J U L Y 2 0 2 1

Flagship Project 1: Enhanced Genetic Resources

LUIS AUGUSTO BECERRA • RTB ISC ANNUAL F2F MEETING

CGIAR RESEARCH PROGRAM ON Roots, Tubers and Bananas

Alliance

LED BY CIP INTERNATIONAL POTATO CENTER · Bioversity International · CIAT International Center for Tropical Agriculture · cirad AGRICULTURAL RESEARCH FOR DEVELOPMENT · IITA Transforming African Agriculture

OUTLINE

KEY SCIENTIFIC ACHIEVEMENTS 2020 & 2021

GENDER OVERVIEW

PROGRESS TOWARDS OUTCOMES & IMPACT

OPPORTUNITIES, PLAND AND TRANSITIONAL RECCOMENDATIONS

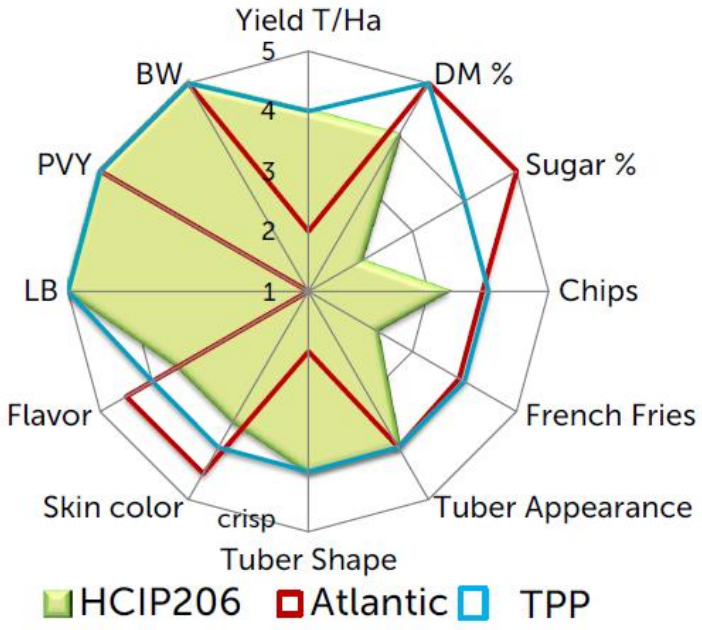
Key scientific achievements 2020-2021

DI1.1: Breeding Community of Practice

Product Advancement Meeting
HCIP 206 variety

syngenta foundation for sustainable agriculture

Targeted product profile potato



1 is poor, 5 is best

Processing / crisp segment
Data collected in Dalat, High Land in Nov18-Feb19 and Sep19-Dec19

	TPP score	HCIP206	Atlantic
Yield T/Ha	4	35.7	11.1
DM %	5	20.7	21.6
Sugar %	4	0.38	0.17
Chips	4	1.7	1.1
French Fries	4	2.7	1.1
Tuber Appearance	4	good	good
Tuber Shape	4	Uni / Large	Inter-Mid
Skin color	4	Yellow	White
Flavor	4	Inter	good
LB	5	Res	Susc
PVY	5	Res	Res
BW	5	Res	Res

TAP-5 Target Product Profile
Variety HCIP206: high yield and Late Blight tolerant

CIP INTERNATIONAL POTATO CENTER

HZPC Growing with our potatoes

DI1.1: Breeding Community of Practice

GI: Market intelligence and product profiling

International Journal of Food Science & Technology — Institute of Food Science & Technology ifst

- From cassava to gari: mapping of quality characteristics and end-user preferences in Cameroon and Nigeria

  Robert Ndjouenkeu, Franklin Ngoualem Kegah, Béla Teeken, Benjamin Okoye, Tessy Madu, Olamide Deborah Olaosebikan, Ugo Chijioke, Abolore Bello ... See all authors

- The East African highland cooking bananas 'Matooke' preferences of farmers and traders: Implications for variety development

  Kenneth Akankwasa, Pricilla Marimo, Robooni Tumuhimbise, Moreen Asasira, Elizabeth Khakasa, Innocent Mpirirwe, Uli Kleih, Lora Forsythe, Geneviève Fliedel ... See all authors

- Prioritising quality traits for gender-responsive breeding for boiled potato in Uganda

  Netsayi Noris Mudege, Sarah Mayanja, John Nyaga, Mariam Nakitto, Samuel Edgar Tinyiro, Damali Babirye Magala, Janet Cox Achora, Sarah Kisakye ... See all authors

- Understanding cassava varietal preferences through pairwise ranking of gari-eba and fufu prepared by local farmer-processors

  Béla Teeken, Afolabi Agbona, Bello Abolore, Olamide Olaosebikan, Emmanuel Alamu, Michael Adesokan, Wasiu Awovale, Tessy Madu, Benjamin Okoye ... See all authors

- End-user preferences for plantain food products in Nigeria and implications for genetic improvement

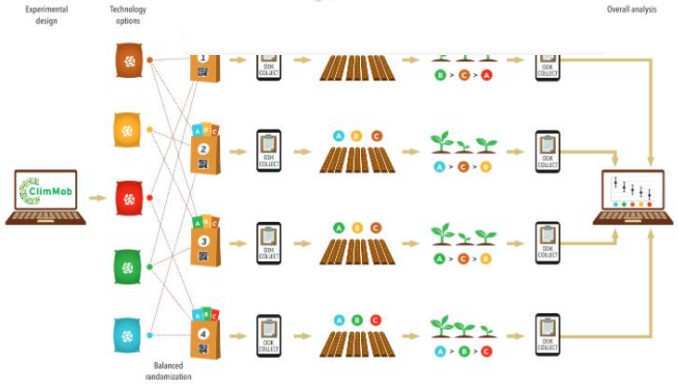
  Delphine Amah, Esmé Stuart, Djana Mignouna, Rony Swennen, Béla Teeken

- Development of a food product profile for boiled and steamed sweetpotato in Uganda for effective breeding

  Robert O. M. Mwanga, Sarah Mayanja, Jolien Swanckaert, Mariam Nakitto, Thomas zum Felde, Wolfgang Grüneberg, Netsayi Mudege, Mukani Moyo ... See all authors

GI: Farmer preferred crop varieties

ClimMob



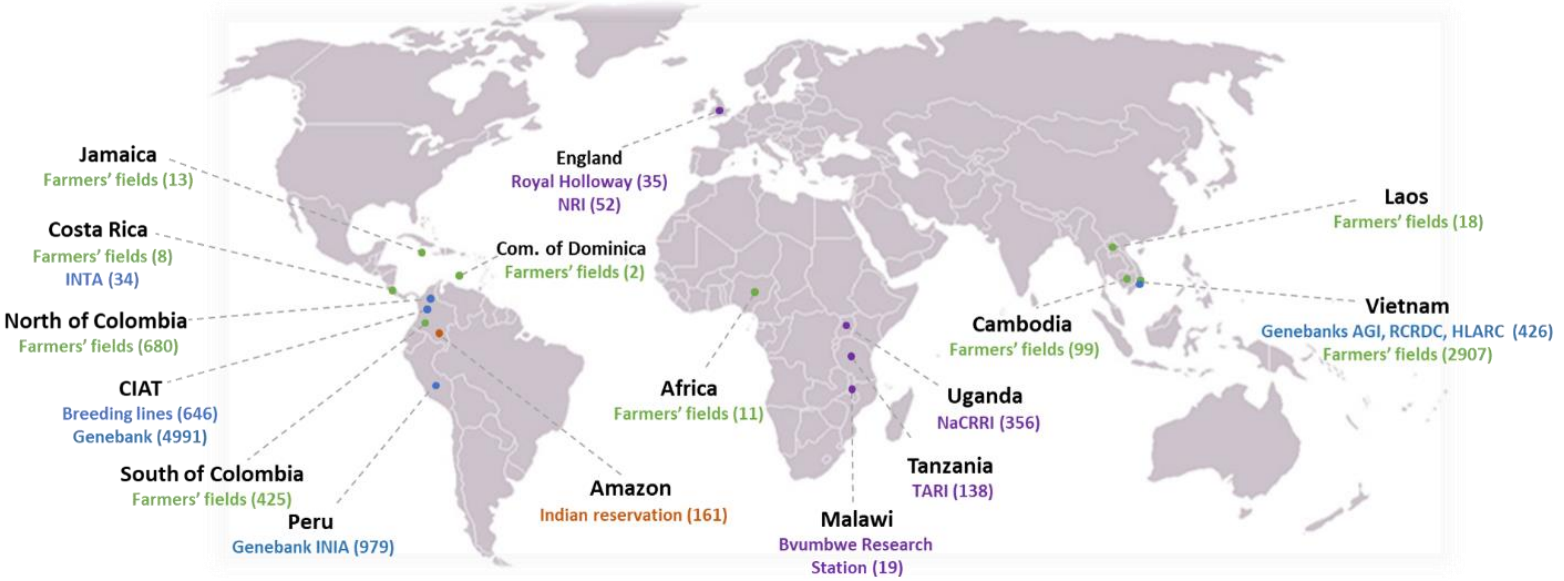
GI: Breeding innovation, modernization and services

