems.

Climate Adaptation & Mitigation

3 4 5 · Equip 500 million small-scale producers to be more resilient to climate shocks, with climate adaptation solutions available through national innovation systems.

Environmental Health & Biodiversity

1 5 6 · Stay within planetary and regional environmental boundaries: consumptive water use in food production of less than 2500 km3 per year (with a focus on the most stressed basins), zero net deforestation, nitrogen application of 90 Tg per year (with redistribution towards low-input farming systems) and increased use efficiency, and phosphorus application of 10 Tg per year.

Genome-edited rice, banana and cassava plants.
Credit: Francisco Sánchez, Erwin Arcillas, Inez Slamet-Loedin

Summary of progress against the theory of change

Work Package 1: Research Enabling and Oversight (REO) Unit

Progress towards End of Project outcomes (EOPO 1)

Over the past year, WP1 made ambitious progress across participating Centers to identify and address social, policy and regulatory challenges associated with the release and widespread acceptance of genome-editing (GEd) products. This Science Group Project (SGP) uses a product life cycle approach which identifies the regulatory and policy changes required at each stage of product development to ensure smooth transition toward development and market deployment of GEd products. These have followed the logical progression listed in the TOC. WP1 focuses on the use of collective action and management best practices to foster innovation enabling conditions, capacity development for CGIAR and NARS target product development teams and staff, and consensus frameworks to promote product safety and acceptance. The REO functions to provide strategic leadership on the development and future deployment of GEd spanning the variety issues that impact product life cycle decision-making.

An analysis and assessment of current capacity and capacity gaps at the Centers was done to define existing and need capacities for the CGIAR and NARS partners, allowing Centers to share expertise and conserve resources. Workshops were organized by IRRI, the CGIAR System Office (SO), IITA, and CIAT that focused on genome editing tools and potential collaborations and methods as well as regulatory and licensing requirements. Similarly, the need for common IP and stewardship practices across Centers resulted in IP discussions with a designated technology provider, facilitated by the SO. Due to SO legality issues, Centers decided to pursue IP independently based on an agreed template. One Center has completed the signing. Collaborative efforts were initiated across Centers. IRRI (which recently received the second Excellence Through Stewardship [ETS] certification) conducted an internal stewardship audit for IITA to ensure adherence to compliance requirements. As an example of shared expertise, IRRI researchers shared their knowledge during the audit. One of the priorities of the REO Unit was to develop a common strategy across Centers on internal and external communications. A joint Internal Plan for Coordination of Communications for Genome Editing has been completed, and a draft gateway decision point document has been developed.

These developments show progress in addressing two major outputs for WP1. The outputs are also in line with testing the assumptions that there exist substantial gaps across Centers in terms of capacity and a common platform is required for achieving long-term gains in genome edited products research and deployment within CGIAR.

PRIMARY CONTRIBUTION OF WORK PACKAGES TO TO END OF PROJECT OUTCOMES

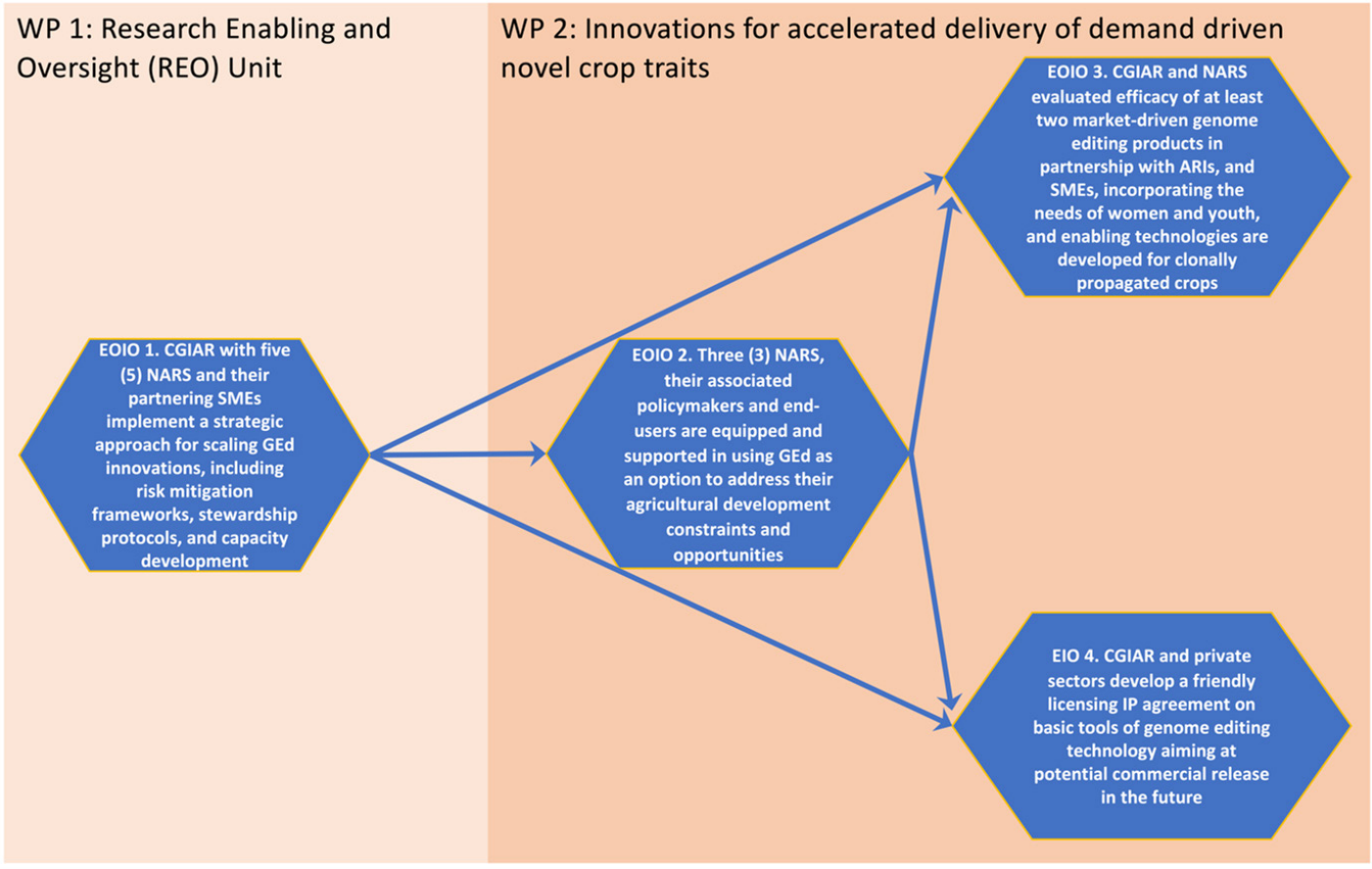


Figure 2 illustrates how each Work Package supports EOIOs and their interconnections. In its first year, WP1 made significant progress in capacity building, framework development, and strategic scaling of GEd products. Capacity gap analysis identified lead Centers for training, regulation, and communication. Over the next year, IP and stewardship capacities will be standardized across Centers to meet commercial standards. Meanwhile, regulatory efforts through bilateral projects will help create a supportive environment for market access and commercialization of GEd products.

Work Package 2: Genome editing enabling technologies and Innovations for accelerated delivery of demand-driven traits

Progress towards End of Project outcomes (EOPOs 2, 3 and 4)

Genome editing in elite lines offers significant advantages in speed and precision for plant breeding, eliminating the need for multiple generations of backcrossing to reduce “linkage drag”. The technical benefits of GEd enable a swift and effective response to global challenges such as food security and future-ready crops, including enhanced disease resistance and reduced fertilizer dependence.

