Cassava

ANNUAL REPORT

2020

Alliance

Bioversity International

CIAT
International Center for Tropical Agriculture
Since 1967 Science to cultivate change

CGIAR

RESEARCH PROGRAM ON
Roots, Tubers and Bananas

The Alliance of Bioversity International and the International Center for Tropical Agriculture (CIAT) delivers research-based solutions that address the global crises of malnutrition, climate change, biodiversity loss, and environmental degradation.

The Alliance focuses on the nexus of agriculture, environment, and nutrition. We work with local, national, and multinational partners across Africa, Asia, and Latin America and the Caribbean, and with the public and private sectors and civil society. With novel partnerships, the Alliance generates evidence and mainstreams innovations to transform food systems and landscapes so that they sustain the planet, drive prosperity, and nourish people in a climate crisis.

The Alliance is part of CGIAR, the world's largest agricultural research and innovation partnership for a food-secure future dedicated to reducing poverty, enhancing food and nutrition security, and improving natural resources.

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CIAT. 2021. Cassava Annual Report 2020. International Center for Tropical Agriculture (CIAT). Cassava Program. Cali, Colombia. 70 p.

Corresponding authors:

Luis Augusto Becerra, Cassava Program Leader
Crops for Nutrition and Health
l.a.becerra@cgiar.org

Jonathan Newby, Cassava Program, Asia Coordinator
Crops for Nutrition and Health
j.newby@cgiar.org

Design and layout: Daniel Gutiérrez
Alliance of Bioversity International and CIAT, Communications Unit

Unless otherwise indicated, the photos used for this handbook are credited to the International Center for Tropical Agriculture (CIAT) and Jonathan Newby.

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Contents

Introduction

Cassava cultivation, though labor intensive and often subsistence oriented, provides smallholders and landless farmers as well as processors and traders across the tropics with a vital entry point for creating employment and income. Outperforming other crops in poor soils and under unpredictable rainfall, cassava is also crucial for enhancing the resilience of crop production systems in the face of climate change. However, cassava will become more susceptible to pests and diseases, as climate change likely increases their range of mobility. Moreover, production costs and postharvest losses remain high; technology uptake is limited; and producers' market links are weak, even though cassava serves as a feedstock for numerous industrial uses, including food, feed, and starch.

The newly formed Alliance of Bioversity International and the International Center for Tropical Agriculture (CIAT) recognizes the vital contribution that cassava makes to poverty reduction, and this is reflected in the objectives and outcomes of the Cassava Sub-Lever's recently developed strategy. In addition, we have prepared a multidisciplinary workplan across our six strategic Research and Service Areas (RSAs). Listed below, the RSAs help integrate work on cassava with the Alliance's strategy and lever structure, and also provide us with an overarching framework for prioritizing investments and delivering impacts in Latin America and the Caribbean (LAC)and Southeast Asia (SEA), while supporting the work of the International Institute of Tropical Agriculture (IITA) in sub-Saharan Africa:

RSA-1: Enhancement of Genetic Resources
Improved varieties (breeding and prebreeding)

RSA-2: Agronomy and Soil Management
Optimized fertility solutions

RSA-3: Crop Protection
Enhanced plant health and insect control (including monitoring techniques)

RSA-4: Seed Systems and Harvesting
Increased access to clean seed material

RSA-5: Post-harvest and Enhanced Nutrition
Better nutrition and income

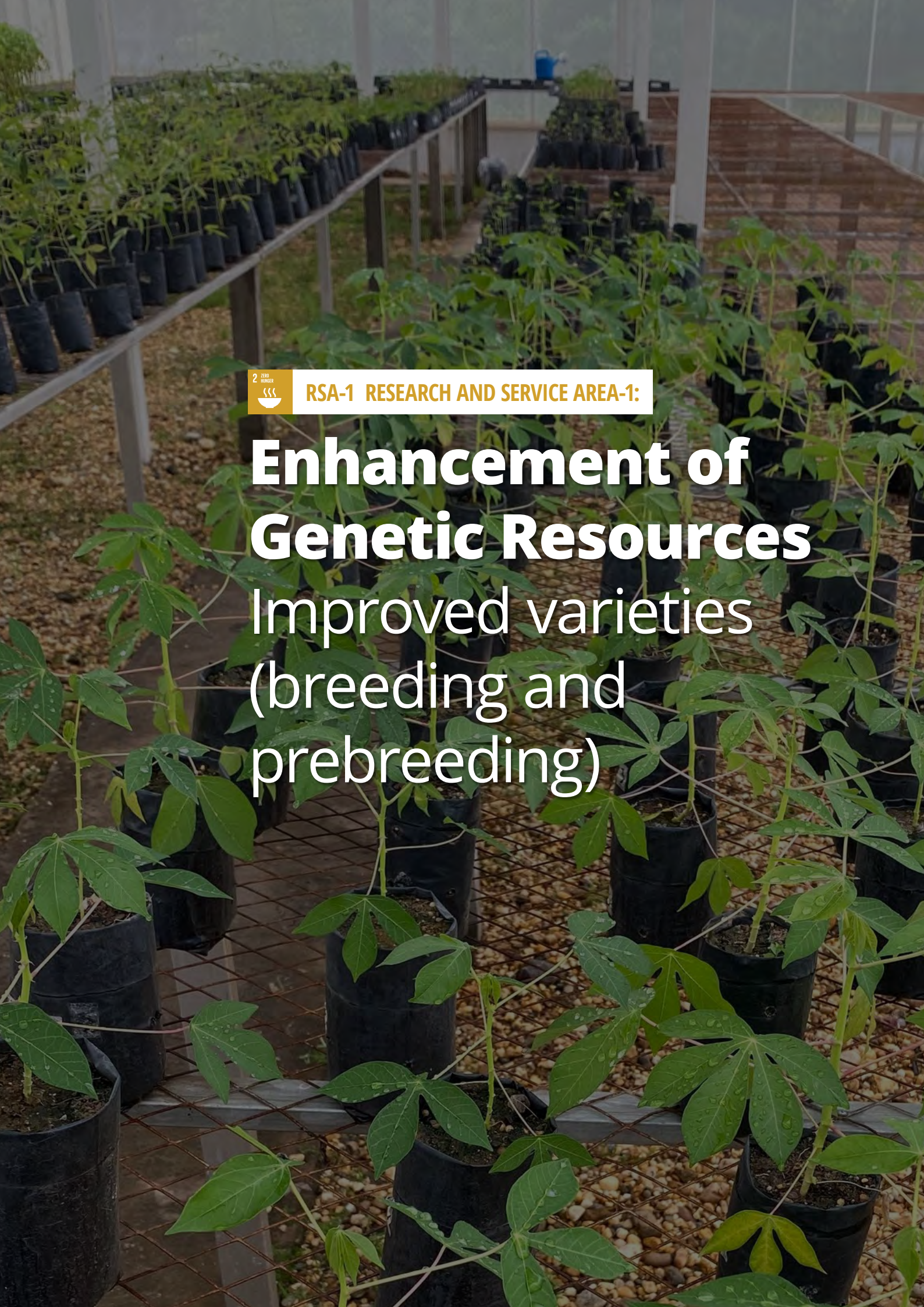
RSA-6: Value Chain, Markets and Policy
Unlocking new market growth (in conjunction with all RSAs)

The following table demonstrates clearly and succinctly how the Cassava Sub-Lever's objectives, outcomes and RSAs are related, and maps these to individual Sustainable Development Goals (SDGs), with the aim of helping donors see how our work on cassava meets their expectations.

Objectives	Longer term objectives – building the future and foundational outcomes – core business	RSAs mapped	SDGs mapped
A. Enhance local resilience and address climate change challenges faced by smallholders growing cassava. (longer term)	1. Diversify cassava for specific landscape uses.	RSA1, RSA2, RSA5, and RS6	**1.** End poverty in all its forms everywhere. **2.** Achieve zero hunger. **5.** Achieve gender equality and empower all women and girls. **9.** Build resilient infrastructure, promote sustainable industrialization and foster innovation. **10.** Reduce inequality within and among countries. **12.** Ensure sustainable consumption and production patterns. **13.** Take urgent action to combat climate change and its impacts. **15.** Improve life on land.
	2. Restore degraded agricultural lands and improve soil health.		**1.** End poverty in all its forms everywhere. **15.** Improve life on land.
B. Boost productivity and create opportunities for smallholders to grow cassava as part of their farming system. (longer term)	3. Maintain yield potential in changing farming systems, with minimal yield gaps.	RSA1, RSA2, RSA5 and RS6	**1.** End poverty in all its forms everywhere. **2.** Achieve zero hunger.
	4. Promote more sustainable resource use.	RSA1, RSA2, RSA4, RSA5 and RS6	**2.** Achieve zero hunger. **5.** Achieve gender equality and empower all women and girls. **10.** Reduce inequality within and among countries. **12.** Ensure sustainable consumption and production patterns. **13.** Take urgent action to combat climate change and its impacts. **15.** Improve life on land.

Objectives	Longer term objectives – building the future and foundational outcomes – core business	RSAs mapped	SDGs mapped
A. Enhance local resilience and address climate change challenges faced by smallholders growing cassava. B. Boost productivity and create opportunities for smallholders to grow cassava as part of their farming system.	5. Deliver smarter, more affordable solutions across the breeding and value chain.	RSA1, RSA2, RSA3, RSA4 and RSA5	**1.** End poverty in all its forms everywhere. **2.** Achieve zero hunger. **5.** Achieve gender equality and empower all women and girls. **9.** Build resilient infrastructure, promote sustainable industrialization and foster innovation. **10.** Reduce inequality within and among countries. **12.** Ensure sustainable consumption and production patterns. **13.** Take urgent action to combat climate change and its impacts. **15.** Improve life on land.
	6. Achieve more effective pest and disease management.	RSA1, RSA2, RSA3, RSA4 and RSA6	**1.** End poverty in all its forms everywhere. **2.** Achieve zero hunger.
	7. Target research and interventions to beneficiaries and technology adoption pathways.	RSA1, RSA2, RSA3, RSA4, RSA5 and RSA6	Strategic targeting of interventions to increase research impacts for target beneficiaries and make research delivery more efficient.