BREEDBASE

https://cassavabase.org
https://sweetpotatobase.org
https://musabase.org
https://yambase.org
https://potatobase.org
potatobase coming soon…

DI1.2: Next Generation Breeding

Identification of cassava varieties in ex-situ collections and global farmer's fields

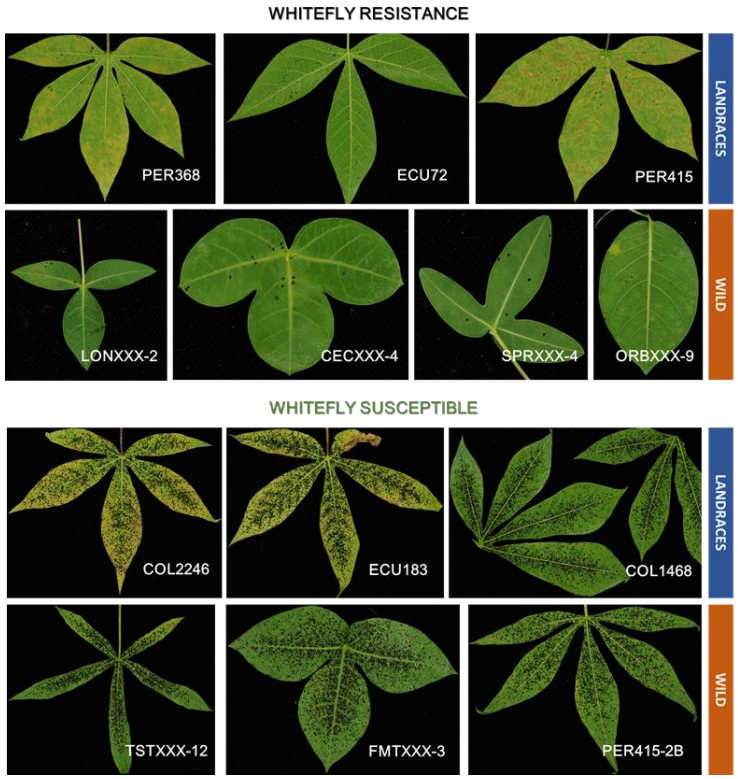


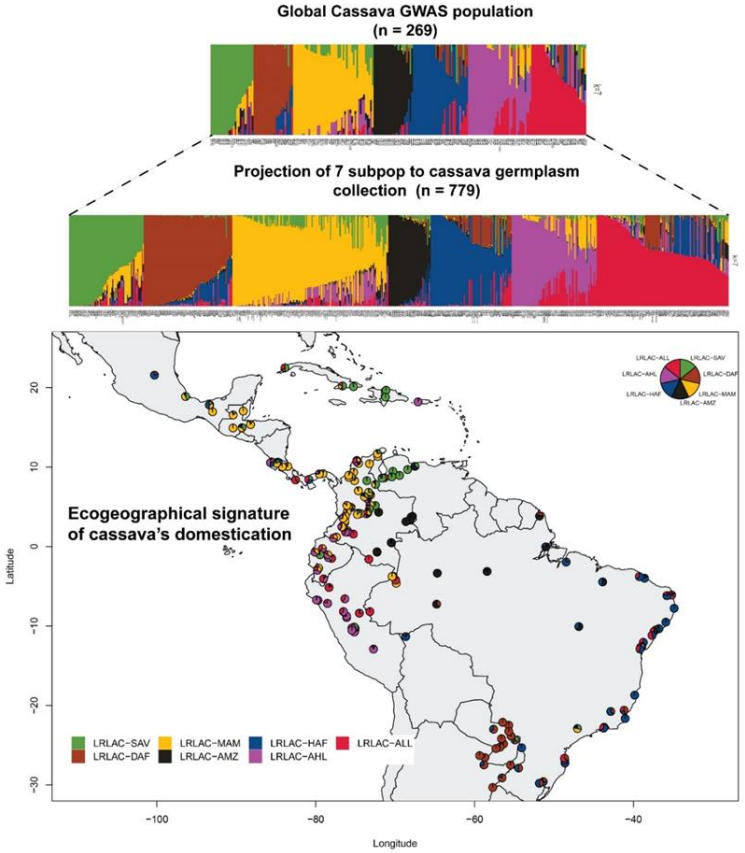
Nearly half of the samples analyzed in last 30 years (11,734) has been processed in the past 4 years using the SNPY-Chip approach 4-fold faster that former molecular methods

DI1.2: Next Generation Breeding

Identification of cassava varieties in ex-situ collections and global farmer's fields

Whitefly Resistance





DI1.2: Next Generation Breeding

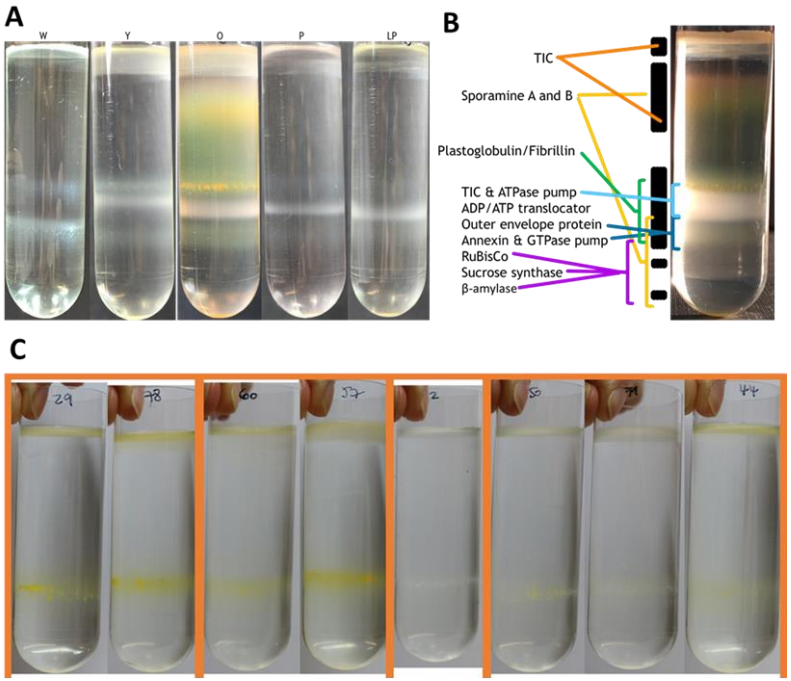
RTB metabolomics platform 2021 progress data acquisition

- Banana population with integrated sensory data.
- Cassava PPD and carotenoid with integrated genomics.
- Sequestration of carotenoids and starch
- Genotyped yam population metabolite profiled.

Technical advances to the RTB metabolomic platforms

- Added **lipidomics**, **volatile** analysis and quantitative **phytohormone** analysis to existing platforms.