The Global Market Intelligence Targeted Product Profile Overview Survey (GloMIP) identified abiotic stress tolerance and disease resistance as farmers’ top breeding priorities. Additionally, consultations were held with CGIAR breeding leads, crop breeders from various Centers, and representatives from partner countries, including Kenya (Kenya Agriculture and Livestock Research Organization [KALRO], National Biosafety Authority); India (Indian Council of Agricultural Research); Ghana (Crops Research Institute); Burkina Faso (Environmental Institute for Agricultural Research); the Philippines (Philippine Rice Research Institute [PhilRice]); Indonesia (National Research and Innovation Agency); and French agricultural institutions across three separate events.

The crop target product profile confirms the relevance of ongoing innovation portfolios funded by the Genome Editing project. However, new breeding priorities have been suggested, including drought and salinity tolerance, improved nutrition across multiple crops, traits to enhance hybridization in cereals, and creation of apomixis in rice.

Summary the progress of innovation research toward achieving the target traits.

OBJECTIVE	APPROACH	KEY FINDINGS	CHALLENGES AND NEXT STEPS
Cassava: Resistance to cassava bacterial blight (CBB)			
Develop cassava varieties resistant to CBB caused by Xanthomonas phaseoli (Xpm), which can result in total yield losses.	• CRISPR-Cas9 was used to edit SWEET gene promoters in cassava variety 60444. • To confirm successful edits, around 100 edited plants were analyzed using PCR, Southern blot, and sequencing.	• Four MeSWEET edited lines were selected for infection assays. • Edited lines are ready for crossing and field validation to assess resistance levels.	• Further validation is needed to assess the long-term stability of edited lines. • Conduct additional trials to confirm effectiveness of mutations in conferring durable resistance to CBB.
Potato: Enhancing resistance to Potyvirus Y (PVY)			
Develop a PVY-resistant potato variety using genome editing to modify the eIF4E gene, mimicking a naturally resistant allele.	• Prime editing guide RNAs (pegRNAs) were designed to introduce precise mutations in the eIF4E gene. • Protoplast isolation and transfection were performed to validate the gene editing approach.	• Protocols for protoplast isolation and culture were developed. • Mini-calli formation was observed.	• Challenges remain in obtaining regenerable calli. • Experimenting with different growth regulators and culture conditions.
Transgene-free genome editing in banana (clonally propagate plants)			
Develop transgene-free disease-resistant banana using advanced genome editing technologies.	• Combined Cas-CLOVER genome editing with PiggyBac transposase to ensure precise edits and subsequent removal of transgenic elements. • Used Agrobacterium-mediated transformation to edit the PUB22/23 genes, associated with disease susceptibility.	• Generated over 100 genome-edited events in banana cultivar Sukali Ndiizi. • Estradiol-induced PiggyBac excision was applied to remove transgenes and sequencing confirmed successful edits.	• Further refinement is needed to improve transgene elimination efficiency. • Ongoing trials aim to assess Cas-CLOVER technology in producing transgene-free banana plants.
Improving nitrogen utilization and prime editing in elite rice varieties			
Enhance nitrogen remobilization in rice to improve resource efficiency under low-input conditions. **Establishment of prime editing techniques for elite rice varieties for allelic replacement.**	• CRISPR/Cas9 genome editing targeted a nucleoside catabolism gene in nitrogen remobilization. • Edited plants were evaluated across T0, T1, T2, and T3 generations. • Prime editing technique was tested for 4 cultivars.	• 13 homozygous mutants (HPT-free) identified in T1 progenies. • T2 mutant lines showed increased biomass and protein content (pot and hydroponic). • Preliminary T3 data indicate improved biomass and chlorophyll content under low nitrogen and water stress conditions. • Prime editing protocols were established for African elite cultivars and direct-seeded rice.	• Field trials are needed to confirm enhanced nitrogen utilization.
Wheat: Developing resistance to rust and powdery mildew			
Enhance wheat resistance to rust and powdery mildew by editing key susceptibility genes.	• CRISPR/Cas9 genome editing was used to target: • Lr67 (TaSTP13-D): A hexose transporter gene linked to resistance against rust and powdery mildew. • MLO gene: Associated with susceptibility to powdery mildew.	• Successfully generated 12 edited lines with Lr67 edits. • MLO-specific edits were introduced, explants currently under selection. • Prime editing is being tested to mimic Lr67 mutations.	• Conducting resistance screening for rust and powdery mildew. • Further optimization of prime editing for wheat.

African genome editing in crops workshop

Genome editing holds great promise for African agriculture, but its success depends on collaboration between scientists and regulators. To bridge knowledge gaps, IITA and IRRI hosted a hands-on workshop in Nairobi on genome editing, regulations, IP, and stakeholder engagement.

Participants from seven African countries discussed patenting, licensing, and biosafety to streamline approvals while emphasizing science-based communication to combat misinformation. This effort aims to enhance agricultural innovation by fostering collaboration and informed decision-making, ensuring safer and more accessible genome-edited crops for African farmers.

Standard operating procedure for transgene free

A standardized protocol for detecting transgene-free lines was developed in rice based on quantitative polymerase chain reaction (qPCR) and polymerase chain reaction (PCR) as an option. The standardized protocol was applied to target traits in rice Enhanced NItrogen Remobilization (ENR) and disease resistance through prime editing. The development of the dossier for non-transgenic status applications is underway. These protocols are being shared with other research Centers to facilitate the broader adoption of genome-edited improvements in rice breeding.

Each crop's progress, capacity development in Africa, and development of a standard operating procedure for transgene-free application align with steps to achieve EOIO targets to evaluate at least two market-driven genome editing products in target countries

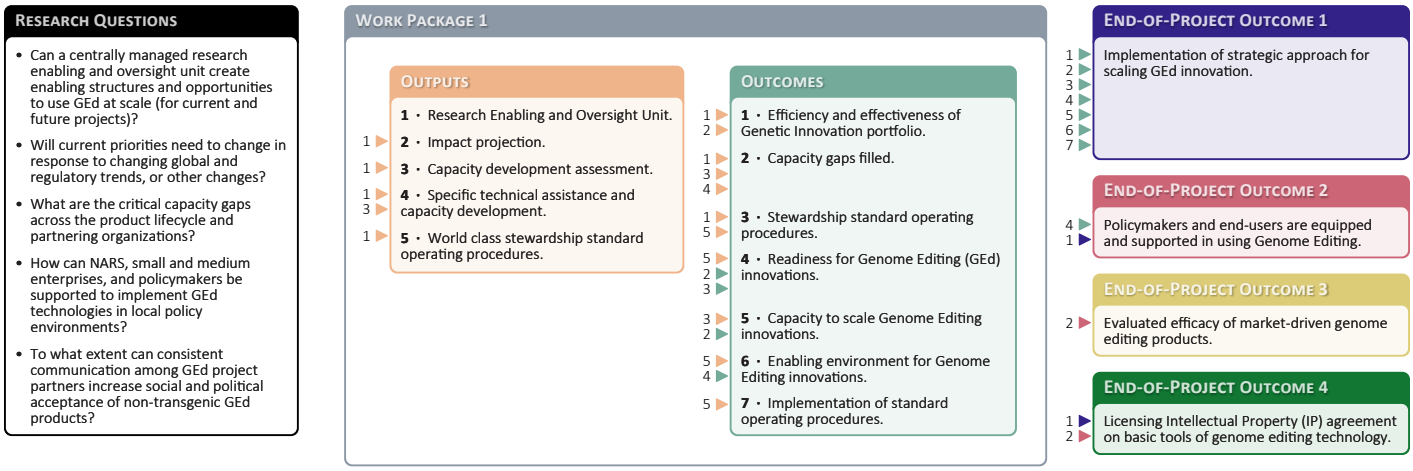
Progress in obtaining freedom to operate on IP rights of editing tools

The CGIAR SO developed a friendly licensing template with a designated technological provider; however, CGIAR legal mandates require representation and signatory authority by individual Centers, although use of the template with individual modifications is encouraged. A potential open-source option with a university has also been explored. Post-FTO analysis, an initial joint research activity will be conducted to test the system in one crop. The progress above aligns with the EOIO "CGIAR and private sectors obtain friendly licensing of essential editing tools".