Guided by this strategic framework, the Cassava Sub-Lever relies on multiple strengths to fulfill its mission of improving the livelihoods of smallholder farmers through genetic solutions to global problems that are fit for purpose within agricultural-economic-social-ecological systems. In operational terms, the RSAs create logical groupings of work around key themes and areas of expertise. In the sections that follow, we report on some of the noteworthy results that the Cassava Sub-Lever achieved in 2020 through its six RSAs.



RSA-1 RESEARCH AND SERVICE AREA-1:

Enhancement of Genetic Resources

Improved varieties (breeding and prebreeding)

In 2020, RSA-1 continued targeting problems in cassava production that have genetic or agronomic solutions, with the aim of increasing the productivity, sustainability and use of this crop in the LAC and SEA regions. Through research on prebreeding, breeding, next-generation breeding, carotene expression profile, waxy cassava variation, cassava mosaic disease sequencing, cassava ORANGE protein characterization, doubled-haploid induction, genetic transformation and gene editing, we continued to advance the genomic-based characterization and identification of genetic diversity in the global cassava reference collection.

RSA-1 research results and the scientists contributing are detailed in the sections that follow.

Scientists contributing to RSA-1:

Xiaofei Zhang
Adriana Bohórquez
Luis Augusto Becerra
Paul Chavarriaga

Other contributing scientists and staff:

Adriana Vásquez
Alejandro Brand
Anestis Gkanogiannis
Angélica Jaramillo
Anibal Peñaloza
Carlos A. Ordóñez
Carmen Bolaños
Daniel Álvarez
Daniel Encarnación
Diana Victoria Marín
Didier Marín
Eugenio Bolaños
Francisco Sánchez
Gerardino Pérez
Hernán Camilo Vargas
Jairo Rodríguez
Janneth Patricia Gutiérrez
Jhon Larry Moreno
John Belalcázar
Jorge I. Lenis
Juan P. Arciniégas
Katherine Castillo
María Isabel Gómez
Nelson Morante
Orlando Vacca
Sandra Salazar
Tatiana Melissa Ovalle
Thierry Tran
Thuy Cu Thi Le

XIAOFEI ZHANG

Prebreeding, breeding, and next-generation breeding

Collaborators: Sandra Salazar, Nelson Morante, Hernán Camilo Vargas, Jorge Iván Lenis and Thierry Tran.

Clones with dual resistance to cassava mosaic disease (CMD) and cassava brown streak disease (CBSD)

We generated and shared 18 full-sib families of CBSD x CMD clones with Dr. Stephan Winter to screen for CBSD resistance. We observed segregation within full-sib families, indicating that the CBSD resistance could be dominant. Moreover, we identified seven clones with dual resistance to CMD and CBSD, and shared these with african national programs. These clones serve as CBSD donors for breeding programs, thus providing a new solution to the CBSD pandemic in Africa.

Understanding target population of environment (TPE) in global cassava production regions

In collaboration with the Alliance's Climate Action Team, we analyzed the climate similarity of global cassava production regions. We observed that the Caribbean coast of Colombia (in the sub-humid and semi-arid lowland tropics) represents cassava growing regions that account for about 50% of global production, including Africa and SEA. Thus, we are focusing our breeding activities in this area to develop cassava varieties or populations that are adapted to major cassava production regions, especially in Asia and Africa.

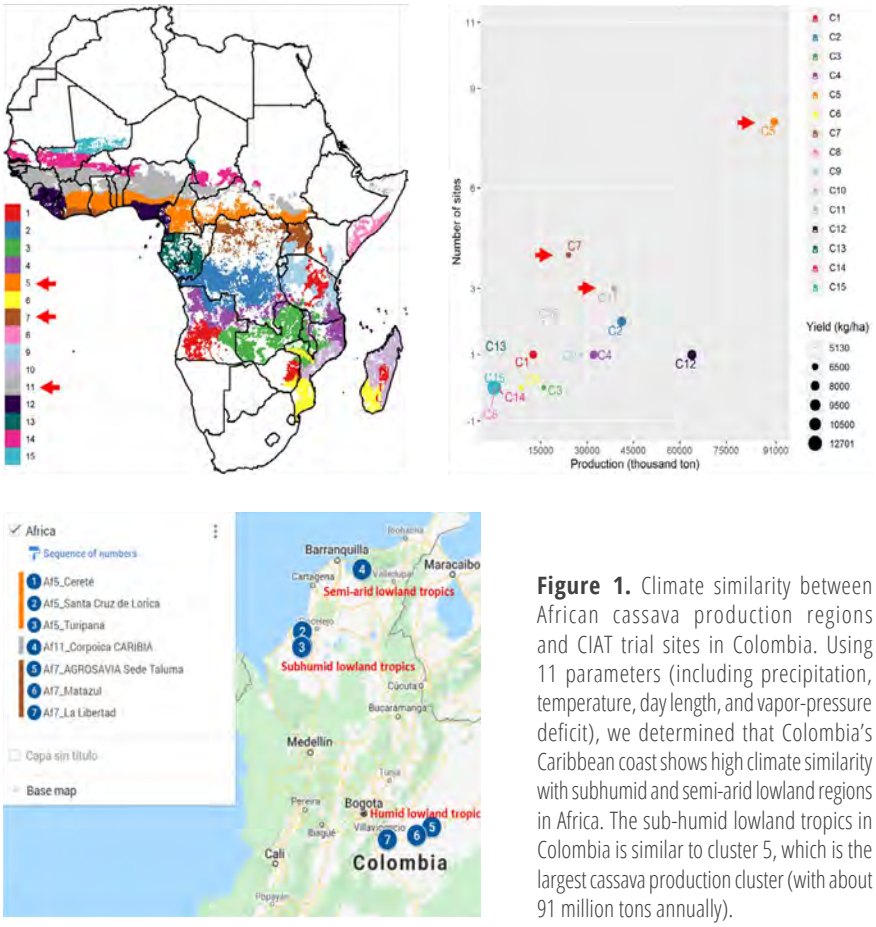


Figure 1. Climate similarity between African cassava production regions and CIAT trial sites in Colombia. Using 11 parameters (including precipitation, temperature, day length, and vapor-pressure deficit), we determined that Colombia's Caribbean coast shows high climate similarity with subhumid and semi-arid lowland regions in Africa. The sub-humid lowland tropics in Colombia is similar to cluster 5, which is the largest cassava production cluster (with about 91 million tons annually).

Validating *CMD2* markers in breeding populations

We screened a multi-parental population and the elite parents using S12_7926132 and S14_4626854. The two markers explained 51% of the population variance in CMD severity. These two markers will be used for marker-assisted selection (MAS) to develop CMD-resistant varieties for Africa and Asia. We also observed a high rate of co-segregation (73%) between two markers on different chromosomes (12 and 14), which requires further investigation.

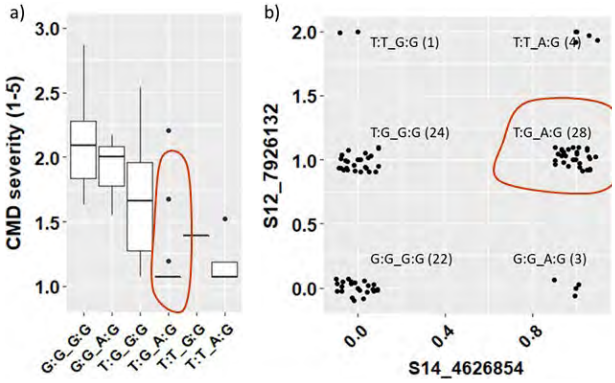


Figure 2. The combined effect and similarity of markers S12_7926132 and S14_4626854 in a multi-parental population. The two markers provided better prediction of CMD resistance. The genotypes with resistant alleles T and A showed high resistance to CMD (red circle).

A) the CMD score of the clones grouped based on the two CMD markers. The x-axis shows the genotype combination. For example, T:G_G:G means the clones had T:G alleles of S12_7926132 and G:G alleles of S14_4626854.

B) genotypic similarity between the markers S12_7926132 and S14_4626854. The two markers matched well for the susceptible genetics, G:G_G:G (22 vs. 25), and the clones with T:G were divided into two groups (24 vs. 28). The 28 clones with both resistant alleles A and T showed high CMD resistance.

Implementing genomic prediction

We developed the training population, using seeds harvested from the 2019-2020 pollination season. The training population was derived from 22 progenitors with high dry matter, nine progenitors with good cooking quality, and 12 progenitors with CMD or whitefly resistance. In total, 392 clones from 44 full-sib families were increased for stake production. We established replicated yield trials in two target environments in May 2021 to develop genomic prediction models.

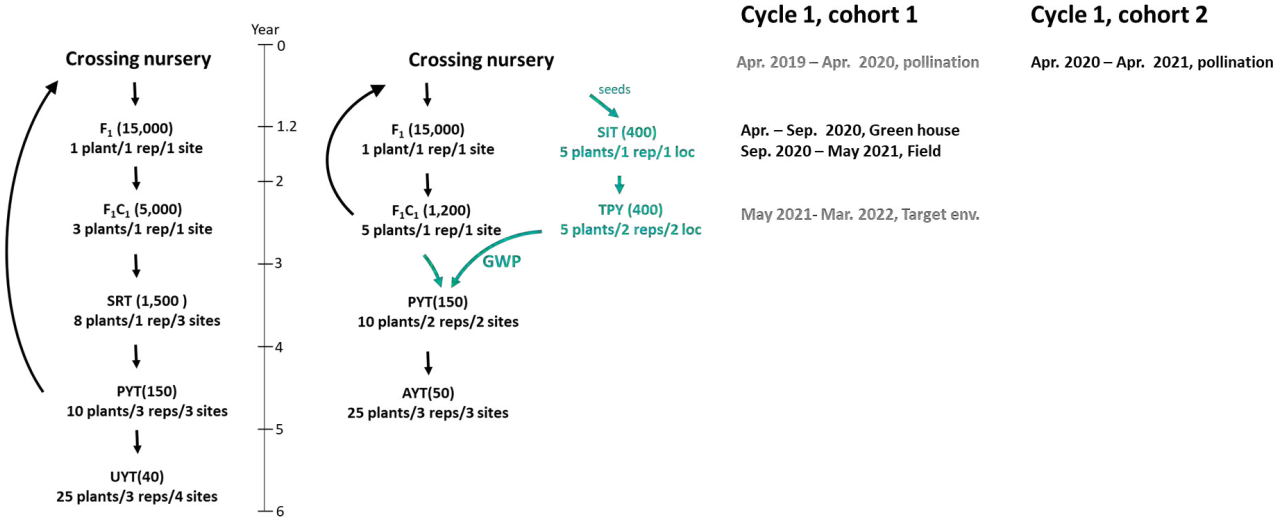


Figure 3. Genomic selection-based breeding scheme implemented at CIAT.The training population was selected from the breeding population based on pedigree.