- Developed **spatial metabolomics** and used to address **carbon allocation** between **starch** and **carotenoid** formation**.**
- Metabolomics and proteomics on **sub-amyloplast cellular fractions** of sweet potato (A,B) and cassava (C) varieties. Proteins in sweet potato fractions were identified (B).



DI1.3: Game changing traits

3*R* potatoes

Extreme and durable resistance to late blight disease

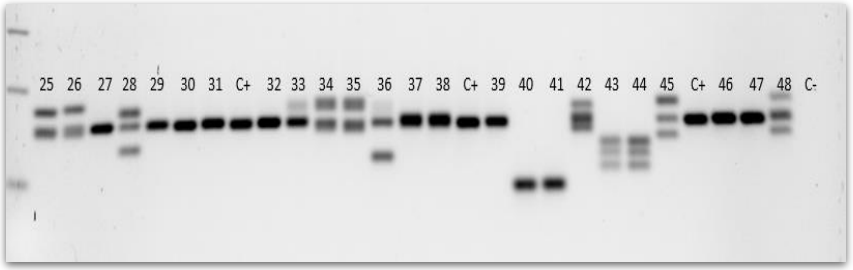
- A stack of 3*R* genes (*RB* (*Rpi-blb1*) and *Rpi-blb2* from *S. bulbocastanum* and *Rpi-vnt1.1* from *S.venturi)* was transferred to farmer preferred varieties (Desiree, Victoria/Asante, Tigoni and Shangi).
- Bioassays of the transgenic events showed complete resistance to late blight.
- Confined field trials (CFTs) conducted for 12 transgenic events from Desiree and 5 transgenic events from Victoria showed complete resistance to late blight.
- T-DNA insertion characterization by targeted sequencing identified **Vic.172** as the best lead transgenic event.
- 2 season multi-location CFTs conducted on Vic.172 for regulatory studies.
- Regulatory study reports for Vic.172 under review for dossier preparation and submission for release.

77 days after planting

DI1.3: Game changing traits

CRISPR-derived New Alleles for *Xanthomonas* Resistance in Cassava

TTCTTCAAAAAAAATAATAAAAGAAACAAGGCCACTGTTACATTGACATATTTTATTCACTTTAATCATGCATGCAAC
TTGACTTCATTCCGTTCCCTGGATTCCTCCCCTATATAAACGCTTCTCGCCCATCCATCATTGCACAACATAGCTAGAG
TTTCCTCTTGAGAAAGAGAGTTTCCTCTGCACAAGGGAAAGAGAGTCTCTACTATAGCCGGAGAATGGCCTTGCACT
TGTCATTGGACTTCGTTTTTGGCGTTTTAGGTACTAATTAAGTTGATAAAGGCTTCATTGCTTTTCTGTTTTGCTTTTG
CTTTCCATCGCTAAACTAATTTGCTCTGTCTTCTTTTTTGCTC



5'-3' Promoter sequence of *MeSweet10a* gene, displaying the EBE for TAL20 Xam668 (red sequence), targeted by two sgRNAs:

- XamEBE-1 highlighted yellow
- XamEBE-2 highlighted blue

First exon highlighted purple.

New alleles for the promoter region of *MeSweet10a* gene.

Multiple band shifts in several CRISPR-edited lines, some possibly homozygous (i.e., 40,41,43,44) with large INDELS, required for durable resistance. Lines may serve as non-GM parental lines after first generation.

C+ represents the wild type allele of TMS6044 variety.

DI1.3: Game changing traits

Genome editing of potato for PVY resistance using prime editing

	Edit in amino acid seq.
pegRNA1	WFDNPIAKSRQTAWGSSL
pegRNA2	WFDSPTAKSRQTAWGSSL
pegRNA3	WFDSPIARSRQTAWGSSL
pegRNA4	WFDSPIAKSRQIAWGSSL
pegRNA5	WFDSPIAKSRQTDWGSSL
pegRNA6	WFDNPTARSRQIDWGSSL
pegRNA7	WFDNPTARSRQIDWGSSL

- Gateway LR reaction with pDePPE transformation vector (INRAE, France) and construct specific pTwist_ENTR vectors
- Protoplast transfection and Agrobacterium mediated transformation
- Regeneration of plants, screening
- Infection assays to evaluate PVY susceptibility in transformed plants

Amino acids targeted for editing using prime editing approach

Amino acids targeted for editing using per construct

DI1.4: Key achievements on RTB crops genetic diversity

- **Collection of novel genetic diversity of RTB crops** from farmers' fields in hotspot areas. Including catalogues of banana in SE Asia and cassava in Peru, Pasco region published. [Surveys were undertaken in RTB crop (banana, cassava, potatoes and yam) hotspot areas in Benin, Peru, and PNG and on farm diversity of crop varieties were collected for genotyping in collaboration with national partners.]

- **Genotyping work of collected samples undertaken**; results indicate that on farm diversity is not fully covered in *ex situ* collection. [116 accessions of banana, 109 yams, 355 casava; 544 different genotypes of native potato varieties identified]

- **The potential of CWR for breeding** in sweet potato tested for improved drought tolerance using potential short-term memory induction.

- **In situ Conservation Knowledge Base** developed as a repository for in situ diversity datasets of RTB crops and providing extensive genetic resources information on diversity of RTB crops and their wild relatives, complemented with Musa In situ tool for the monitoring of natural biodiversity of Musa.

Gender Overview

Gender and Breeding work is housed in FP1

Through RTB-EiB collaboration, the G+ tools for gender responsive breeding were piloted in four breeding programs, findings were used to inform product profile development.

RTB

- Sweetpotato – Uganda (CIP)
- Banana – Uganda (Alliance Bioversity-CIAT)

EiB

- Cassava – Nigeria (IITA)
- Beans- Zimbabwe (Alliance Bioversity-CIAT)

Cassava Nigeria

Banana Uganda

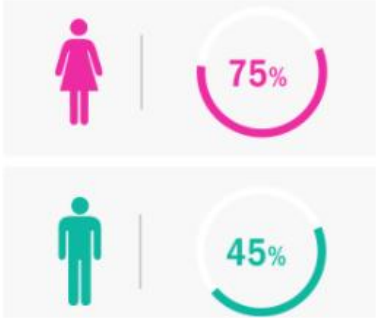
Sweetpotato Uganda

Adapted gender tools and new evidence available

G+ tools highlighted as one of the CGIAR at 50 innovations to change the world

G+ tools published, showcased as an RTB golden egg

Gender and breeding innovations included in the "Market Intelligence" initiative



Inclusion of quality traits in ontologies and trait prioritization for RTB crops

11 journal articles present theoretical framing and practical implications for breeding

International Journal of Food Science and Technology 2021, 56, 1076–1092

Progress towards outcomes & Impact

Innovations & Progress in FP1

Type	Stage 1: Discovery/ proof of concept	Stage 2: Successful piloting	Stage 3: Available/ ready for uptake	Stage 4: Uptake by next user
Biophysical Research	3	1		1
Genetic (variety and breeds)	2	2		1
Production systems and Management practices				
Research and Communication Methodologies and Tools		1	1	
Social Science				
Total	**5**	**4**	**1**	**2**

Development of transgenic potato variety resistant to late blight in Uganda

From Stage 1 to Stage 2:

Completion of proof-of-concept was achieved after 3 seasons in the field, 2 seasons of multilocational confined field trials and 1st draft of regulatory dossier in preparation

Plant Biotechnology Journal

Plant Biotechnology Journal (2019) **17**, pp. 1119–1129 doi: 10.1111/pbi.13042

Stacking three late blight resistance genes from wild species directly into African highland potato varieties confers complete field resistance to local blight races

Marc Ghislain[1,*], Arinaitwe Abel Byarugaba[2], Eric Magembe[1], Anne Njoroge[1], Cristina Rivera[3], Maria Lupe Roman[3,†], José Carlos Tovar[3,‡], Soledad Gamboa[3], Gregory A. Forbes[3], Jan F. Kreuze[3], Alex Barekye[2] and Andrew Kiggundu[4]