Doctor Paula Díaz (UNAL-IBUN) and Francisco Sánchez (The Alliance) inoculating cassava plants with Xpm strains in the level-2 biosafety greenhouse at The Alliance.

Credit: Paul Chavarriaga

WP1: Research Enabling and Oversight (REO) Unit



Work Package 1 progress against the theory of change

WP1 provides strategic oversight across the product life cycle of genome editing projects within CGIAR. To ensure a unified approach, we brought together key stakeholders during the inception meeting to establish a shared understanding of project objectives. The REO Unit was formed to guide research and decision-making on genome editing policies, regulatory compliance, and stakeholder engagement as part of this project.

A capacity needs assessment and survey were conducted across CGIAR Centers to identify gaps in expertise and infrastructure. The findings informed the development of targeted training programs to enhance technical and regulatory capabilities. A training workshop, jointly organized by IITA and IRRI in Kenya for African scientists and regulators, focused on building genome editing expertise, incorporating technical skills and regulatory knowledge. Participants also received IP management training, stakeholder mapping, and engagement strategies from relevant experts, ensuring a holistic understanding of the genome editing landscape.

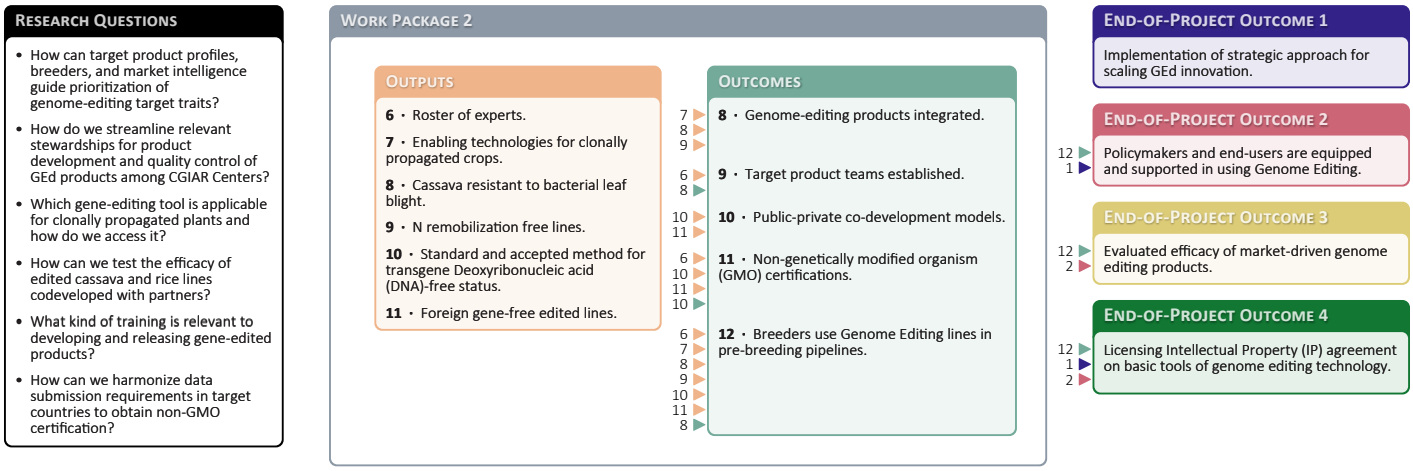
In addition to the Kenya workshop, CIAT organized specialized training sessions on gene editing techniques for Colombian researchers. On stewardship, IRRI, which received the second Excellence to Stewardship (ETS) certification, served as an internal auditor to ensure adherence to stewardship best practices. CIMMYT conducted its internal audit, by an invited expert from ETS, and will train a field partner in Kenya for stewardship. These efforts contribute to a harmonized compliance and risk management approach across Centers.

PROGRESS RATING & RATIONALE

On track

WP1 completed 3 out 5 expected outputs.

WP2: Building Partnerships that Realize the Benefits of Precision Genetics



Work Package 2 progress against the theory of change

WP2 utilized the WP1 frameworks and guidance in developing prioritized GEd products to address critical challenges in agriculture. Significant achievements have already been made across various research areas.

At CIP, genome editing was employed to enhance resistance to Potyvirus Y (PVY) in the potato cultivar Shangi by modifying the susceptible eIF4E gene to resemble the Eva1 PVY-resistant allele found in its wild relative. Seven prime editing constructs were used to transfect protoplasts isolated from Shangi. The regeneration of transfected protoplasts is currently being optimized to establish an efficient gene editing protocol for developing PVY-resistant potato varieties in Kenya.

IITA, partnering with Deemetra, used Cas-CLOVER and PiggyBac to develop a transgene-free genome-edited banana. More than 100 edited events were created in the "Sukali Ndiizi" cultivar background targeting disease susceptibility genes. Efforts are now directed toward optimizing construct design and integrating technologies for transgene removal.

Meanwhile, the Alliance of Bioversity International and CIAT used CRISPR-Cas9 to edit the promoter of SWEET genes in cassava variety 60444 to confer resistance to cassava bacterial blight caused by Xanthomonas (Xpm). Around 100 edited cassava lines were obtained and analyzed and three were selected for infection assays.

IRRI utilized CRISPR/Cas9 to target a gene involved in nucleoside catabolism in rice, aiming to improve nitrogen remobilization. Progress has been made in characterizing T1 and T2 lines. Preliminary data from ongoing T3 yield assessment in screen house paddy soil showed increased biomass and chlorophyll content under low nitrogen and reduced water conditions. A protocol for detecting transgene-free lines was also developed, and results are being complied for non-transgenic status application.

Additionally, IITA and IRRI held a workshop in Nairobi to train gender balanced participation and regulators from seven African countries on technical and regulatory aspects of genome editing to streamline crop approval and public trust.

PROGRESS RATING & RATIONALE

On track — WP2 showed significant progress toward all outputs and on track to complete them within 3 years.

Definitions

On track

- Progress largely aligns with Plan of Results and Budget and Work Package theory of change.
- Can include small deviations/issues/delays/risks that do not jeopardize success of Work Package.

Delayed

- Progress slightly falls behind Plan of Results and Budget and Work Package theory of change in key areas.
- Deviations/issues/delays/risks could jeopardize success of Work Package if not managed appropriately.

Off track

- Progress clearly falls behind Plan of Results and Budget and Work Package theory of change in most/all areas.
- Deviations/issues/delays/risks do jeopardize success of Work Package.

Section 4: **Quantitative overview of key results**

This section provides an overview of results reported and contributed to, by the CGIAR project on Genome Editing from 2023 to 2024. These results align with the CGIAR Results Framework and and Genome's Editing theory of change. Further information on these results is available through the CGIAR Results Dashboard.