Quick clonal propagation was performed in the greenhouse to obtain four plantlets from one seedling. The plantlets were transplanted in the field for stem cutting increase (or seed increase). In the spring of 2021, yield trials of the training population will be established at two locations to collect phenotypic data, including plant type, dry matter, yield and disease resistance. The breeding population was planted in the field in 2020, and based on field performance, the population size will be dramatically reduced from 15,000 to about 1,200 clones. These will be planted for seed increase in 2021, and genotypic data will be collected for genomic prediction. Clones selected based on the predicted value will be planted in preliminary yield trials for variety development and in crossing nurseries as progenitors to produce seeds for the next selection cycle. CET, Clonal Evaluation Trial; PYT, Preliminary Yield Trial; AYT, Advanced Yield Trial; SIT, Seed Increase Trial; TPY, Training Population Trial.

Using CassavaBase to manage yield trial data

In 2020, we had a total of 64 trials covering all stages of seven breeding pipelines, three of which are dedicated to Africa. All the agronomy and quality data were managed in CassavaBase. We continue to use the same three to four checks for all breeding pipelines, thus allowing connectivity between trials within and among breeding populations, which facilitates the calculation of genetic gains.

Identifying CMD-resistant clones for SEA

Among cassava clones introduced in Vietnam from CIAT and IITA, we identified nine clones showing CMD resistance and giving 30% more starch yield than KU50 (10.5 vs. 7.9 t/ha at two locations in Vietnam), the predominant variety in SEA. These clones were advanced to regional yield trials at seven locations under medium or high CMD pressure. The best clones will be released as the first generation of varieties for cassava farmers in the region.

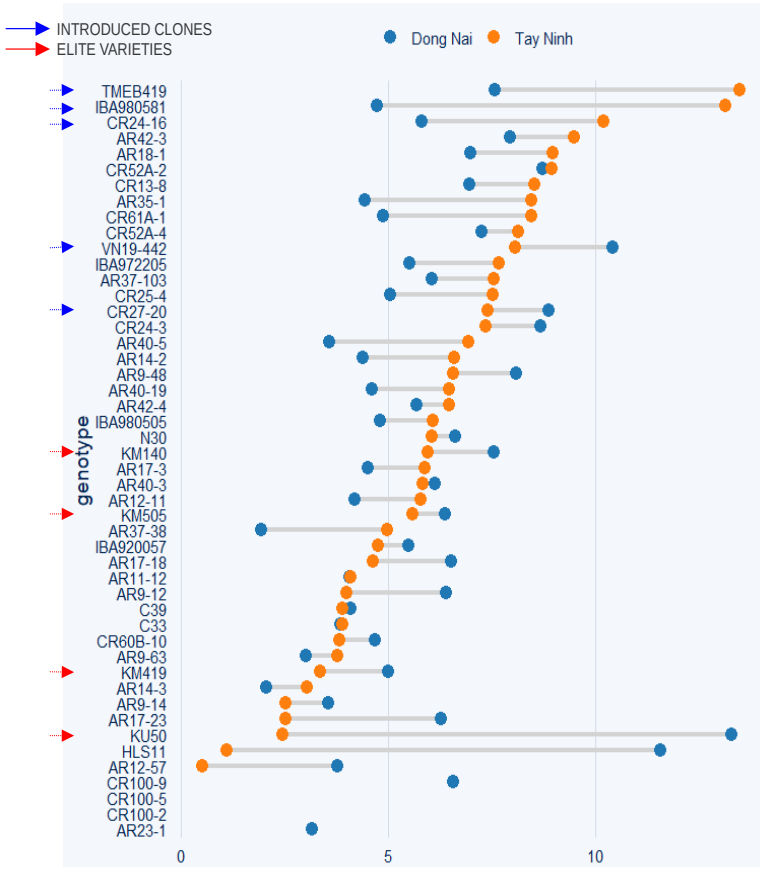


Figure 4. Starch yield of elite varieties and clones introduced from CIAT and IITA. Yield trials were established in 2020 at two locations, Dong Nai with low CMD pressure and Tay Ninh with high CMD pressure. Under high CMD pressure, the elite varieties (orange arrows) showed a dramatic yield reduction. The introduced clones (blue arrows) have the potential to be released as the first generation of CMD-resistant varieties in SEA.

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- Pineda M; Morante N; Salazar S; Cuásquer J; Hyde PT; Setter TL; Ceballos H. 2020. Induction of earlier flowering in cassava through extended photoperiod. Agronomy 10: 1273. Doi:10.3390/agronomy10091273
- Hackathon to develop market segments and product profiles for breeding programs. https://cgspace.cgiar.org/handle/10568/110979

Partners: IITA, Cornell University, Leibniz Institute-DSMZ, KU, BMGF, Ingredion; Perú Yanesha: UNDAC, IBC, INIA, FECONAYA; NRI, UCR, RHUL, AGI, AGROSAVIA, NTA, INIA, TARI, NaCRRI, AGI, RCRDC, HLARC, URG-CIAT.

ADRIANA BOHÓRQUEZ CHAUX AND LUIS AUGUSTO BECERRA

Prebreeding, breeding, and next-generation breeding

Collaborators: María Isabel Gómez, Anestis Gkanogiannis, Carmen Bolaños, Carlos Ordóñez, Adriana Vásquez, Gerardino Pérez, Daniel Encarnación and Janneth Patricia Gutiérrez.

Cassava Genetics Molecular Lab)

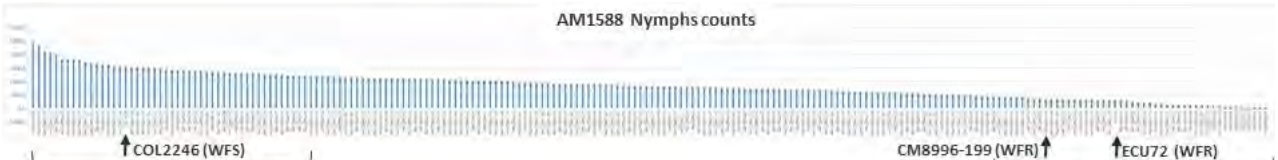
The cassava genetics group supported the cassava research community in maintaining high-quality control of population development. The Ugandan 5CP lines (Mkumba, NASE3 and NASE14 and their susceptible comparators, Sauti and Mkuranga) are the parental materials for crosses directed towards improved whitefly resistance (WFR), associated with virus-disease resistance. Although the genetic/biochemical pedigree of the selected 5CP genotypes is poorly understood, they are likely to be hybrids or non-"true-breeding." Sequence polymorphisms and molecular features were identified to enable their use as quantitative trait markers in breeding programs. The 5CP accessions (510 samples from Africa) were processed using a SNP-chip for the Nanofluidic Dynamic Arrays (SNPY-Array; Fluidigm®, USA) developed by our group, which contain 96 single nucleotide polymorphisms (SNPs) for genotyping in cassava (Becerra Lopez-Lavalle, personal communication). The technique allowed simultaneous collection of both endpoint and real-time data from a unique chip cell with 97% confidence.

Genetic duplicates test: In our variety identification test, all samples that are genetic duplicates belong to the same group. In total, we found 40 genetic duplicate groups (GD-groups) that represent 40 different genotypes. This set of duplicate samples contains 508 samples from Uganda, Tanzania and Malawi.

African Cassava Whitefly Project (ACWP)

Advanced intercrossing to create pre-breeding cassava progenies homozygous for the whitefly resistance (WFR) loci and possessing superior WFR to ECU72 (the original WFR donor): Cassava lines homozygous for WFR are needed to transfer superior resistance into regionally preferred, African-adapted cassava germplasm. In phase I of the project, we generated two advanced crosses (CM8996 and GM8586). From these F1s, we developed twelve "advanced crossover" F2 populations for whitefly resistance: The seeds of these offspring were planted in the soil, multiplied on cuttings, and phenotyped in the greenhouse. Analysis of the progeny harboring all three WFR regions, a subset of these regions and another completely lacking these regions will determine the ability of these markers to identify the superior WFR seen in the F2 progeny of the CM8996 and GM8586 crosses.

We developed a high-throughput, quantitative phenotyping method for whitefly resistance in cassava, which consists of a greenhouse experimental design and an ImageJ plugin, called Nymphstar, for automated counting of nymphs. For 4 years, we phenotyped the progeny (F1) of ECU72 (WFR) and COL2246 (whitefly susceptible or WFS) crosses (CM8996) as well as ECU72 (WFR) and TMS60444 (WFS) crosses (GM8586). We have completed the phenotyping for five of these F2s (AM1588, GM12200, GM12201, GM12202 and GM12199). The true-F2 AM1588 (CM8996-199 x CM8996-199) was selected for its high resistance to the whitefly Aleurotrachelus socialis (Fig. 5). DNA was extracted from 200 offspring of true-F2 AM1588, and a RAD-sequencing approach was used. The 15 top resistant and 15 top susceptible offspring of this F2 were selected for assessment. Whitefly infestations were performed, and paired samples split for RNA and metabolite analyses. RNAs were sent to UCR for RNA-seq (eQTLs), while tissue was sent to RHUL for chemotyping. The SNPs analysis, mapping and quantitative trait locus (QTL) analysis are in process. We will then identify molecular markers within the cassava WFR genes. The advanced intercrossed cassava will next be used to: (i) identify, verify and refine QTLs for WFR, and (ii) identify potential epistasis for WFR QTL.