Innovations & Progress in FP1

Nigeria releases five cassava varieties developed with genomics-assisted breeding and consumer preference studies

FP1

Genomics-assisted breeding used by IITA and NRCRI to develop new improved varieties (stage 4)

Support / Collaborate

FP2

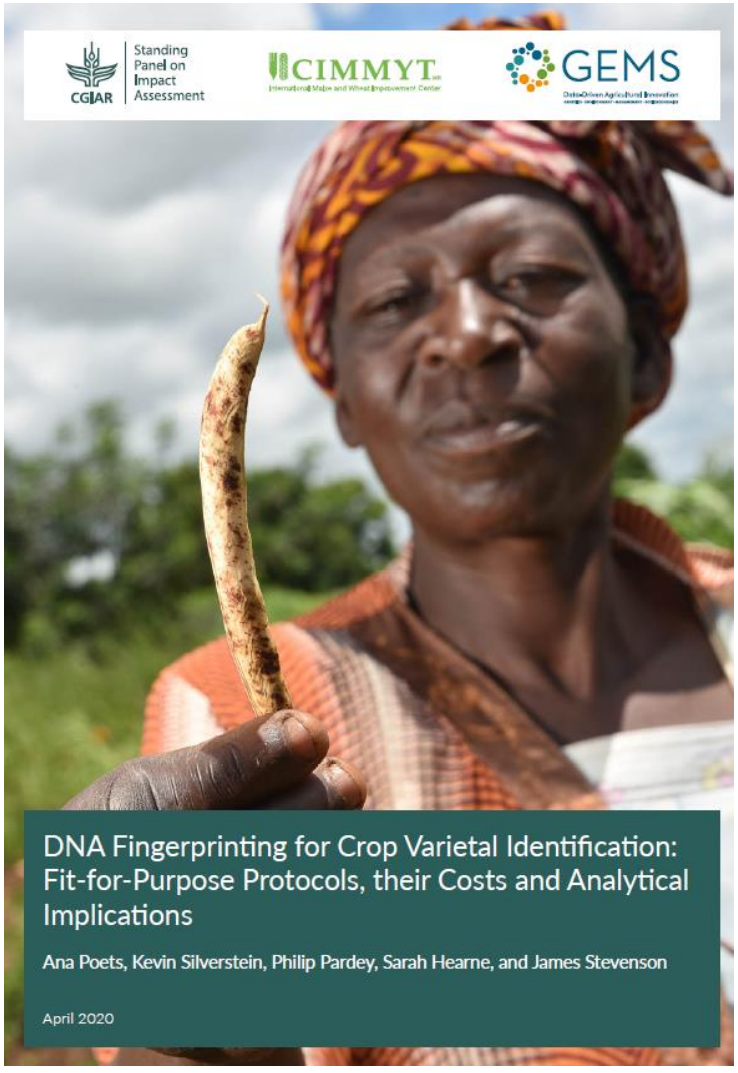
High yielding, virus resistant cassava varieties released in Nigeria (stage 3)

Photo credit: Ismail Rabbi, IITA

Innovations & Progress in FP1

DI1.4: Assessment of on-farm diversity

- DNA fingerprinting for Global Crop Varietal identification and pedigree reconstruction.



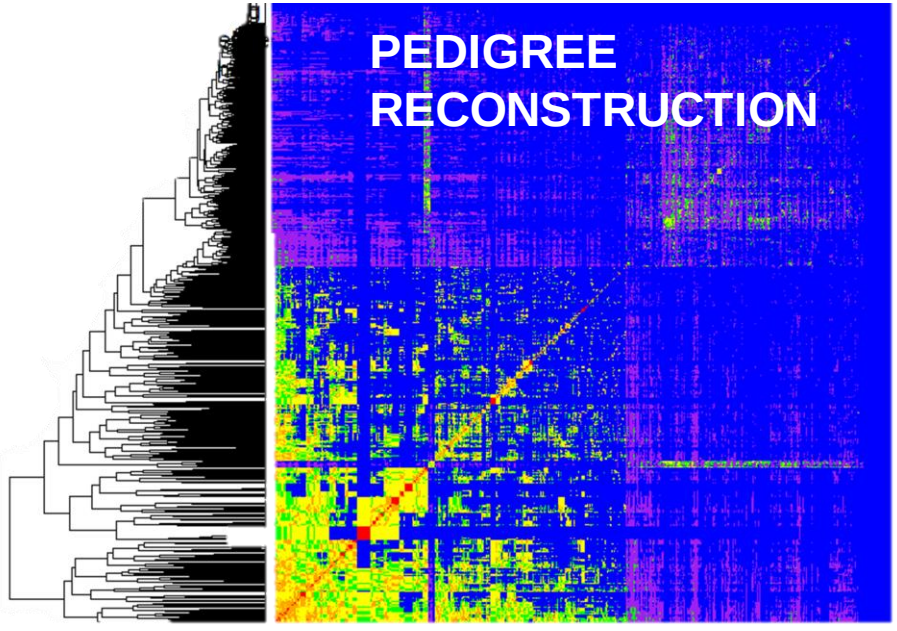
Plant Molecular Biology
https://doi.org/10.1007/s11103-021-01124-0

Check for updates

DNA fingerprinting reveals varietal composition of Vietnamese cassava germplasm (*Manihot esculenta* Crantz) from farmers' field and genebank collections

John Ocampo[1,2] · Tatiana Ovalle[1] · Ricardo Labarta[1] · Dung Phuong Le[1] · Stefan de Haan[1] · Nguyen Anh Vu[3] · Le Quy Kha[4] · Luis A. Becerra Lopez-Lavalle[1]

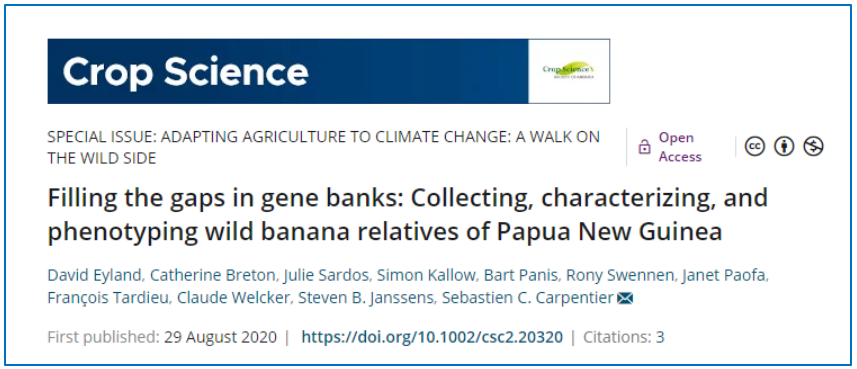
Received: 1 December 2019 / Accepted: 29 January 2021



Innovations & Progress in FP1

DI1.4 Progress towards outcome

Publications on the genotyping work on the *in situ* collected materials from the hotspots of the 4 RTB crops



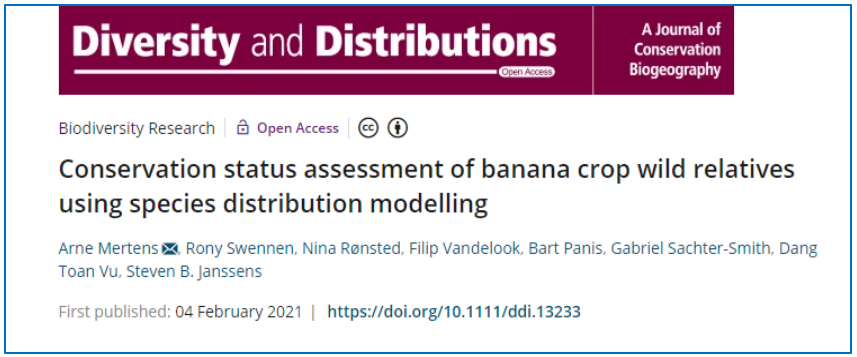
Crop Science

SPECIAL ISSUE: ADAPTING AGRICULTURE TO CLIMATE CHANGE: A WALK ON THE WILD SIDE | Open Access

Filling the gaps in gene banks: Collecting, characterizing, and phenotyping wild banana relatives of Papua New Guinea

David Eyland, Catherine Breton, Julie Sardos, Simon Kallow, Bart Panis, Rony Swennen, Janet Paofa, François Tardieu, Claude Welcker, Steven B. Janssens, Sebastien C. Carpentier

First published: 29 August 2020 | https://doi.org/10.1002/csc2.20320 | Citations: 3



Diversity and Distributions — A Journal of Conservation Biogeography

Biodiversity Research | Open Access

Conservation status assessment of banana crop wild relatives using species distribution modelling

Arne Mertens, Rony Swennen, Nina Rønsted, Filip Vandelook, Bart Panis, Gabriel Sachter-Smith, Dang Toan Vu, Steven B. Janssens

First published: 04 February 2021 | https://doi.org/10.1111/ddi.13233

Publication of blogs on the diversity and distribution of wild banana and yam species in hotspot areas.