The data used to create the graphics in this section were sourced from the CGIAR Results Dashboard on 04 April 2025. These results are accurate as of this date and may differ from information in previous Technical Reports. Such differences may be due to data updates throughout the reporting year, revisions to previously reported results, or updates to the theory of change.

Contributions to the CGIAR results framework: number of results by level and category

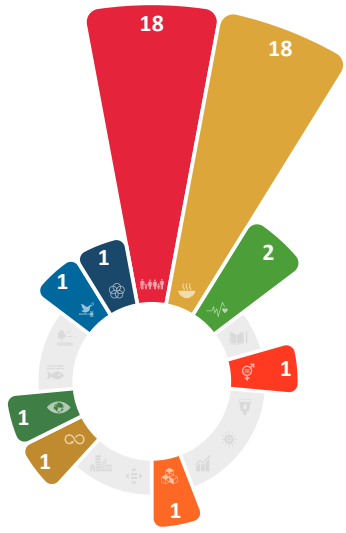
Outputs

- Innovation development 9
- Other outputs 5
- Capacity sharing for development 4
- Knowledge products 2

Outcomes

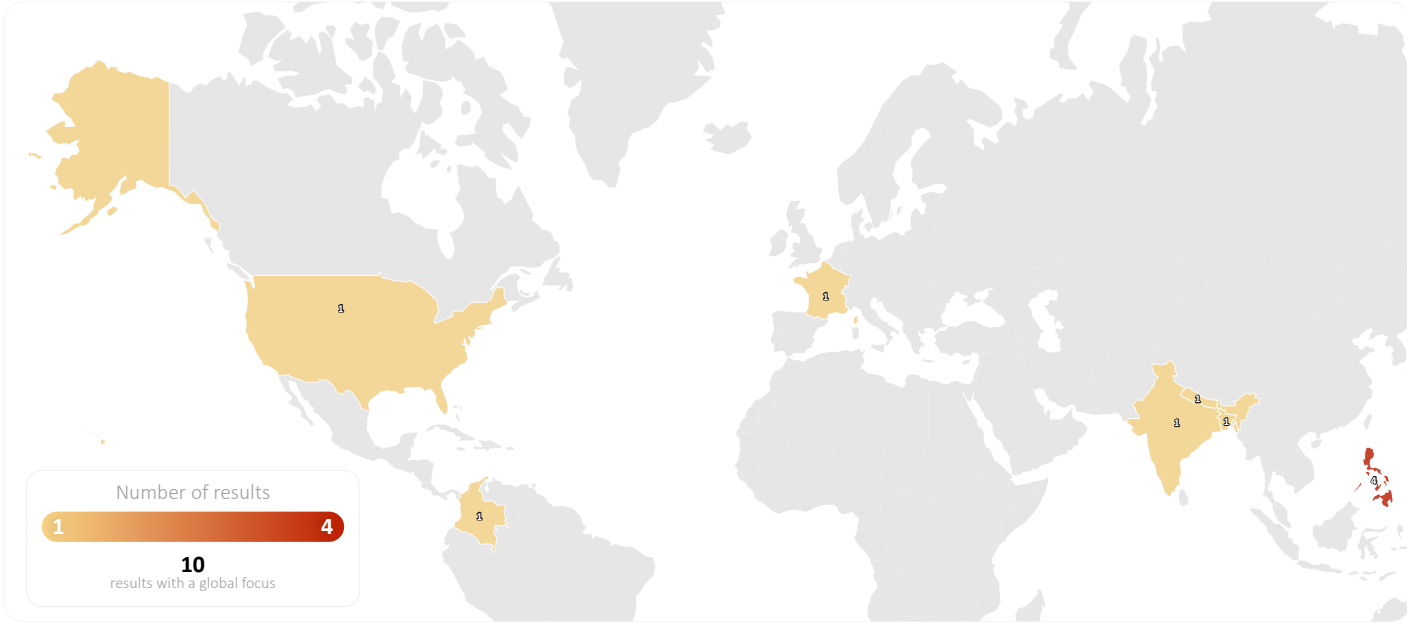
- Other outcomes 1

Contribution of results to the UN Sustainable Development Goals (SDGs)

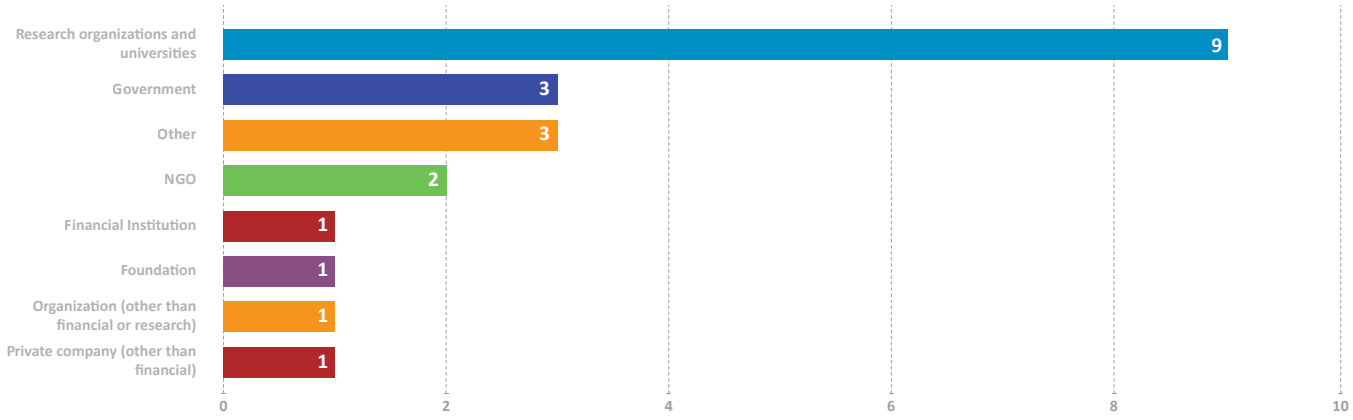


1	No Poverty	18
2	Zero Hunger	18
3	Good Health and Well-Being	2
4	Quality Education	-
5	Gender Equality	1
6	Clean Water and Sanitation	-
7	Affordable and Clean Energy	-
8	Decent Work and Economic Growth	-
9	Industry, Innovation and Infrastructure	1
10	Reduced Inequalities	-
11	Sustainable Cities and Communities	-
12	Responsible Consumption and Production	1
13	Climate Action	1
14	Life Below Water	-
15	Life on Land	-
16	Peace, Justice and Strong Institutions	1
17	Partnership for the Goals	1