Top 16 S	Clone	Mean # Nymphs	# replicas
1	AM1588-212	376.9	5
2	AM1588-190	323.8	5
3	AM1588-29	309.5	9
4	AM1588-90	292.6	5
5	AM1588-18	279.1	6
6	AM1588-228	274.5	4
7	AM1588-169	268.0	6
8	AM1588-168	267.7	7
9	AM1588-205	247.7	8
10	AM1588-207	243.8	5
11	AM1588-140	236.6	8
12	AM1588-89	234.7	5
13	AM1588-45	226.6	5
14	AM1588-13	226.1	6
15	AM1588-214	210.3	5
16	AM1588-134	209.3	7

Top 16 R	Clone	Mean # Nymphs	# replicas
1	AM1588-83	23.9	6
2	AM1588-24	27.2	5
3	AM1588-44	27.4	3
4	AM1588-15	32.1	6
5	AM1588-175	35.0	6
6	AM1588-127	42.0	6
7	AM1588-69	42.6	6
8	AM1588-142	47.7	5
9	AM1588-121	49.2	5
10	AM1588-68	50.4	4
11	AM1588-36	51.7	6
12	AM1588-178	53.6	4
13	AM1588-21	54.0	6
14	AM1588-129	54.2	7
15	AM1588-136	58.3	6
16	AM1588-186	59.2	3

Figure 5. Phenotyping of true-F2 AM1588 (CM8996-199 xCM8996-199) and selection of the top resistant and top susceptible offspring.

Cassava Genetics Tissue Culture Lab

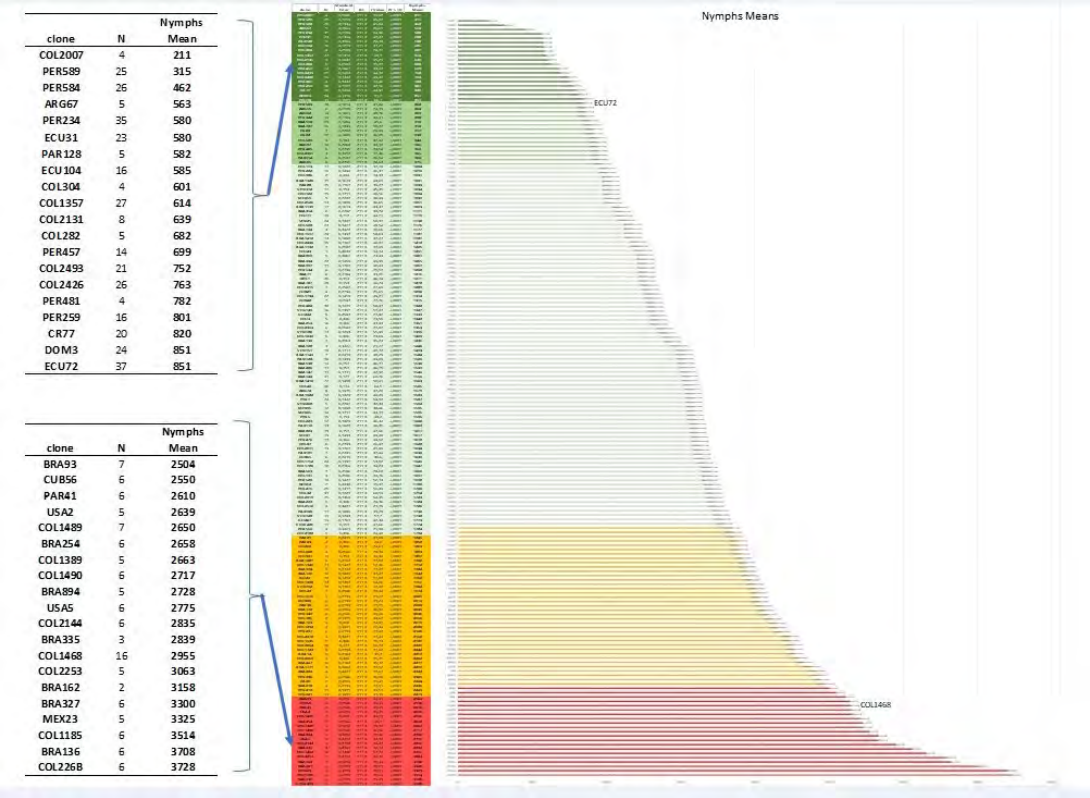
We have successfully conserved two families (GM13489 and GM13494) from cassava breeding, with 300 individuals showing CMD-CBSD resistance, in support of CIAT-Asia breeding programs. We also established in vitro 104 individuals from advanced selection for beta-carotene (2019) content in support of the cassava breeding program.

Analysis for whitefly resistance in a unique cassava germplasm array comprising seven sub-populations identified in the Latin American *Manihot esculenta* tribe

Of the 6,240 cassava accessions held at CIAT, nearly 20% (1,200) were selected for genetic profiling, primarily at random, based on the georeference information and frequency of cassava landraces used to generate CIAT elite breeding lines or cultigenes. In all, 18,286 SNPs were obtained from 292 LAC cassava landraces. Seven sub-populations were resolved, and 219 unique individuals were identified and will be phenotyped for whitefly resistance to undertake a genome-wide association study. This approach will allow us to unravel the genomic regions that may be responsible for whitefly resistance in cassava.

Of the 219 unique genotypes, 172 were evaluated using the phenotyping method described, including Nymphstar image analysis. The COL1468 genotype, commonly called CMC40, was used as a susceptible check to later perform genome wide association studies (GWAS) with data on the SNPs (this analysis will be presented in 2021).

The independent-samples t-test (LSD) showed five significantly different groups in the response to *A. socialis* (Figure 6). Highlighted in dark green, 20 unique genotypes (11,6%) showed the highest levels of resistance to the whitefly *A. socialis*, according to the parameter measured (nymph count), including the ECU72 genotype, known for its high levels of resistance. Highlighted in red, 20 of these genotypes (12%) showed the highest levels of susceptibility, including the susceptible check COL1468. Fourteen genotypes showed intermediate levels of resistance (green), and 37 (yellow) showed intermediate levels of susceptibility. In light green, there are many genotypes (83), amounting to nearly half (48%), that would be classified as intermediate in their response to whitefly.



clone	N	Nymphs Mean
COL2007	4	211
PER589	25	315
PER584	26	462
ARG67	5	563
PER234	35	580
ECU31	23	580
PAR128	5	582
ECU104	16	585
COL304	4	601
COL1357	27	614
COL2131	8	639
COL282	5	682
PER457	14	699
COL2493	21	752
COL2426	26	763
PER481	4	782
PER259	16	801
CR77	20	820
DOM3	24	851
ECU72	37	851

clone	N	Nymphs Mean
BRA93	7	2504
CUB56	6	2550
PAR41	6	2610
USA2	5	2639
COL1489	7	2650
BRA254	6	2658
COL1389	5	2663
COL1490	6	2717
BRA894	5	2728
USA5	6	2775
COL2144	6	2835
BRA335	3	2839
COL1468	16	2955
COL2253	5	3063
BRA162	2	3158
BRA327	6	3300
MEX23	5	3325
COL1185	6	3514
BRA136	6	3708
COL226B	6	3728

Figure 6. Phenotyping for whitefly resistance of 172 unique genotypes. LSD (least significant difference) α = 5%.

We collaborated with Agrosavia (Colombia), so far sending plants of 54 varieties (for "Zonas Llanos Orientales") through the cassava breeding program.

COVID-19 contingency

During the COVID-19 contingency (March-December 2020), logistical support was provided to organize the staff, coordination with the leader and supervisors, monthly schedules and permits to enter the campus. All these activities were necessary for maintaining the essential activities of the Cassava Program.

We sent in vitro seedlings of 11 genotypes with CMD resistance (AR, CR, C families and others) to Dr. Stephan Winter (Head, Plant Virus Department) with the Leibniz Institute-DSMZ in Germany for collaborative projects with the cassava breeding program.

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Irigoyen, M.L., Garceau, D.C., Bohorquez-Chaux, A. et al. Genome-wide analyses of cassava *Pathogenesis-related (PR)* gene families reveal core transcriptome responses to whitefly infestation, salicylic acid and jasmonic acid. *BMC Genomics* 21, 93 (2020). https://doi.org/10.1186/s12864-019-6443-1

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Partners: Agrosavia, RHUL, UCR, NRI, University of Greenwich, Leibniz Institute-DSMZ-German Collection of Microorganisms and Cell Cultures.

LUIS AUGUSTO BECERRA

Cassava genomics, cassava metabolomics, carotene expression profile, waxy cassava variation, CMD sequencing, and cassava OR protein characterization

Collaborators: Diana Katherine Castillo, Tatiana Melissa Ovalle, Diana Victoria Marín, Janneth Patricia Gutiérrez, Daniel Álvarez, Angélica Jaramillo, Jairo Rodríguez.

Carotene expression profiling

This work aims to characterize the expression of nine candidate genes associated with high carotene content in cassava. The expression patterns of six cassava carotene-associated genotypes were evaluated monthly. In 2020, we recorded expression data from all samples at different growing stages. One of the candidate genes, *BETA6* (Manes.09G032800), showed a 200-fold expression change in the six genotypes evaluated relative to a calibrator (PAR39). This gene could be a good candidate for identifying in a cassava population genotypes with high carotene content (Figure 1). Six primer sets belonging to genes regulated by *BETA6* were designed and standardized to evaluate its contribution to *BETA6* expression (Table 1 and Figure 2).

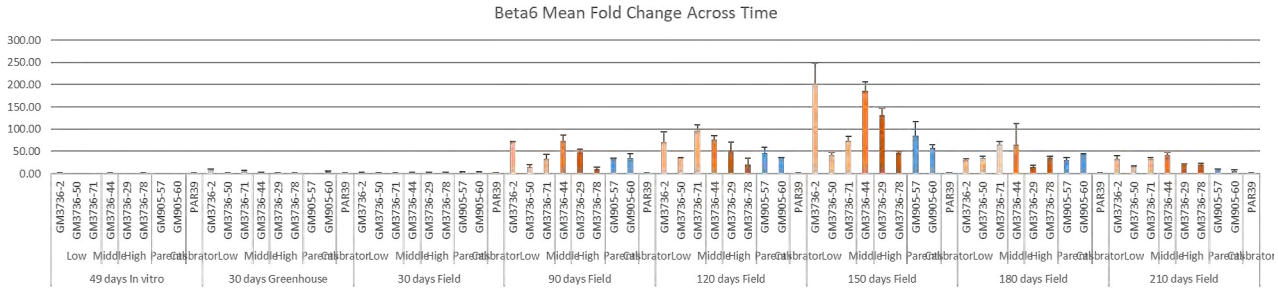

Figure 7. Expression profile of the BETA6 candidate gene in six cassava carotene families over time. The expression pattern was evaluated using housekeeping genes (Ubiquitin and Histidine) as expression normalizers and relative to PAR39. Light orange indicates cassava genotypes with low carotenoid content, middle orange those with medium carotenoid content and dark orange those with high carotenoid content.