Looking ahead: opportunities, plans and transitional issues for One CGIAR

DI1.4 In situ Conservation Golden egg

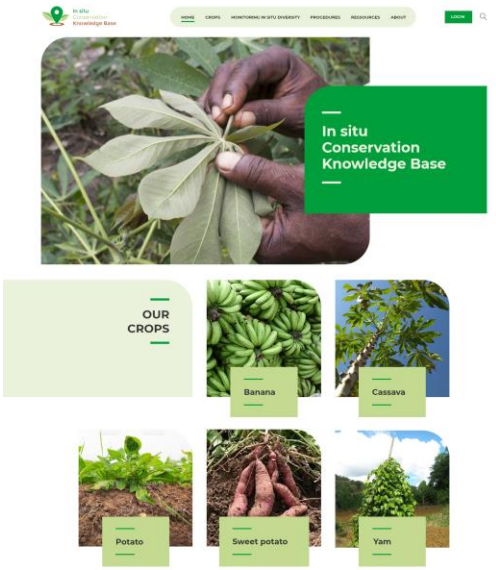
In-situ conservation information system for monitoring RTB crop genetic diversity

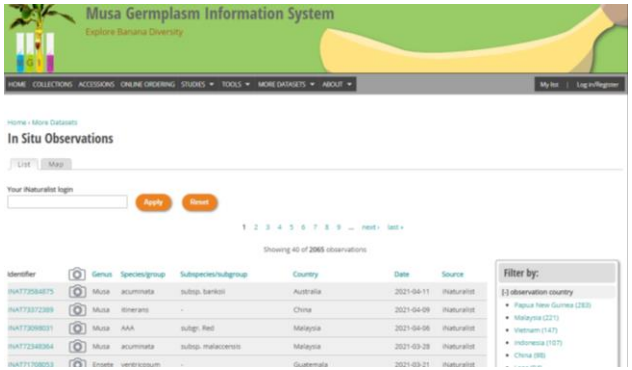
Purpose

Provides a set of in-situ/ex-situ diversity tools and protocols for identifying biodiversity hotspot areas, characterizing genetic diversity and agronomic traits, and for monitoring changes in biodiversity conservation status.

Expected impact

To document and locate local diversity and unlock its broad utilization, while also supporting the conservation of genetic resources to address upcoming climate, social, and economic challenges.





RTB FP1 transitioning to One CGIAR

FOOD, LAND & WATER SYSTEMS LEVEL

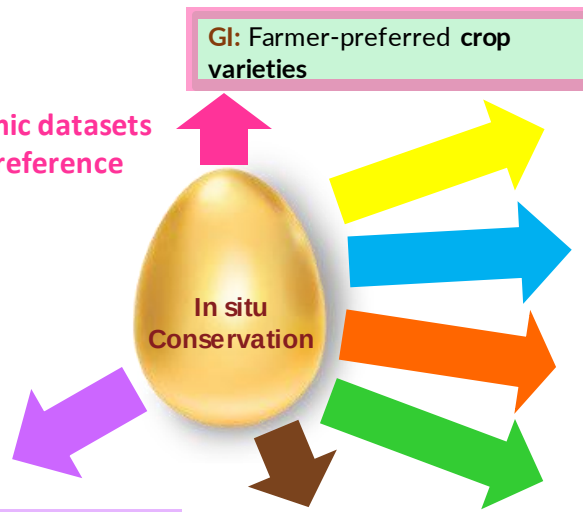
- **ST:** Transforming food systems from **net carbon sources to sinks**
- **ST:** Realizing gains across the **water-energy-food-forest-biodiversity** nexus
- **ST:** **Agroecology** across food, land & water systems
- **ST:** Building systemic resilience to **climate extremes**
- **ST:** Food systems transformation for **healthy, safe & affordable diets**
- **ST:** **Informing sustainable development pathways** with foresight & metrics
- **ST:** **National strategies & policies** for driving transformation
- **ST:** **Rethinking markets and value chains** for inclusion & sustainability
- **ST:** Levering **gender & social equality** in agrifood systems

FARMING SYSTEMS LEVEL

- **RAFS:** **Climate-resilient dryland** crop-tree-livestock systems – dealing with climate variability & risks
- **RAFS:** **Urban and peri-urban** agri-food systems – delivering safe healthy food sustainably
- **RAFS:** **Sustainable intensification** of mixed crop-tree-livestock systems – reducing environmental footprint & improving livelihoods
- **RAFS/ST/GI:** Harnessing **digital technologies** for timely decision-making across food, land, & water systems

THEMATIC LEVEL (COMPONENTS OF SYSTEMS)

- **RAFS:** Protecting human health through a **One Health** approach
- **RAFS:** **Resilient aquatic foods** in food, land & water systems
- **RAFS:** **Climate smart livestock** – policy & practice
- **RAFS:** **Nature-positive agriculture** for agrobiodiversity, water & environment
- **RAFS:** Sustainably improving **livestock productivity** for improved livelihoods
- **RAFS:** Excellence in **agronomy**



RESEARCH PROGRAM ON Roots, Tubers and Bananas

CGIAR

Thank you

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Bioversity International

CIAT

cirad

IITA

DI1.2: Next Generation Breeding

RTB Metabolomics platform outputs 2020/21

Publication outputs

- Drapal, M., Amah, D., Schöny, H., Brown, A., Swennen, R., & Fraser, P. D. (2020). Assessment of metabolic variability and diversity present in leaf, peel and pulp tissue of diploid and triploid Musa spp. ***Phytochemistry***, 176, 112388. https://doi.org/https://doi.org/10.1016/j.phytochem.2020.112388
- Drapal, M., Lindqvist-Kreuze, H., Mihovilovich, E., Aponte, M., Bonierbale, M., & Fraser, P. D. (2020). Cooking dependent loss of metabolites in potato breeding lines and their wild and landrace relatives. ***Journal of Food Composition and Analysis***, 88, 103432. https://doi.org/https://doi.org/10.1016/j.jfca.2020.103432
- Drapal, M., Ovalle Rivera, T. M., Becerra Lopez-Lavalle, L. A., & Fraser, P. D. (2020). Exploring the chemotypes underlying important agronomic and consumer traits in cassava (Manihot esculenta crantz). ***Journal of Plant Physiology***, 251, 153206. https://doi.org/https://doi.org/10.1016/j.jplph.2020.153206
- Price, E. J., Drapal, M., Perez-Fons, L., Amah, D., Bhattacharjee, R., Heider, B., Fraser, P. D. (2020). Metabolite database for root, tuber, and banana crops to facilitate modern breeding in understudied crops. ***The Plant Journal***, 101(6), 1258–1268. https://doi.org/10.1111/tpj.14649
- Enfissi, EMA., Drapal, M., Perez-Fons, L., Nogueira, M., Berry, HM., Almeida, J and Fraser, PD. 2020. New Plant Breeding Techniques and their regulatory implications: An opportunity to advance metabolomics approaches, ***J. Plant Physiol***. doi: 10.1016/j.jplph.2021.153378.