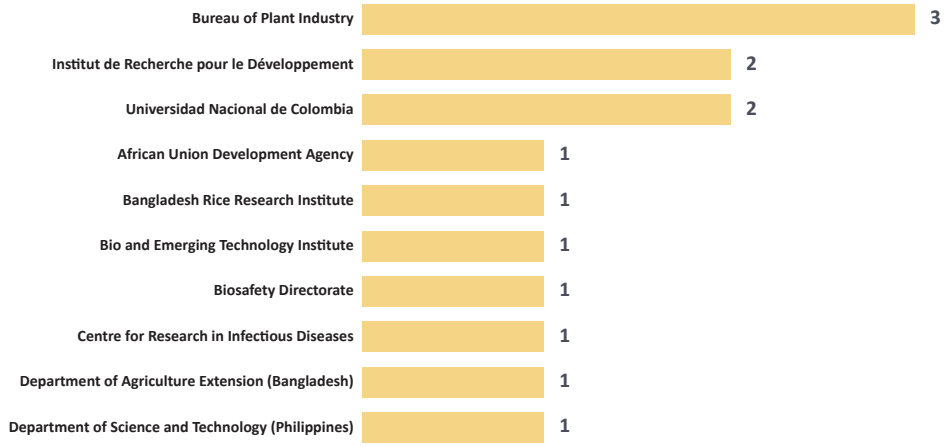
Geographic focus of results



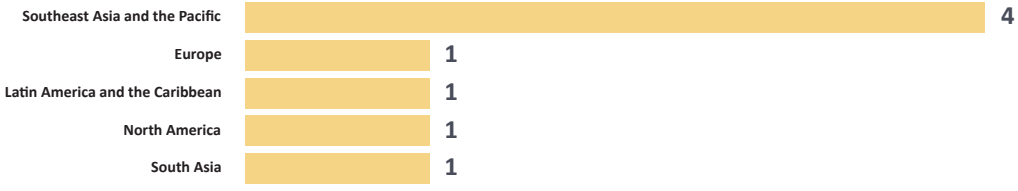
RESULTS BY PARTNER TYPE



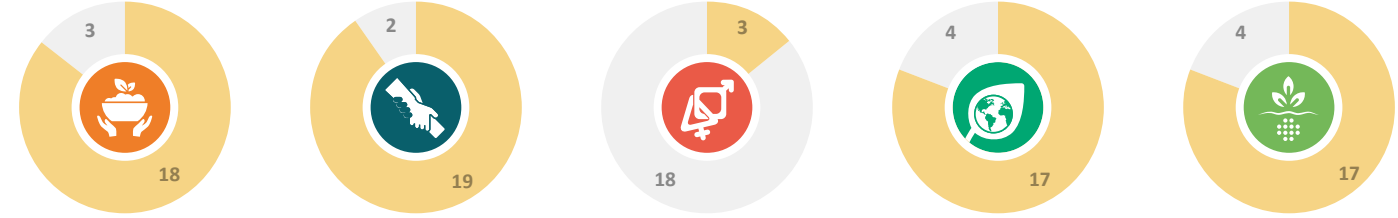
RESULTS BY CONTRIBUTING PARTNERS



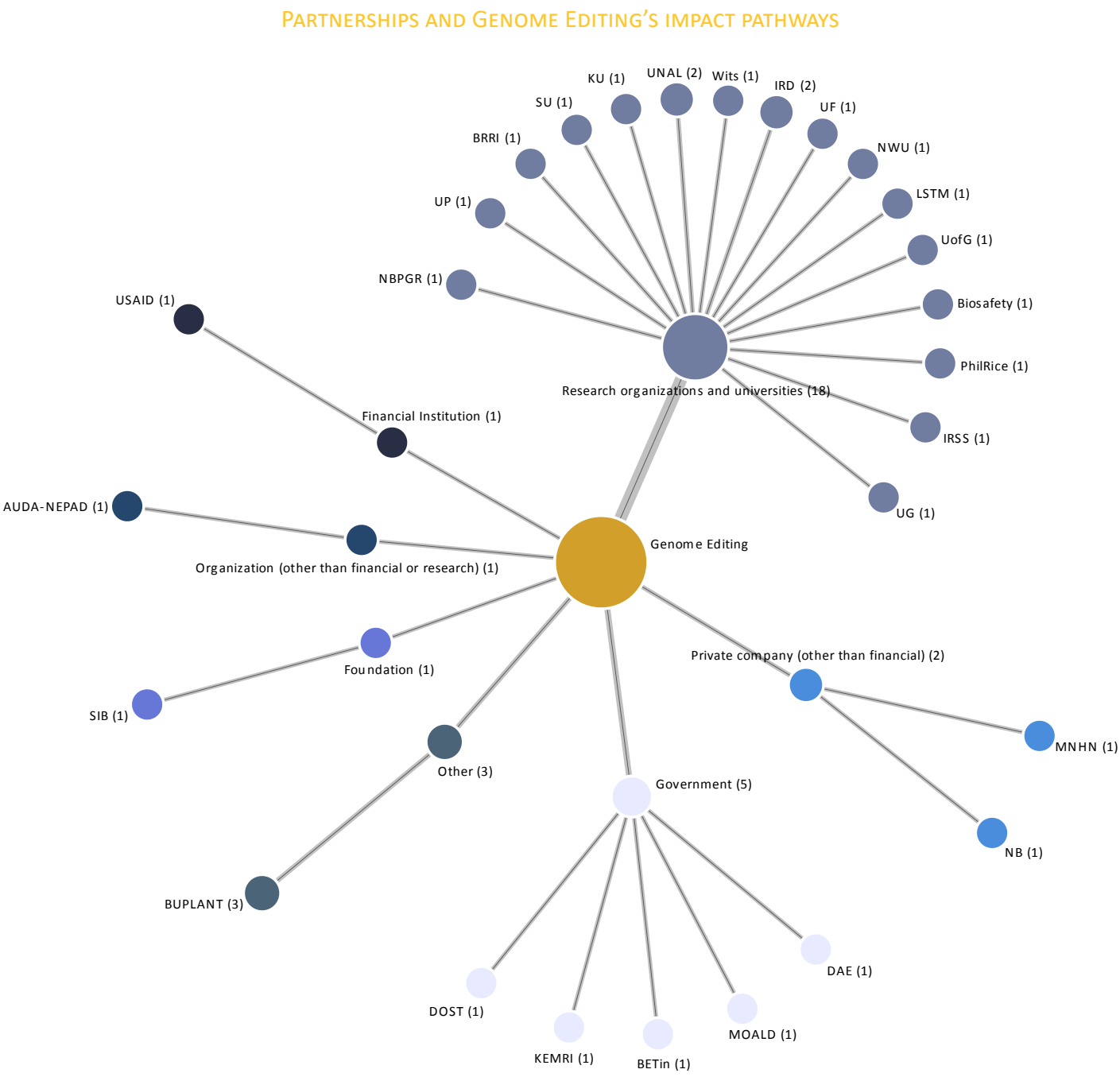
RESULTS BY REGION



CONTRIBUTION OF RESULTS TO THE CGIAR IMPACT AREAS



- **2 = Principal:** Contributing to one or more aspects of the Impact Area is the principal objective of the result. The Impact Area is fundamental to the design of the activity leading to the result; the activity would not have been undertaken without this objective.
- **1 = Significant:** The result directly contributes to one or more aspects of the Impact Area. However, contributing to the Impact Area is not the principal objective of the result.
- **0 = Not targeted:** The result has been screened against the Impact Area, but it has not been found to directly contribute to any aspect of the Impact Area as it is outlined in the CGIAR 2030 Research and Innovation strategy.
- **Not applicable:** Pertains to 2022 reported results when only information on Gender and Climate impact area tagging was available.



Recap of the Genome Editing project's partnership network with the private and public sectors, in particular, the significant number of NARS partners and universities.

Partnerships and Genome Editing's impact pathways

Recap of the Genome Editing project's partnership network with the private and public sectors, in particular, the significant number of NARS partners and universities.

The Genome Editing project emphasizes the importance of partnerships in achieving its EOIOs and contributing to its broader theory of change. These collaborations enhance research capacity, extend reach, and maximize the impact of genome editing innovations for smallholder farmers. By strategically prioritizing partners according to the project stage and regulatory environment, the project ensures equitable access to the technologies while fostering sustainable agricultural solutions and minimizing future technological disparities.