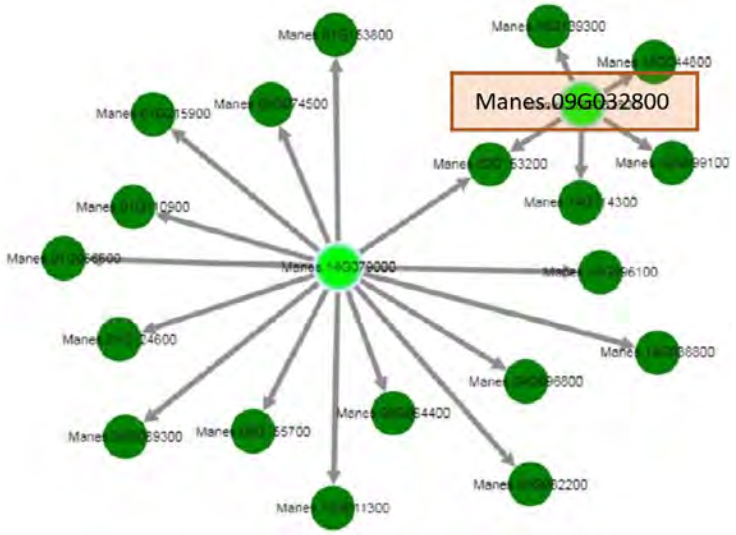

Figure 8. Expression network for BETA6. In the orange box, Beta6 appears, with circles representing the genes under its regulation..

Table 1. List of genes regulated by *BETA6* and the designed primer sequences.

Genes regulated by Beta 6				
Manes.18G139300	PTHR13902:SF49 - SERINE/THREONINE - PROTEIN KINASE WNK4-RELATED	Beta6_18G139300F	Forward	AGCAGTGATCCAACAAGGACGGACA
		Beta6_18G139300R	Reverse	TCCATTGCAACATCCGGTGGCG
			Forward	TTGGATTGCCGGAAACGGACGC
			Reverse	CTGCAAGTTCTCGGGTGAACGAAGC
Manes.18G044800	PTHR33181:SF6 // NO NAMED	Beta6_18G044800F	Forward	CGTGGGAGAACCTACTTTGCAGCG
		Beta6_18G044800R	Reverse	CAGCTTCAGTAGCCCTCGTTTGCG
			Forward	TGGGATTCGCAAACGAGGGCTACT
			Reverse	AGTGCACCTAGCCCAGCTAAAGCA
Manes.12G099100	PTHR12313:SF12 - RING ZINC FINGER PROTEIN-RELATED	Beta6_12G099100F	Forward	TCAATACCTCCCAGCGCACCGT
		Beta6_12G099100R	Reverse	TGCAGCCCATTTCTCAGCAAGGC
			Forward	GGTGGACAGAGAGTTGGGCAGGAA
			Reverse	ACGGTGCGCTGGGAGGTATTGA
Manes.02G153200	PTHR12537:SF64 - PUMILIO HOMOLOG 11-RELATED	Beta6_02G153200F	Forward	TCTCGCAGCGATCACCAGAGCA
		Beta6_02G153200R	Reverse	TGCCCATGTTCTCTGCCACCCT
			Forward	AGCTTCTTGGTTCTCCCGCGACT
			Reverse	ACCTGGCGTGTGATACTTGGCCT

Cassava waxy variation

Based on evidence collected in 2019, we designed an amplification protocol (Table 2) to obtain by conventional polymerase chain reaction (PCR) the full length of the GBSSI gene (3500bp) (Figure 9). The fragment obtained was sequenced as a trial, using the MinION sequencing platform. The aim is to capture internal variation of the gene by sequencing the full fragment without overlapping segments. The sequencing of candidate genotypes will be conducted in 2021, using the MinION sequencing platform in the Cassava Genetics Lab.

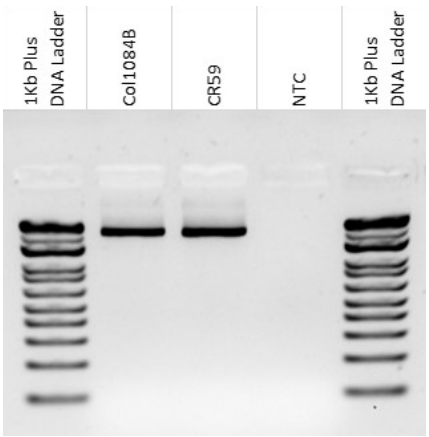

Figure 9. Quality gel of the full amplification of *GBSSI* gene by conventional PCR.

Table 2. Amplification conditions standardized for *GBSSI* full length fragment.

Taq Platinum amplification			**50 µL**	
1X rxn	**Initial Conc.**	**Reagents**	**3 X**	**Final Conc.**
39.00 µL		Injection or PCR grade water	**117.00** µL	
5.00 µL	10 X	Platinum Taq® (Invitrogene) buffer	**15.00** µL	**1** X
1.50 µL	10 mM	dNTP's	**4.50** µL	**0.3** mM
2.00 µL	50 nM	Mg^{2+}	**6.00** µL	**2** nM
1.00 µL	10 pmol/µL	Primer - Fwd	**3.00** µL	**0.2** pmol/µL
1.00 µL	10 pmol/µL	Primer - Rev	**3.00** µL	**0.2** pmol/µL
0.50 µL	5 U/µL	Platinum Taq®	**1.50** µL	**2.5** Unit
50.00 µL			**50.00 µL**	
	Change stock concentrations as required			Change final concentrations as required
PCR program		**STAGE 01** STEP 01: 95° C x 2 min **STAGE 02** STEP 01: 94° C x 30 sec STEP 02: 52° C x 1 min STEP 03: 72° C x 4.5 min **STAGE 03** STEP 01: 72° C x 5 min Hold at 4° C	35 cycles	
		1.5% [w/v] agarose gel at maximun voltage		

CMD sequencing

CMD is caused by a virus belonging to the Geminiviridae virus family and can be transmitted in two ways: first, through stakes from infected cassava mother plants and second, through the whitefly (*Bemisia tabaci*) insect vector. The plant material for this study consisted of 42 crosses between a Nigerian variety TME-3 that represents a new source of CMD resistance and an improved disease-tolerant line (TMS 30555), which was classified as susceptible for this cross given the extreme nature of the resistance under study. The amplification conditions for these samples were improved and standardized (Table 3 and Figure 10), and then cloned and sequenced (Figure 11). The aim was to obtain information on each band at the sequence level. The sequences obtained were assembled, and sequence analysis will be conducted in 2021.

Table 3. Amplification conditions standardized for the CMD gene.

1X rxn	Initial Conc.	Reagents	10 X	Final Conc.
15.30 µL		Injection or PCR grade water	**153.00** µL	
2.00 µL	10 X	Platinum Taq® (Invitrogene) buffer	**20.00** µL	**1** X
0.60 µL	10 mM	dNTP's	**6.00** µL	**0.3** mM
1.20 µL	50 nM	Mg^{2+}	**12.00** µL	**3** nM
0.40 µL	10 pmol/µL	Primer - Fwd	**4.00** µL	**0.2** pmol/µL
0.40 µL	10 pmol/µL	Primer - Rev	**4.00** µL	**0.2** pmol/µL
0.10 µL	5 U/µL	Platinum Taq®	**1.00** µL	**0.5** Unit
20.00 µL			**200.00 µL**	
	Change stock concentrations as required			Change final concentrations as required

RAPD PCR program	**STAGE 01**	STEP 01: 95° C x 2 min	
	STAGE 02	STEP 01: 94° C x 30 sec	
		STEP 02: 53 - 54 - 55° C x 1 min	35 cycles
		STEP 03: 72° C x 1 min	
	STAGE 03	STEP 01: 72° C x 5 min	
		Hold at 4° C	

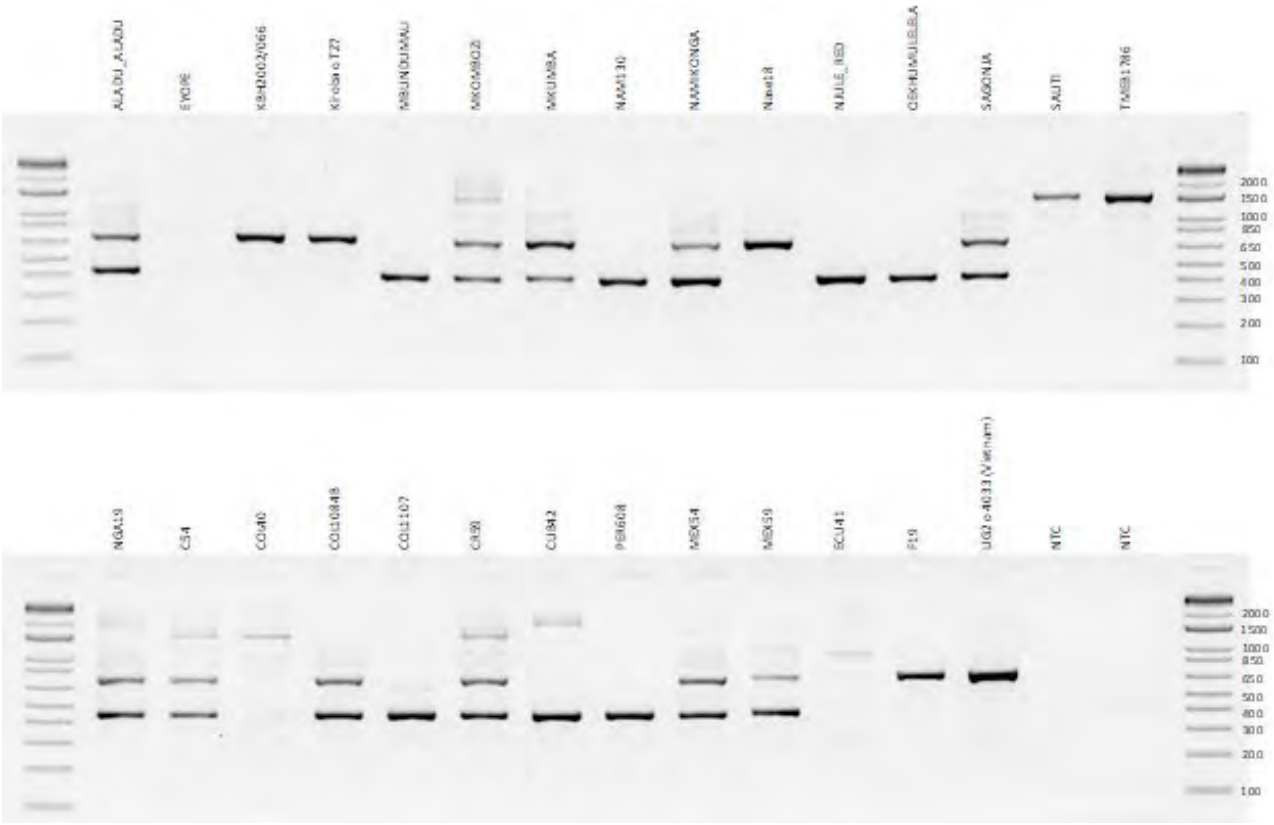

Figure 10. Quality gel of the standardized full amplification of the CMD gene.