IITA Transforming African Agriculture

CIP INTERNATIONAL POTATO CENTER

Alliance

Bioversity International

CIAT International Center for Tropical Agriculture

ROYAL HOLLOWAY UNIVERSITY OF LONDON

nature methods

PERSPECTIVE

https://doi.org/10.1038/s41592-021-01197-1

Check for updates

Mass spectrometry-based metabolomics: a guide for annotation, quantification and best reporting practices

91. Price, E. J. et al. Metabolite database for root, tuber, and banana crops to facilitate modern breeding in understudied crops. *Plant J.* **101**, 1258–1268 (2020).

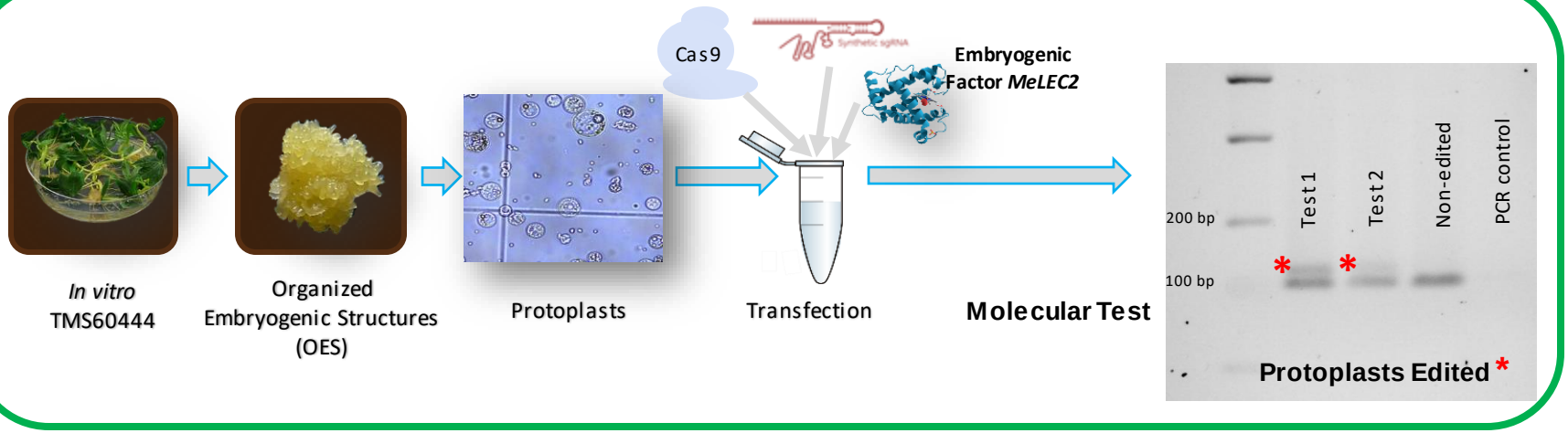
Recent exemplary documentation of a metabolomics experiment that evaluated metabolite levels in crop species, providing not only an extensive database but moreover an excellent example of how to correctly investigate understudied species.

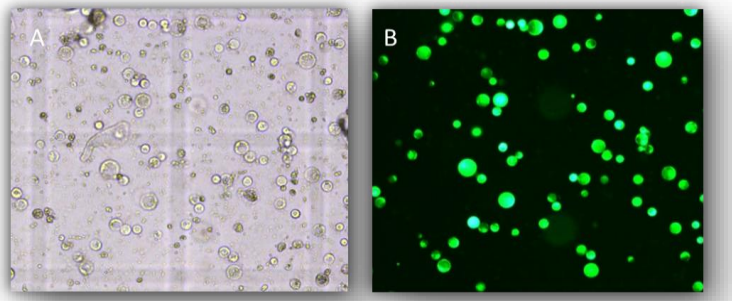
Solid foundation to build on

- **In progress** - 2021 has witness a high input of samples yielding further - **Banana, cassava, sweet potato, potato** & **yam** publications
- Added value funding **BBSRC GCRF** "NutriFood: The production and promotion of nutrient rich foodstuffs to address the double burden of malnutrition" RHUL with IITA **(Dr Ranjana Bhattacharjee)** & **CIAT (Dr Augusto Becerra Lopez-Lavalle**) partners ~ £1.0 million.

DI1.3: Game changing traits

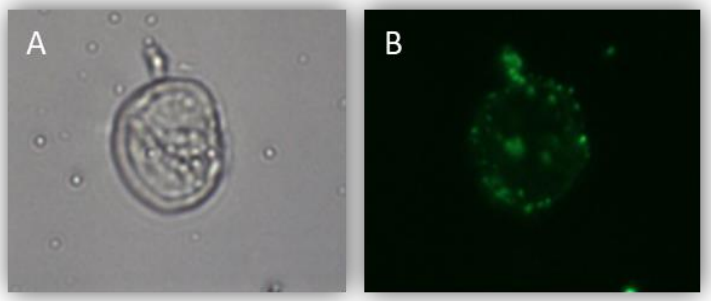
DNA-free Gene Editing **Protocol** for Cassava Protoplasts





Protoplasts isolated from cassava embryogenic tissue.

A. Protoplasts in Neubauer chamber.
B. Protoplasts+FDA under GFP filter.

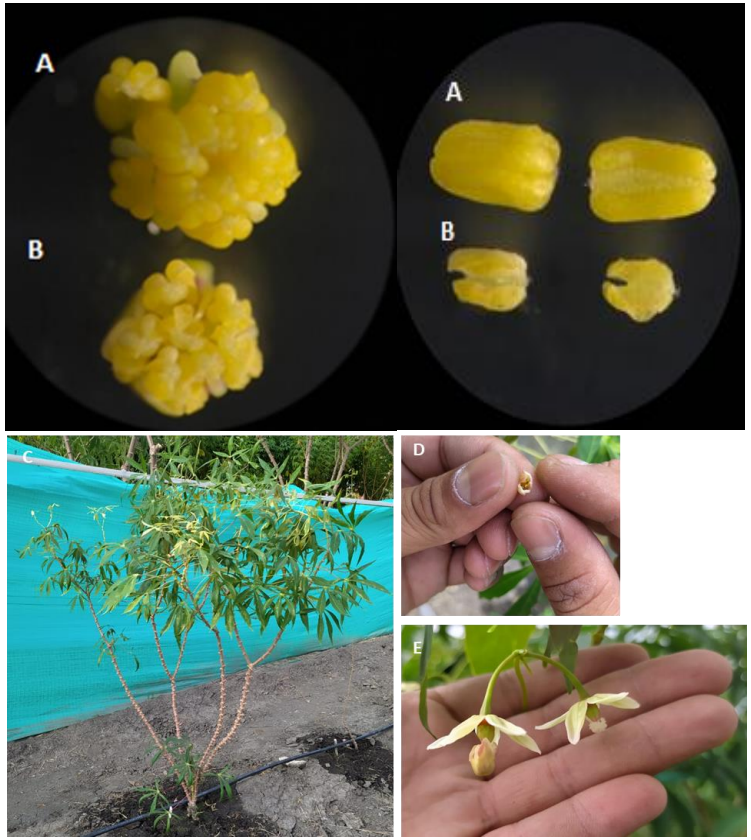


Protoplasts transfected with *MeLEC2* embryogenic protein fused to GFP.