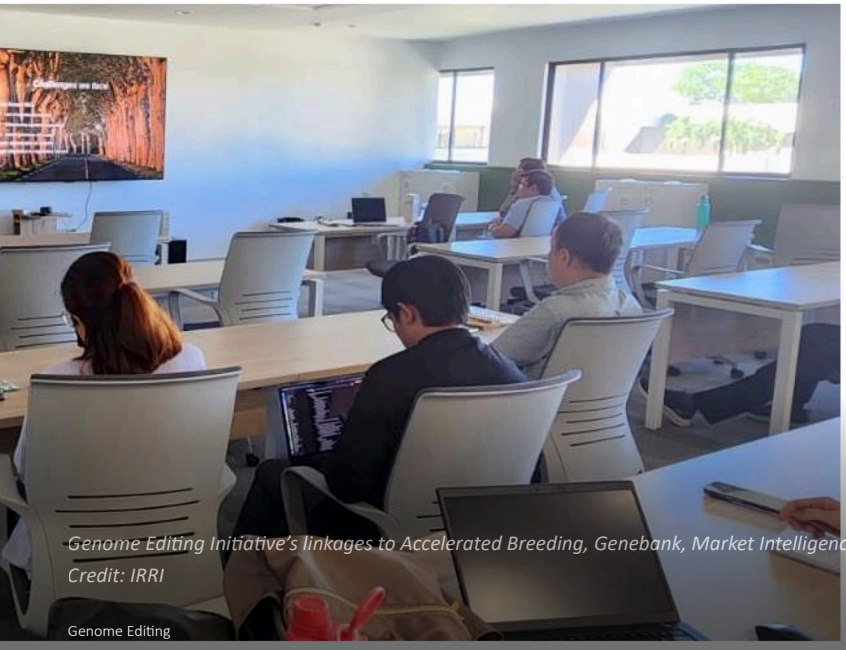
Key collaborations with NARS such as KALRO, in Kenya; the National Root Crops Research Institute, in Nigeria; Universidad Nacional de Colombia; PhilRice, in the Philippines; and the Bangladesh Rice Research Institute are fundamental to the research, validation, and deployment of genome-edited technologies. These partnerships enhance the relevance, scalability, and sustainability of research outputs, nurturing local innovation ecosystems. By working closely with NARS institutions, the project supports evidence-based decision-making and aligns research outputs with national agricultural priorities. Additionally, these partnerships contribute to policy dialogues and regulatory advancements, creating an enabling environment for genome-edited innovations.

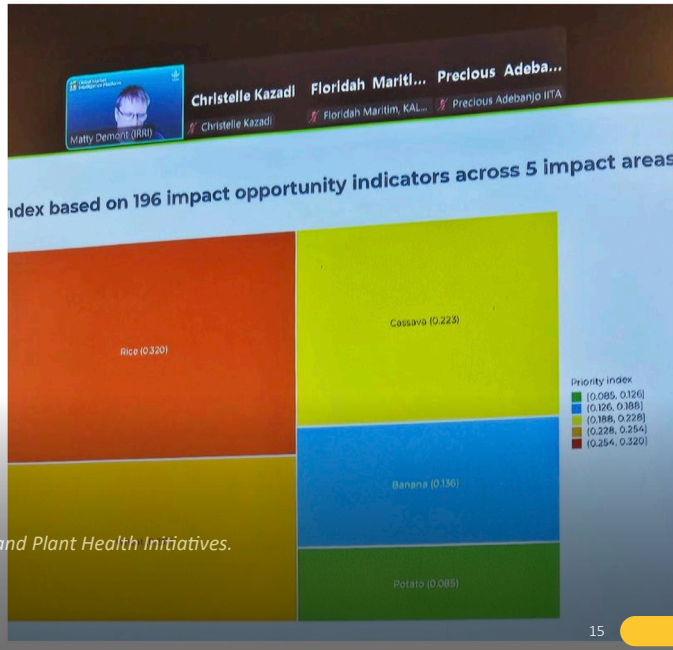
Partnerships with global research leaders, including the Vlaams Institute of Biotechnology Institute for Plant Biotechnology Outreach, French National Research Institute for Sustainable Development (IRD), and other advanced institutions, strengthen genome editing knowledge within CGIAR Centers and NARS. This fosters cross-institutional learning and ensures that genome editing applications are informed by the latest scientific advancements. Through these collaborations, the Genome Editing project makes significant strides toward its EOIOs while ensuring equitable access to technological advancements.

Engagement with private-sector entities like Demeetra and other technology providers is essential for developing genome editing tools tailored to clonally propagated plants and other crops. These collaborations not only advance precision breeding but also address IP challenges associated with genome editing technologies. By leveraging the expertise and resources of the private sector, the Genome Editing facilitates the responsible and sustainable deployment of genome editing innovations.

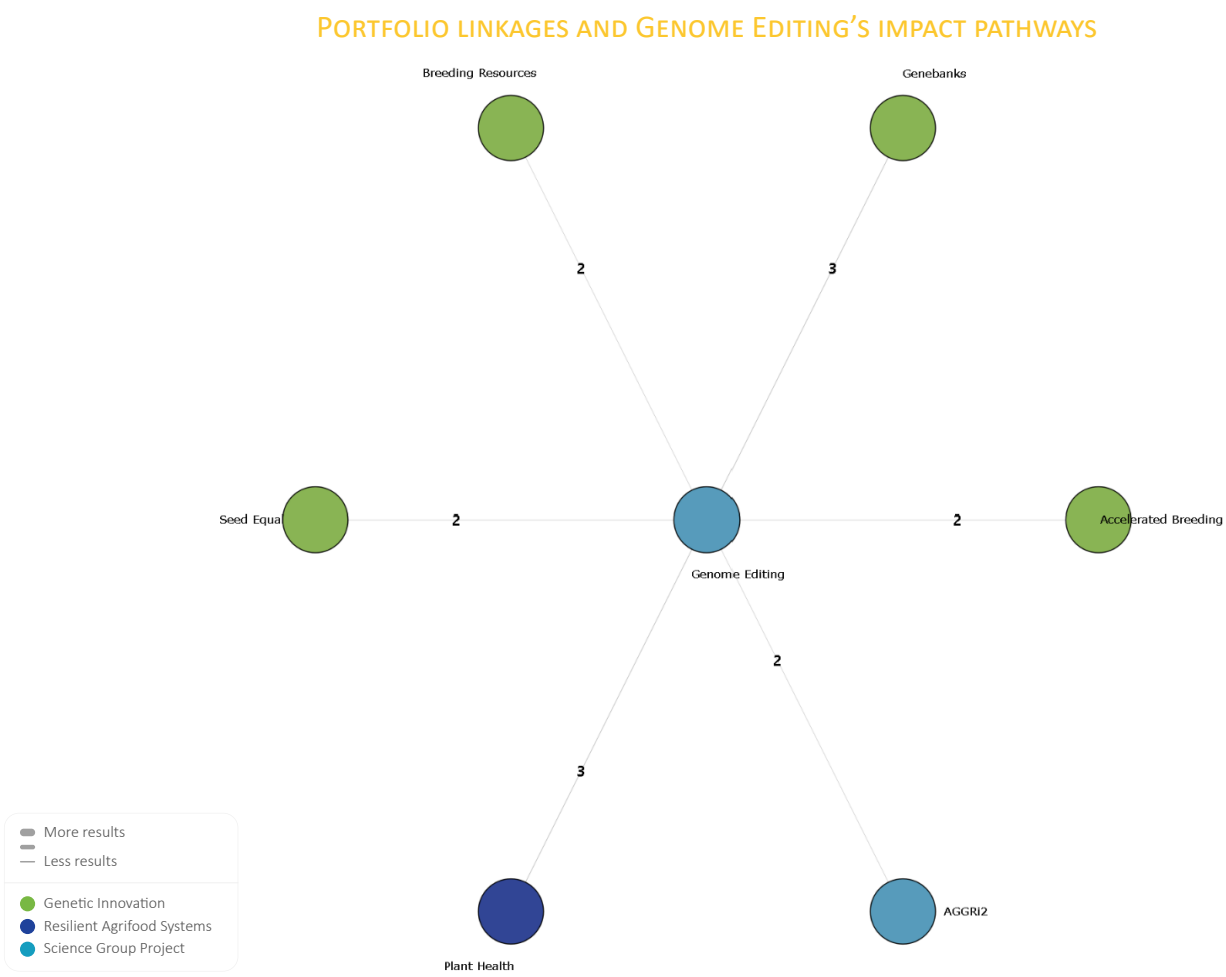
Genome Editing's partnerships are carefully selected based on their capacity to contribute to direct outputs (sphere of control) and their potential to influence key stakeholders expected to adopt best practices (sphere of influence). For example, collaborations with Keystone and the Global Stewardship Group were instrumental in shaping a strategic approach for scaling genome editing innovations during the inception phase. Similarly, the involvement of organizations such as the International Service for the Acquisition of Agri-biotech Applications AfriCenter, Morrison & Foerster Law Firm, and the American Seed Trade Association during the Africa Workshop underscores the project's commitment to fostering global partnerships that support the adoption and scaling of genome editing technologies. Additionally, IRD, CGIAR Science Outreach, and several French institutions co-organized a workshop focusing on genome editing tools and potential future collaborations.