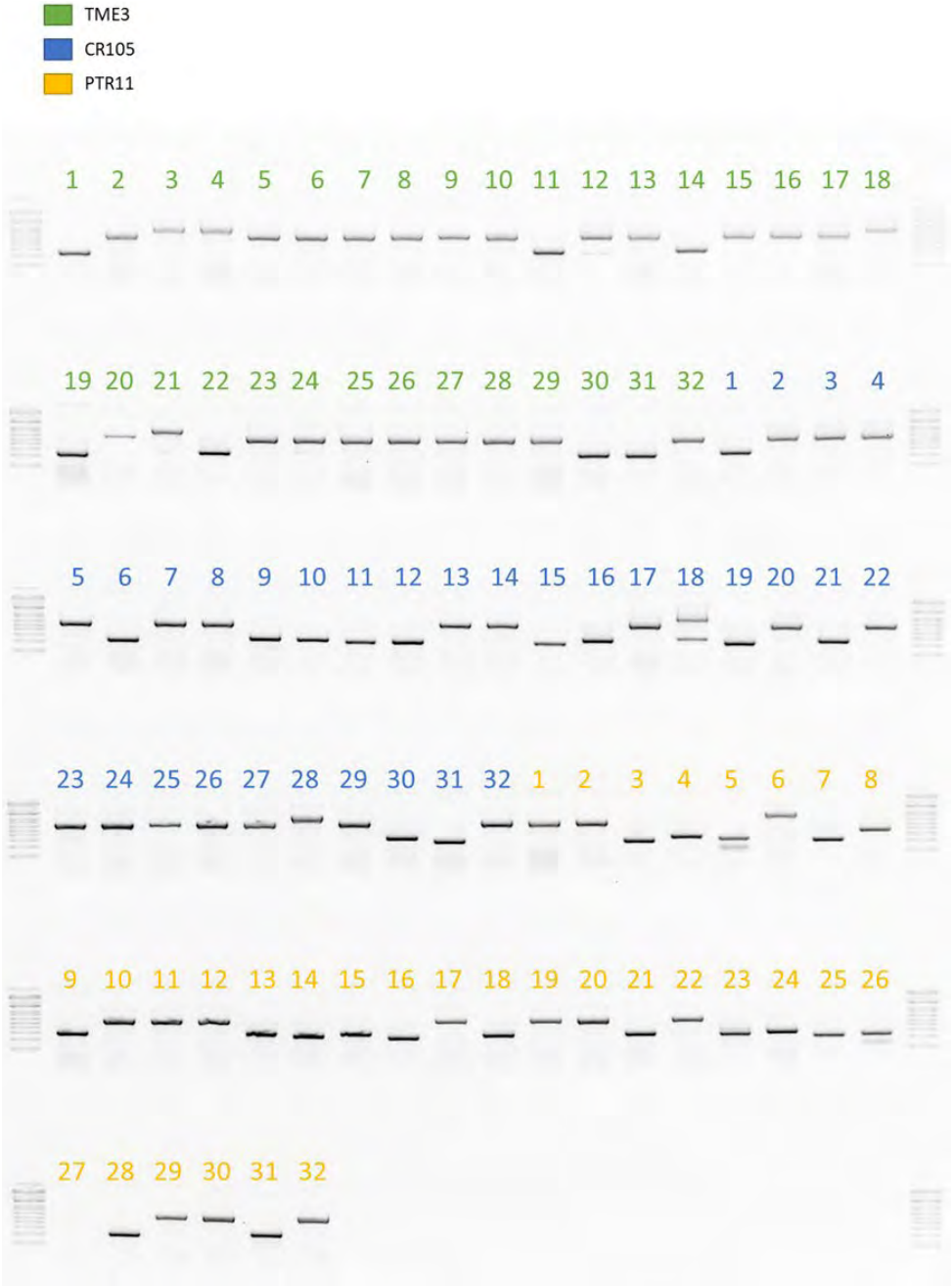

Figure 11. Quality gel of the PCR colony of DH5a carrying the CMD fragment to be sequenced.

Cassava ORANGE protein characterization

In recent years, ORANGE protein (OR) has received special emphasis. This is a chaperone not belonging to the carotenoids biosynthesis pathway, which promotes the accumulation of carotenoids and their stability in several plants. In the absence of information about this protein, we aimed to identify, characterize and investigate its role in the biosynthesis and stabilization of carotenoids in cassava and its relationship with PSY, the rate-limiting protein of the carotenoids biosynthesis pathway. The gene and protein characterization of OR, expression levels, protein amounts, and carotenoids levels were evaluated in roots of one white (60444) and two yellow cassava cultivars (GM5309-57 and GM3736-37). Four OR variants were found in yellow cassava roots. Variants 1 (MeOR_X1) and 2 (MeOR_X2) expression levels remained

unchanged, but significantly higher OR protein amounts were observed in the yellow varieties (Figure 12). Cassava PSY1 gene expression was significantly higher in the yellow cultivars, although PSY protein amount did not vary.

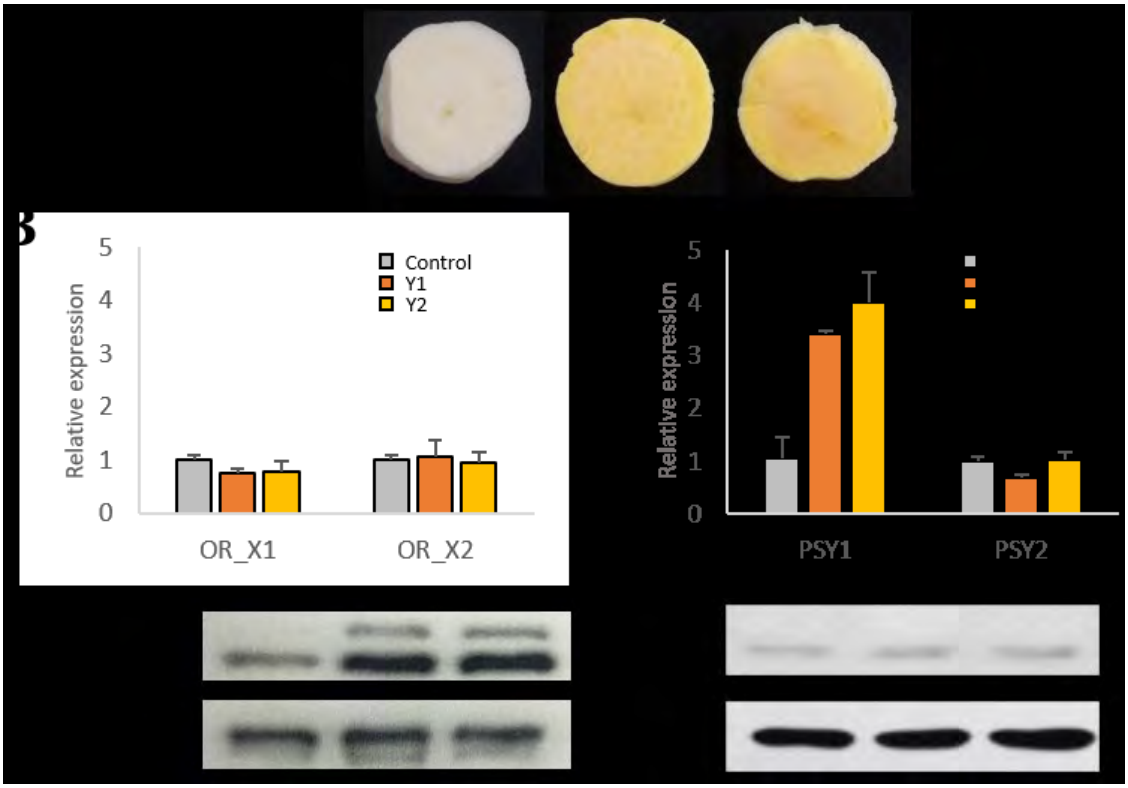

Figure 12. Expression and protein levels of OR and PSY in cassava roots. A) cross sections of white-fleshed control, and yellow-fleshed Y1 and Y2 cassava root genotypes; B) relative expression levels of MeOR_X1, MeOR_X2 and PSY in control, Y1, and Y2 genotypes by real-time qRT-PCR; and C) MeOR protein levels in the roots of control, Y1, and Y2 genotypes. Actin protein was used as a loading control. Values are the average ± SD of three biological replicates. *Significant difference when compared to the control ($p<0.05$, $n = 3$). MeOR_X1, cassava variant 1; MeOR_X2, cassava variant 2; MePSY1, cassava phytoene synthase 1; MePSY2, cassava phytoene synthase 2.

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PAUL CHAVARRIAGA

Next-generation breeding, gene editing

Collaborators: Franciso Sánchez, Juan Pablo Arciniegas, Didier Marín, Orlando Vacca, Anibal Peñaloza, Jhon Larry Moreno, Thierry Tran, Anestis Gkanogiannis and Alejandro Brand.