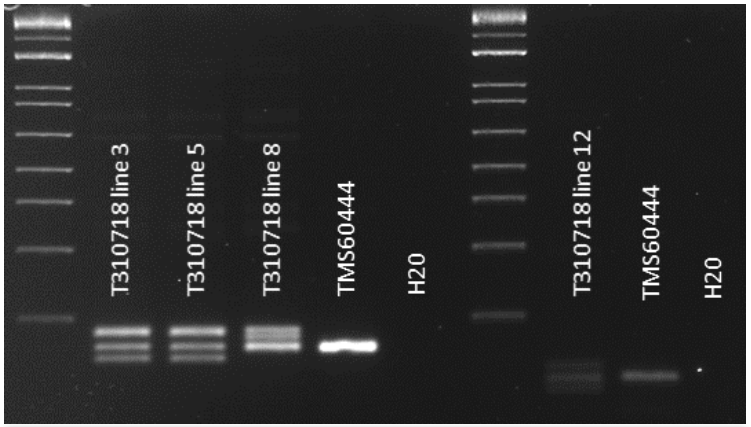
A. Protoplast under white light.
B. Protoplast under GFP filter.

In vitro gene editing test in cassava protoplasts

CRISPR-derived Haploid-Inducer (HI) Cassava **Lines**



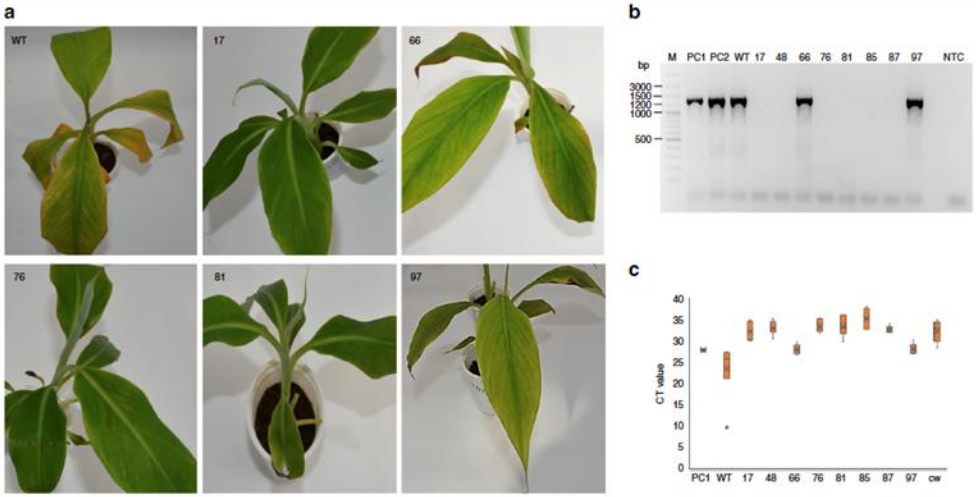
Thirty gene-edited lines were produced by targeting the *pL1a* gene with CRISPR-Cas9 to prevent pollen tube germination. Anthers of wild type TMS60444 (A), and HI-T310718-*pL1a09* (B). HI-lines flowering in the field (C,D,E)



Multiple band shifts in CRISPR-edited HI-lines (T3 series) indicate the presence of INDELS in the *PL1a* gene. HI-lines are to be crossed with wt cassava plants, i.e., TMS6044 or similar, to induce non-GM haploid progeny. The pollen tube shouldn't germinate and therefore transgenes shouldn't be transmitted to progeny.

Gene Editing for Crop Improvement at IITA

- Gene editing platform established for banana and yam.
- Proof of concept established for inactivation of endogenous banana streak Virus (eBSV) integrated in the plantain genome.
- Developing resistance to banana Xanthomonas wilt by knocking down of susceptibility genes (DMR6, SWEET14, Mlo, Bs5).
- Developing resistance for Fusarium wilt.



Evaluation of genome-edited and wild type non-edited control plants of plantain under greenhouse conditions.

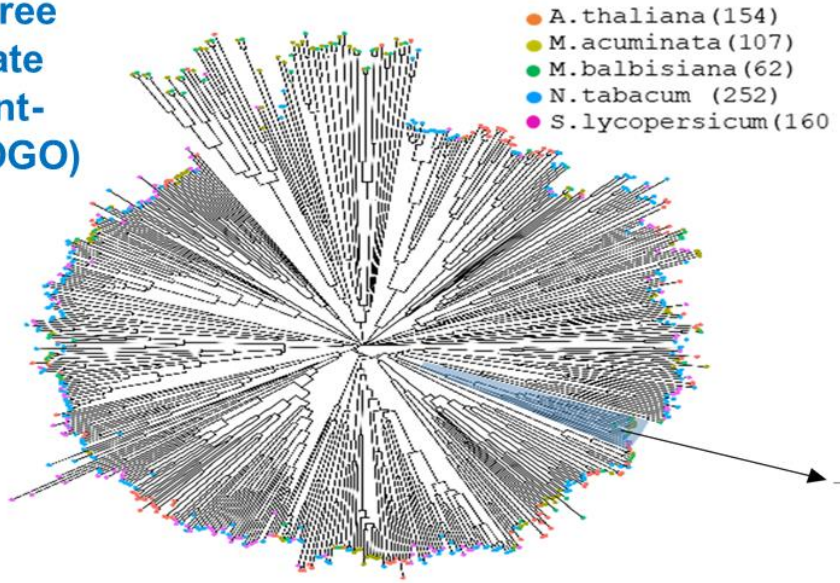
Greenhouse evaluation of *MusaDMR6* mutant and wild-type plants for resistance to banana Xanthomonas wilt disease.

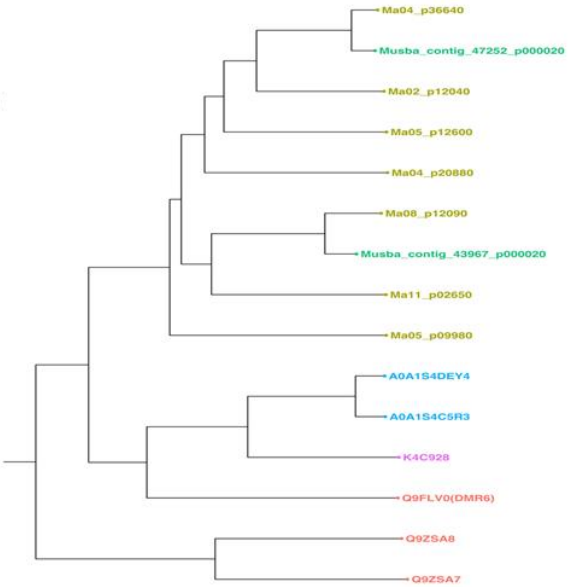
Tripathi *et al.* 2019 Comm Biol
Ntui *et al.* 2020 Cur Biol
Syombua *et al.* Plant Biotech J
Tripathi *et al.* 2021 Plant Biotech J

DI1.3: Game changing traits

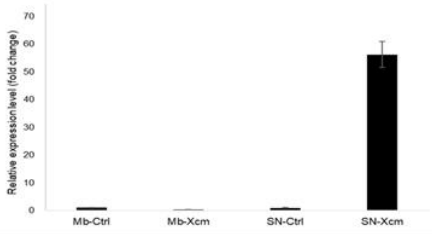
Editing of DMR6 Orthologue in Banana

Phylogenetic tree of 2-oxoglutarate Fe(II)-dependent-oxygenase (2OGO) gene-family





Expression of *MusaDMR6* orthologue (Ma04_p20880.1) in *Musa*



Tripathi et al. (2021) Plant Biotech J

- Downy mildew resistant 6 (DMR6), a susceptibility gene, is activated during host-pathogen interaction.
- DMR6 over-express during pathogen infection and suppress plant immunity.

DI1.3: Game changing traits

Disease resistant Genome-Edited Banana

- Banana genome of Sukali Ndiizi has been edited to knockdown the orthologs of DMR6.
- The banana *dmr6* events showed enhanced resistance to banana Xanthomonas wilt.
- The banana *dmr6* events did not show any detrimental effect on plant growth.