Overall, these partnerships play a pivotal role in research execution, validation, and future deployment of genome-edited technologies, ensuring the relevance, scalability, and sustainability of research outputs. The project's strategic approach to partnership selection and prioritization ensures that partners at the outcome level are best placed to influence actors expected to change practice, contributing to the overall theory of change progress toward EOIOs.





Genome Editing Initiative's linkages to Accelerated Breeding, Genebank, Market Intelligence and Plant Health Initiatives.
Credit: IRRI



These diagrams outline the unique structure of the Genome Editing project being able to link, collaborate, and work together with a high number of Initiatives.

Portfolio linkages and Genome Editing's impact pathways

These diagrams outline the unique structure of the Genome Editing project being able to link, collaborate, and work together with a high number of Initiatives.

The Genome Editing project is unique in its nature. It is CGIAR's only technology-based joint project driven by a strong request from NARS and actively supported by a select group of CGIAR Centers with a proven track record in this field. Additionally, it is currently funded as a bilateral project supported by four donors.

GEd is a powerful emerging technology that enables highly precise modifications to the genetic material of living organisms. While conventional breeding has made significant advancements, delivering impactful genetic solutions for smallholder farmers in developing countries, it alone cannot meet the future demand for high-quality, nutritious food in the face of climate change and the need to expand agriculture into marginal soils.

One of the major limitations of conventional breeding is its inability to precisely select traits without introducing undesirable co-segregating traits from donor lines (known as genetic linkage drag). Efforts to remove these traits are often incomplete, requiring additional breeding cycles that increase costs and delay benefits. Due to this challenge, conventional breeding has traditionally focused on a narrow pool of elite germplasm. However, the increasing availability of diverse sequence information on agricultural crops, combined with climate variability and soil condition expansion, highlights the need for novel approaches to crop improvement.

The widespread availability of sequence data in open access platforms and advancements in bioinformatics tools, in combination with tissue culture technologies, support the application of genome editing in crops. Genome editing allows the team to leverage genetic information from diverse genebank collections, including landraces and wild relatives and repository information from breeding initiatives, to make precise modifications in elite lines — by only a few base pairs or by switching off the barrier genes or by modifying a regulatory element used by a disease agent — without the lengthy process of traditional crossbreeding.

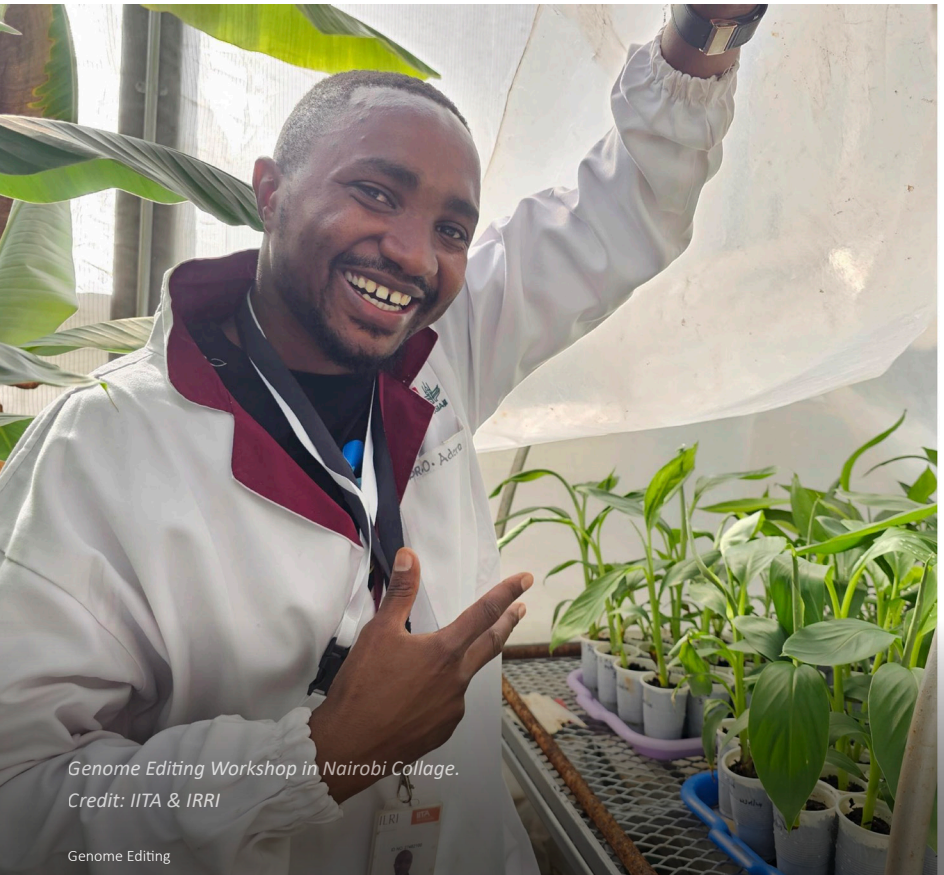
GEd can be directly applied to elite breeding lines and commercial varieties, enabling the rapid and cost-effective introduction of essential traits to farmers. To date, GEd has been successfully applied to more than 40 crops across 25 countries, with several cultivars already released.

As a novel breeding tool, we began by prioritizing key traits through close collaboration with Market Intelligence. Leveraging insights from target product profile consultations with lead breeders from CGIAR's Accelerated Breeding Initiative, we focused on currently intractable traits that classical breeding struggles to address. Additionally, we integrated bioinformatics knowledge as well as phenotyping information from the Breeding Resources and Genebanks Initiatives, utilizing available tools to enhance trait selection precision and then validating expected mutations.

Moving forward, gene-edited products can be integrated into breeding platforms as pre-breeding material for elite lines or directly incorporated into the breeding pipelines of national agricultural research and extension services. This approach will ensure the seamless integration of GEd into global breeding efforts, accelerating the development of high-yielding, climate-resilient crop varieties for smallholder farmers.