Doubled-haploid induction

In maize, haploids are routinely produced using crosses with Stock 6 to a rate of 2-3% maternal haploid seed when it is self-pollinated or outcrossed as a male (Coe et al., 1959). This is 10 to 20 times higher than spontaneous haploid induction rate (HIR) in maize (Zhao et al., 2013), which occurs through a process called gynogenesis, where the male gametes induce haploid embryo formation only from the female chromosomes (Jackson, 2017). Breeding performed with Stock 6-derived inducers produces lines with elevated HIRs up to 20.42% (Cengiz et al., 2016). The genetic mechanism behind Stock 6 haploid induction was characterized in three groups, and the gene implicated (GRMZM2G471240) was named NOT LIKE DAD (NLD) (Gilles et al., 2017), MATRILINEAL (MTL) (Kelliher et al., 2017) or ZmPHOSPHOLIPASE A1 (ZmPLA1) (Liu et al., 2017). This gene was found mutated in Stock 6, and the inducer line HKR showed a 4-bp insertion in the exon 4, which causes a frameshift replacing the last 49 amino acids (aa) of the wild-type protein by an unrelated amino acid sequence of 20 aa, followed by a premature STOP codon (Kelliher et al., 2017). CRISPR/Cas9-mediated genome editing was performed to knock out ZmPLA1 (Liu et al., 2017). Three lines with 1-bp insertion, 11-bp deletion and 1-bp deletion in the target region, which are putative knockout alleles for ZmPLA1, were chosen for pollination assays. In self-pollinated knockout lines, the HIR ranged from 3.7% to 6.67%. In the offspring of breeding male knockout lines with wild-type lines, the chromosomes proceeded only from the maternal genomes. The behavior of ZmPLA1 knockout lines was like the gene mutation in Stock 6.

Target selection and genetic transformation

The GRMZM2G471240 gene was aligned with *Manihot esculenta* v6.1 in Phytozome (https://phytozome.jgi.doe.gov/) to find possible candidate genes. This analysis revealed six candidate ortholog genes (Manes.05G171400, Manes.12G093400, Manes.18G033200, Manes.13G126900, Manes.12G102200 and Manes.18G033400). Multiple alignment with phylogenetic analysis with maximum likelihood (Figure 13) determined that Manes.12G093400 and Manes.12G102200 are the most closely related to GRMZM2G471240 and the most suitable candidate genes to knock out through CRISPR/Cas9 for doubled-haploid induction.

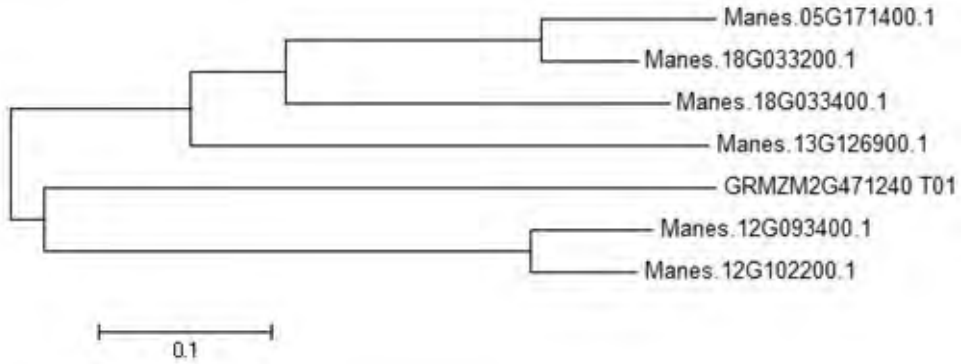


Figure 13. Maximum likelihood phylogeny tree of cassava candidate genes with GRMZM2G471240.

Once the candidate genes were selected, single-guided RNAs (sgRNAs) were designed with CRISPR P 2.0 (http://crispr.hzau.edu.cn/CRISPR2/). The selection was made based on affinity of sgRNAs with the gene, the position in the gene and the off targets present in the whole cassava genome (Figure 14). Then, sgRNAs were cloned independently on pHSE401 vector and introduced on *Agrobacterium tumefaciens* LBA4404.

A

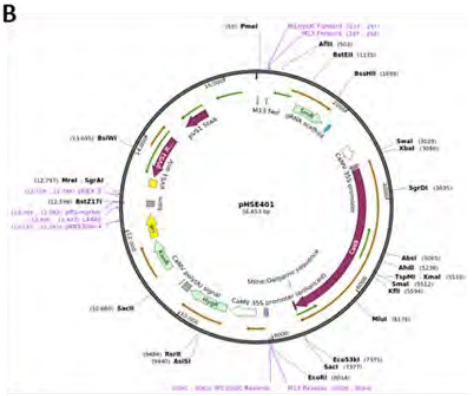
>Manes.12G093400 | Chromosome12:14822139..14823812 forward

ATGGCTACTGGTAATAACATTTCGAAACAATGGAAGAAGATAACAGTGTTGAGCATTGATGGAGGTGGGATCAG
AGGCATCATTCCTGCTACCATTCTTGCTTCTCTTGAATCCAAGCTTCAGGTACTTGAAAAGAAATTTTTCTTTT
TCTTTTTTTTTTTTTTTAAATTTCTTCATCTTAATTTGTTTTGCCTTTTTTCCATGGTGTTAATCATTAGAAT
TTGGATGGACCAGATGCGAGAATTGCAGATTATTTGATGTAATTGCAGGGACAAGTACTGGTGGGCTGGTAAC
CACCATGCTCACAGCTCCAAACAAGGACAAAAGACCTATGTTCCAAGCAAAAGACATCAAGAACTTCTATTTAG
AACACTGTCCCAAGATTTCCCACAGAAAAGGTGAAATCTTTCAAGTTTTACTTAATTTCTTTCTTGTTTTTTT
ATTAAATGGAATTATAATAAATGGGTCTAATTTCATTTCTTCTATGGATTGAGCAGTCGAAATAACTTCATTAG
CTCGATAACAAATTTCTTTGGTGCGGTTATGGGACCAAAGTACGATGGAAAATACCTGAAAGGACTGACAAATG
AGCTGCTAGGGGACTTGACCATAAAAGAGACTTTGGCAGATGTGATTATACCTGCTTTTGATATCAAACTTCTT
CAGCCAGTCATCTTCTCAACTGATGATGTATGTATATATACATGCTAAAGCTGAGCTAGTAAAAATTTTTCCTT
GAATATGAGCTGAGATTTTTACAATTTTCTTGTCTTAACAGGCGAAAGTTGATGCTCTGAGAAATGCTAGACTA
GCTGATATCTGCATCAGCACCTCTGCAGCTCCAACTTTCTTTCCAGCACATTCCTTTACCATCAAGGATGATCA
AAGGACTCGCACTTTTGAACTCATTGATGGTGGGGTTGCAGCCAATAATCCTGTAAGCTACTCTTCTTCTGCAC
ATACACTCGTAGCAATAAAAGGCTTTTCATTGATAATTTGTTGCGCTTGTGACTGAAGACATTACTAGCAATAA

>Manes.12G102200 | Chromosome12:21375405..21377776 forward

ATGGCTGCTGATCATAATAATAATTTGAAGCCAGGAAAGAAGATAACAGTGCTGAGCATTGATGGAGGTGGGAT
TAGAGGTATCATTCCTGGTATCATTCTTGCTTCTCTTGAATCTAAACTTCAGGTAACAGAAATTTTCTTTTTCT
TTTCTTTTATTTTAATTTCTCTTCATCTTAATTAATTTGTTGTGCTGTTCATGGTTGTAATCACAAGGAATTTGG
ATGGACCAGATGCAAGAATTGCATATTATTTTGATATACTTGCAGGGACAAGCACTGGAGGGTTGTTAACCACC
ATGCTTACAGCTCCTAACAAAGACAAAAGACCTATGTTCCAAGCAAAAGACATGGAAAATTTCTATTTAGAACA
CTGTCCCAAGATTTCCCACAGAAAAGGTGAAATCATCTCTGATTTTTTCAATTATTTTTGTTTTACATGGAAT
GATAATAAATGGGTTTTCAATTCTTCCATGGATTAAACAGTCGAAATAACTTCATTGACTCGATCACAAACTTT
TTTGGTGCTGTTTTGGGGCCAAAATATAATGGAAAATATCTGCATGAACTGACAAATCAGTTGTTAGGAGACTT
GACCATAAAGGAAACCTTGGCGAATGTGATTATACCTGCGTTTGACATAAAGCTTCTTCAGCCAGTCATCTTCT
CAACAGATGATGTATGTATATTACATATAGATTACCAAAATTAAGTTAAATTTTGCGTCCATGCATGCGCAAAA
ATATAAGATTTTTAAAAAGTTTATTGTCTTAACAGGCGAAAGTTGATGCTTTGAAAAATGCTAGGCTAGCTGAT
ATCTGCATCAGCACCTCTGCAGCTCCAACTTTCTTTCCACCACATTCCTTTACCACCAAGGATGATCAAAGGAC

B



C

Construct	5'-3' + PAM	Gene	Location
PHSE401-pL1a09	TTGCAGGGACAAGTACTGGTGGG	Manes.12G093400	Exon 2
PHSE401-pL1a10	GTTAGGAGCTGTAAGCATGGTGG	Manes.12G102200	Exon 2

Figure 14. Coding sequences of candidate genes for doubled-haploid induction. Single-guided RNAs (sgRNAs) selected for Manes.12G093400 and Manes.12G102200. Introns not highlighted, exons in blue, Protospacer Adjacent Motif (PAM)in red, sgRNA sequence in green, and primers for molecular evaluation in yellow (800-950 bp fragment) and purple (100-200 bp fragment).

The CRISPR/Cas9 machinery was introduced into TMS60444 friable embryogenic callus (FEC) through *Agrobacterium tumefaciens* genetic transformation, according to Taylor et al. (2012) with modifications

Molecular analysis of regenerated lines

Two genetic transformation essays were named T310718 and T100519. The molecular analysis of regenerated lines detected at least 40 southern-positive transgenic lines with 1 to 5 inserts, few of which were duplicates. Nested PCR was performed on southern-blot-confirmed transgenic lines to detect signs of gene editing. PCR products were run on a Metaphor agarose 3% electrophoresis gel. Bands different than the control (TMS60444) indicated gene editing events. Further confirmation through sequencing INDELS is underway (Figure 15).