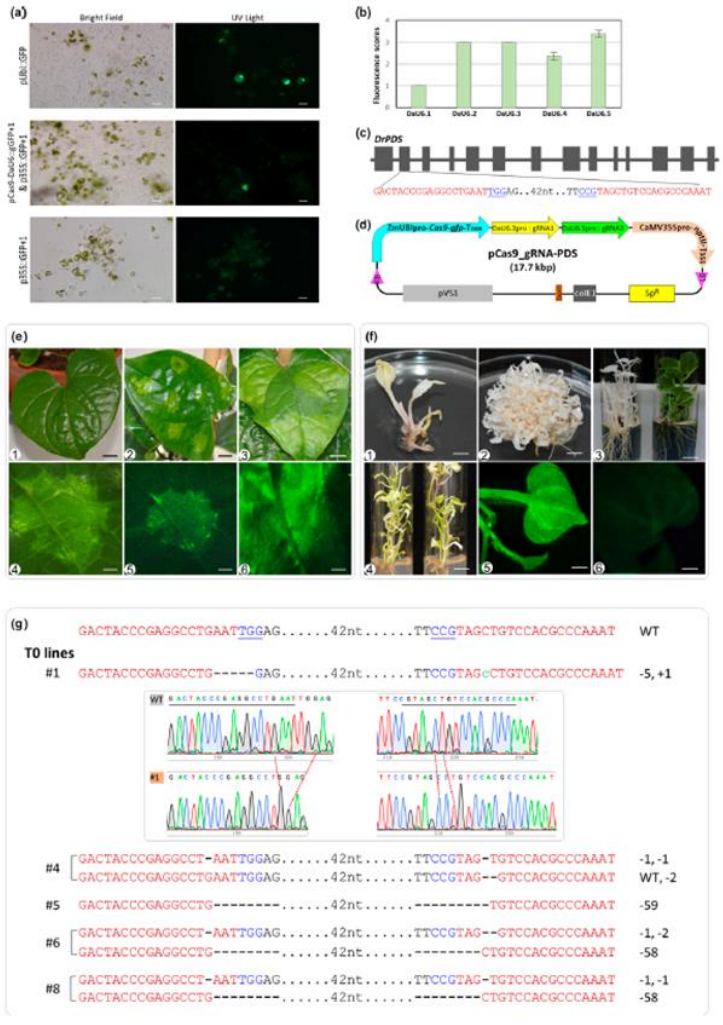
Tripathi et al. (2021) Plant Biotech J

Evaluation of mutants for disease-resistance

DI1.3: Game changing traits

Genome Editing of Yam

- Reference genome for *Dioscorea rotudata* and *D. alata* are available.
- System for genome editing of yam established.
- Supported by NSF and BMGF under BREAD project.
- IITA, Iowa State University and University of Michigan.
- If funds are available, genome editing of yam will target resistance to:
  - Viruses and
  - Anthracnose disease



Syombua *et al.* 2021 Plant Biotech J

DI1.4: Key achievements on RTB crops genetic diversity

- **Collection of novel genetic diversity of RTB crops** from farmers' fields in hotspot areas. Including catalogues of banana in SE Asia and cassava in Peru, Pasco region published. [Surveys were undertaken in RTB crop (banana, cassava, potatoes and yam) hotspot areas in Benin, Peru, and PNG and on farm diversity of crop varieties were collected for genotyping in collaboration with national partners.]

- **Genotyping work of collected samples undertaken**; results indicate that on farm diversity is not fully covered in *ex situ* collection. [116 accessions of banana, 109 yams, 355 casava; 544 different genotypes of native potato varieties identified]

- **The potential of CWR for breeding** in sweet potato tested for improved drought tolerance using potential short-term memory induction.

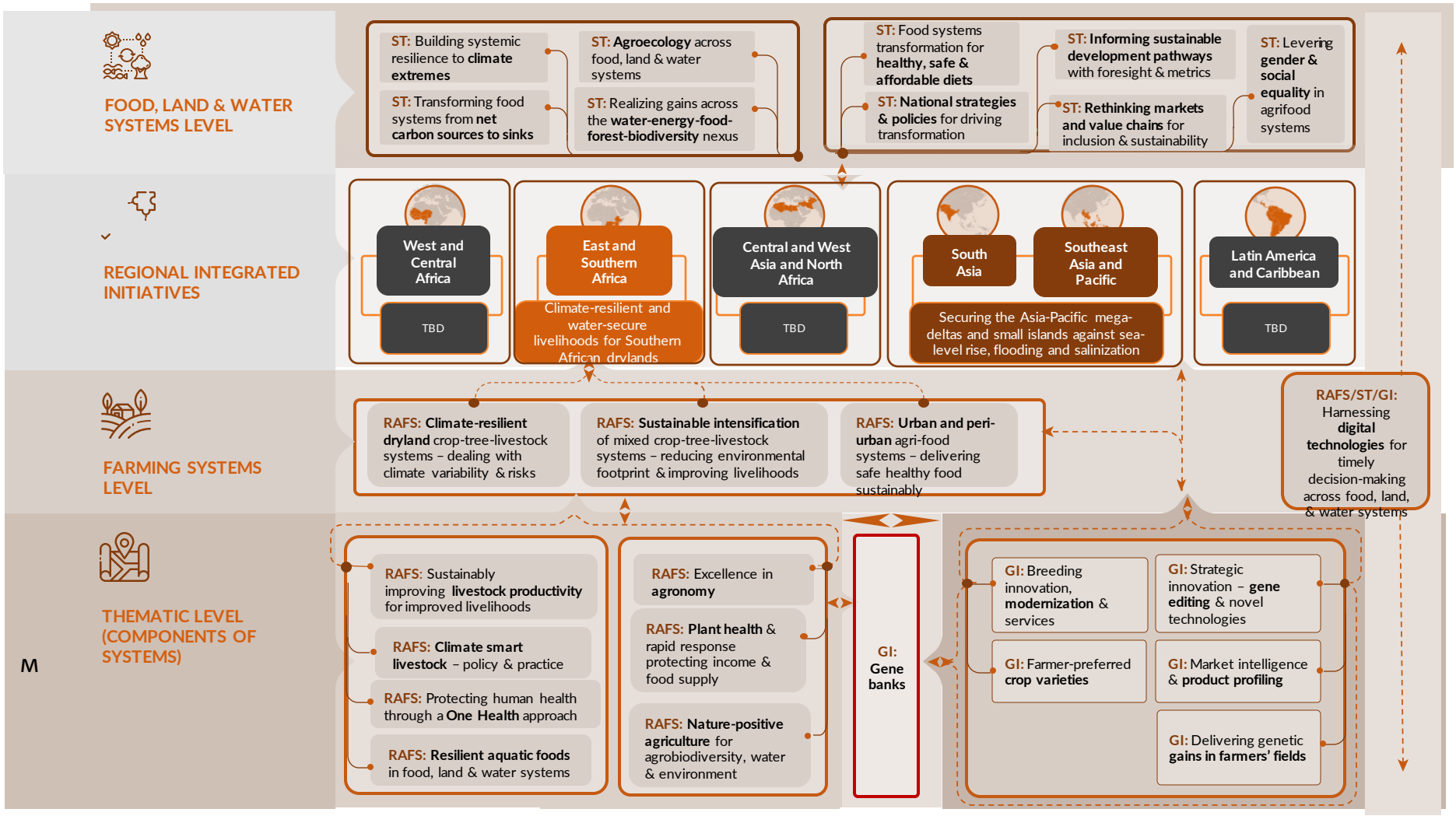
- **Payments for Agrobiodiversity Conservation Services (PACS)**: 24 PACs schemes applied in 4 Latin American countries.

- **In situ Conservation Knowledge Base** developed as a repository for in situ diversity datasets of RTB crops and providing extensive genetic resources information on diversity of RTB crops and their wild relatives, complemented with Musa In situ tool for the monitoring of natural biodiversity of Musa.

- 3000 potato **accessions were successfully cryopreserved**, using improved cryo protocol.

- **Global conservation assessment of banana CWR** undertaken as well as studies on seed storage behavior and population genetics undertaken and published.

Positioning of FP1 in the Initiative Structure



Positioning of FP1 in the Initiative Structure