Section 7: **Adaptive management**

Exploring Alternative Genome Editing Technologies for Clonally Propagated Plants	Research under WP2 on clonally propagated plants has made significant progress for genome editing in crops like potato, banana and cassava. However, a major challenge remains, such as regenerating whole plants from edited protoplasts in potatoes and ensuring the complete removal of introduced transgenes in bananas. Further exploration and testing of alternative genome editing technologies are essential to overcome these challenges.
Expanding Capacity-Building and Strengthening Partnerships with NARS in Latin America, Africa, and Asia	Existing technical training programs and partnerships are crucial to equip CGIAR and NARS partners with essential genome editing skills. Expanding these initiatives through amplified collaborations with advanced institutions while building skills for local project partners from key NARS will advance local innovation, strengthen breeding programs, and support adoption of beneficial genome-edited products. It is essential that capacity building efforts also extend to skills needed to manage the range of innovation-enabling conditions that support and facilitate a stage-gate product development process. These are resource-intensive efforts that require adequate support to work across the range of products and target geographies.
Enhancing Risk Management Strategies Stewardship, Certification, and Outreach and Communication	Proactive risk management, particularly regarding regulatory challenges and the IP landscape in genome editing, is essential for anticipating potential roadblocks and implementing adaptive strategies. This includes not only the transfer of technology but also the dissemination of best practices in process and product stewardship, as well as effective communication strategies, stakeholder engagement, and regulatory implementation and compliance. These efforts will ensure more predictable project execution and impact realization for project beneficiaries and stakeholders.
Expanding Humanitarian and "Friendly" Licensing Portfolio for Genome Editing Technologies and Evaluating the Licensed Technology for CGIAR Crops	While current IP licensing efforts are promising, final negotiations and agreement signings for this technology remain a relatively new process for CGIAR Centers. It is also essential to test novel licensed genome editing tools for CGIAR crops to assess their effectiveness. Ensuring access for NARS and small and medium enterprises is a key priority. Additionally, there is a need to expand the licensing portfolio to keep pace with the rapid advancements in genome editing technologies.



Genome Editing Workshop in Nairobi Collage.
Credit: IITA & IRRI

Section 8: **Key result story**

High-Fidelity Genome Editing Tool for Banana using Cas-CLOVER

The Cas-CLOVER technology enables precise, transgene-free genome editing in bananas, addressing IP challenges and offering a breakthrough in global agricultural biotechnology.

Cas-CLOVER banana plantlets, banana plants and IITA researchers.
Credit: IITA

Primary Impact Area

Other relevant Impact Areas targeted

Contributing Initiative

Genome Editing

Contributing Centers

IITA – International Institute of Tropical Agriculture

Contributing external partners

Foreign, Commonwealth & Development Office (FCDO) · Bill & Melinda Gates Foundation · Australian Centre for International Agricultural Research (ACIAR)

Geographic scope

Regions: Global

Cas-CLOVER technology has revolutionized genome editing in banana by enabling high-precision, transgene-free editing. This innovation addresses critical bottlenecks in clonal crops, where traditional genome-editing methods struggle to eliminate transgenes and face complex IP constraints. Researchers worldwide can now develop resilient and improved banana varieties without the regulatory hurdles associated with transgenic modifications.

Challenge: Overcoming Transgene Persistence and IP Barriers in Clonal Crops

Bananas, a staple for millions, are highly vulnerable to pests and diseases like Xanthomonas wilt. Genome editing offers a promising solution for disease resistance, but conventional methods introduce transgenes that persist in clonal crops, raising regulatory and public concerns. Additionally, navigating CRISPR-related IP rights remains complex, with restrictive licensing limiting access, particularly in developing regions. Alternative genome-editing approaches with clearer IP frameworks are essential for broad adoption.

Objective: A Transgene-Free Future for Banana Genome Editing

IITA scientists aimed to develop a genome-editing approach that maintains the precision of CRISPR-based systems while eliminating transgenic elements and bypassing restrictive IP barriers. This would enable the development of improved, disease-resistant banana varieties that comply with global biosafety regulations and are accessible to smallholder farmers without legal and financial constraints.

Solution: Developing Cas-CLOVER Technology for Banana

IITA researchers adapted Cas-CLOVER as a precise and efficient alternative to CRISPR/Cas9 to address transgene persistence and restrictive IP barriers. Cas-CLOVER uses a dual-guide RNA system, ensuring high specificity by requiring both gRNAs to bind before initiating double-strand breaks, significantly reducing off-target effects. Researchers integrated Cas-CLOVER with PiggyBac transposase, which introduces genetic cargo and later removes T-DNA, ensuring no foreign DNA remains in the final plant. This alternative system offers researchers a viable solution with fewer IP constraints than CRISPR/Cas9., a system that enables scarless transgene removal.

IITA scientists, in partnership with Demeetra, designed a Cas-CLOVER editing system targeting the banana phytoene desaturase (MusaPDS) gene as a visual marker to validate editing efficiency. The resulting albino phenotype confirmed successful gene disruption, with sequencing revealing up to 91 base pair deletions. Unlike CRISPR/Cas9, which cuts at the PAM-proximal site, Cas-CLOVER mutations occurred within L- and R-gRNA sites, demonstrating a distinct editing pattern.

To apply this technology for disease resistance, two constructs, pDMT-CAPES-PUB1 and pDMT-CAPES-PUB2, were developed to target polyubiquitin box proteins (PUB22/23), genes linked to disease susceptibility. Over 100 genome-edited events were generated using Agrobacterium-mediated transformation in the “Sukali Ndiizi” banana variety. These plants underwent transgene elimination assays using estradiol treatment as PiggyBac was regulated by an inducible promoter. Molecular characterization via Sanger sequencing confirmed targeted mutations in PUB22/23, demonstrating the potential of Cas-CLOVER for precise genome editing. However, all edited plants retained transgenic elements, highlighting the need for optimization in construct design and transgene elimination strategies. Ongoing refinements aim to enhance transgene removal efficiency, bringing Cas-CLOVER technology closer to practical applications in banana breeding and improvement.

Users and Beneficiaries

Cas-CLOVER benefits agricultural researchers and breeders by offering a transgene-free, IP-friendly genome-editing approach. Smallholder banana farmers in Africa, Asia, and Latin America stand to gain from disease-resistant varieties developed with this technology, improving productivity and resilience against significant crop threats.

Next Steps

The project will continue its collaboration between IITA researchers and Demeetra. Key activities include Agrobacterium-mediated transformations, transcriptomic analysis, molecular characterization, and transgene elimination trials. Dissemination of findings through scientific publications, policy engagement, and regulatory discussions will be crucial to ensuring the adoption of Cas-CLOVER technology in global banana breeding programs.

Key Activities and Dissemination

The Cas-CLOVER genome editing research findings were presented at international scientific conferences, engaging experts in plant biotechnology and genome editing. Peer-reviewed publications in leading journals provided a platform for knowledge exchange and validation. Moving forward, optimization of transgene elimination strategies and continued engagement with regulatory bodies and partnerships with national agricultural research institutes will accelerate the deployment of Cas-CLOVER technology in banana improvement.

> This breakthrough in genome editing for banana represents a major step forward in developing disease-resistant, high-yielding varieties without transgenic concerns. The Cas-CLOVER technology holds great promise for transforming the future of banana breeding.
>
> Leena Tripathi, Director of East Africa Hub, IITA

CGIAR SO, IRD and Genome Editing Initiative Meeting.
Credit: CGIAR SO

CGIAR

Genome Editing Workshop in Nairobi Collage.
Credit: IITA & IRRI