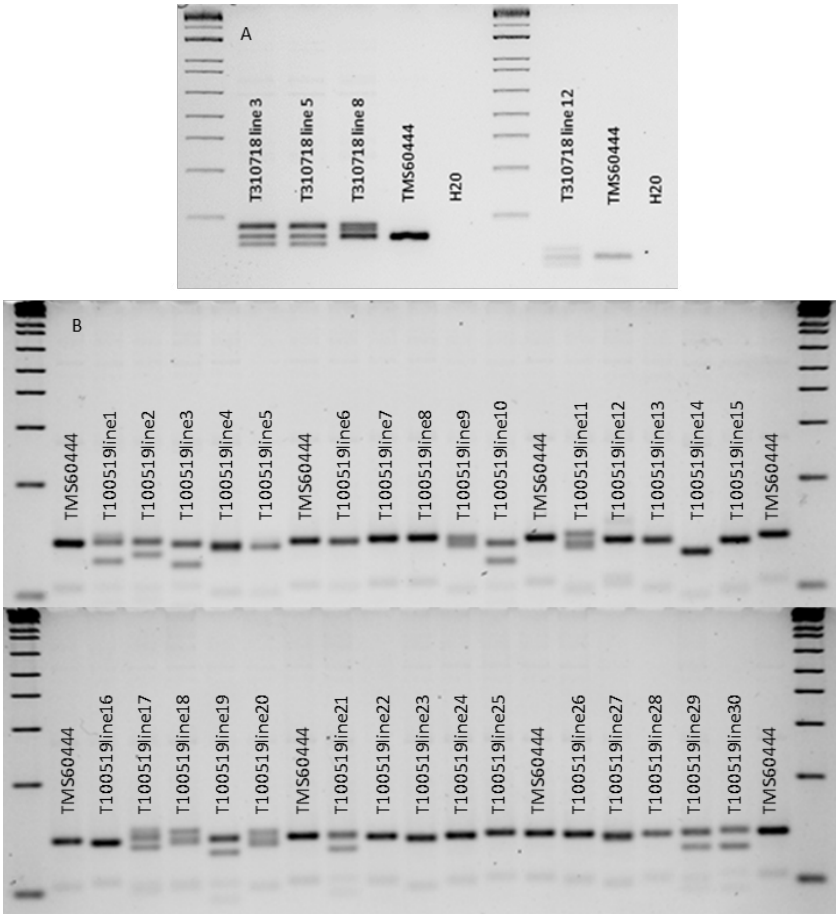


Figure 15. Metaphor agarose 3% electrophoresis of transgenic putative doubled-haploid inducer lines (A) Metaphor agarose 3% electrophoresis of T310718 lines. (B) Metaphoragarose 3% electrophoresis of T100519.

Establishment in the greenhouse and field

T310718 and T100519 lines were transferred from in vitro to the greenhouse and later to the field. As expected for a maize haploid inducer irregular phenotype, regenerated cassava also showed dwarfism, thin stems, and partially wrinkled leaves, with narrow lobes (Figure 16A and 16C). We cannot discard at this point somaclonal variation. Plants showed reduced vigor, as evidenced by delayed transfer to the field, which took at least 90 days, compared to the usual 60 days. Since the main goal of haploid inducers is to produce male flowers for pollinating cultivars, plants were exposed to red light in the greenhouse (Figure 16B) to stimulate early flowering once established in the field. When transferred to the field, plants conserved the altered phenotype. Some lines flowered and produced both female and male flowers. Male flowers from T310718 lines (Figure 16D) were used to pollinate TMS60444 flowers (Figure 16E). Currently, T310718 and T100519 plants established in the field are flowering and ready for use in crossing assays with elite cultivars.

Figure 16. Establishment of T310718 lines in the greenhouse and field. (A) & (B) plants in greenhouse, (C) plant in field, (D) T310718 male flower and (E) TMS60444 female flower.

Waxy starch cassava

The waxy phenotype is due to a mutation or down-regulation of the granule-bound starch synthase I *GBSSI*) gene, a protein responsible for amylose synthesis (Zhao et al., 2011). The cassava clone AM 206-5, with 0% amylose, has been characterized (Ceballos et al., 2007), and it was found that the allelic variant in *GBSSI* consists of a cytosine deletion that generates a frameshift, producing a premature stop codon in the amino acid position 1360 of the sixth exon (Aiemnaka et al., 2012). Evidence that the waxy trait is caused by a point mutation in the *GBSSI* gene suggests the use of gene editing to generate similar knockout alleles in other cassava cultivars. Our objective in this research was to develop new waxy alleles in a model cassava genotype, using CRISPR/Cas9.

CRISPR/Cas9 has become the tool of choice for genome editing, recognized for being highly specific, efficient and reliable for multiple gene edition in a variety of cells and organisms (Ran et al., 2013; Khatodia et al., 2016). CRISPR/Cas9 technology uses the bacterial endonuclease Cas9 to generate double strand breaks (DSB) directly in the DNA sequence of interest, assisted by a molecule of single guide RNA (sgRNA) that pairs with the target sequence. The cleaved DNA sequence is repaired and generates insertions/deletions, or indels (reviewed by Hsu et al., 2014). With the aim of applying this technology to mutate the *GBSSI* gene in cassava, we report the characterization of cassava waxy transgenic-edited lines.

Construction of binary vectors and plant genetic transformation

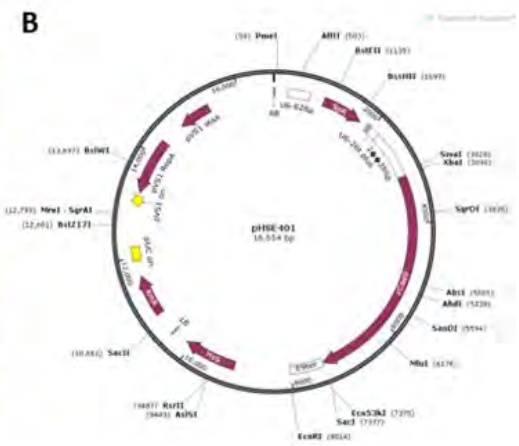
Three sgRNAs were selected using the CRISPR-P online tool (Lei et al., 2014), based on the consensus sequence between the KU50 *GBSSI* coding sequence (GenBank accession JF708948.1) previously reported by Ceballos et al. (2007) and the available sequence of the model genotype 60444. Figure 17A depicts the GBSSI CDS of KU50, indicating exon composition and the location of each gRNA within the sequence (green highlights). Selected gRNAs were integrated independently into the pHSE401 binary vector (Addgene, Figure 17B), according to the protocol of Xing et al. (2014), and electroporated into the *Agrobacterium tumefaciens* strain LBA4404. Details of gRNA sequences are shown in Figure 17C.

A

>KU50_GBSSI_CDS

ATGGCAACTGTAATAGCTGCACATTTAGTTTCCAGGAGCTCACACTTGAGCATCCATGCATTAGAGACTAAGGCTA
ATAATTTGTCTCACACTGGACCCTGGACCCAAACTATCACTCCCAATGGTTTAAGGTCCCTCAACACTATGGATAAA
CTCCAAATGAAGACACAATCAAAAGCTGTGAAAAAGGTCTCTGCCACCGGCAATGGTAGGCCTGCTGCCAAAATT
ATTTGTGGTCATGGAATGAATTTAATCTTTGTTGGAGCTGAAGTTGGTCCCTGGAGCAAAACTGGTGGACTTGGTG
ATGTTCTTGGAGGACTCCCCCCTGCCATGGCCGCAAGAGGGCACCGCGTCATGACAGTGTCTCCCCGCTATGACCA
GTACAAGGATGCTTGGCATACCTCTGTATCCGCTCAGATTAAAATTGGAGATAGAATTGAAACTGTCCGCTTCTTC
CACTCCTACAAAAGAGGAGTTGATCGGGTCTTCGTGGATCATCCAATGTTCCTTGAGAAGGTATGGGGCAAAACT
GGATCTAAAATATATGGCCCAAGAGCAGGTTTGGATTACCAGGACAACCAACTGCGATTTAGCTTGTTATGCCTTG
CTGCTCTGGAGGCACCGAGAGTTCTGAACTTGAACAGCAGCAAAAATTTCTCAGGACCCTACGGAGAAGAAGTTG
CCTTCATTGCCAACGACTGGCACACTGCTCTGCTTCCATGTTATCTAAAAGCCATTTACCAATTATGGGATTTAT
GAAGCAAGGTTGCCTTTTGCATCCACAACATTGCATATCAGGGAAGATTTGCCTTCTCAGACTTCCCACGACT
TAATCTGCCAGATAAATTCAAAAGCTCTTTTGACTTTATCGATGGGTATGAGAAGCCCGTGAAGGGAAGGAAAATC
AATTGGATGAAGGCCGGGATATTGGAATCAGACAGGGTTTTGACTGTGAGCCCATACTATGCCCAAGAAGTCATC
TCTGGAGTTGAAAGAGGCGTCGAGCTGGATAACTTCATTCGTAAAACTGGCATTGCTGGTATTATAAATGGCATG
GACGTCCAGGAGTGGAATCCTGTTACAGATAAATACATTGACATCCACTACGATGCCACAACTGTTATGGACGCAA
AACCTTTGTTGAAGGAAGCCCTTCAAGCAGAAGTCGGATTGCCTGTTGATAGGAATGTTCCTTTGATAGGCTTCAT
TGGTAGATTAGAAGAGCAGAAGGGTTCAGATATTTTTGTTGGAGCTATTTCCCAATTGGTTGAACACAATGTGCAG
ATAGTAATCCTTGGAACTGGCAAAAAGAAATTTGAGAAGCAGATTGAGCATCTGGAGGTTTTGTACCCTGACAAG
GCAAGAGGAGTTGCAAAATTCAATGTGCCGTTGGCGCACATGATCACAGCTGGTGCTGACTTTATGCTGGTTCCAA
GTAGATTTGAGCCCTGTGGTCTCATTCAGTTGCATGCTATGCGATATGGAACAGTTCCCATTGTTGCTTCTACTGGT
GGTCTTGTTGATACTGTTAAAGAAGGTTACACAGGATTCCAAATGGGGGCCTTGCGCGTTGAATGTGACAAAATT
GATTCAGCAGATGTAGCTGCGATAGTTAAAACTGTGGCAAGAGCTCTTGGCACTTATGCTACCGCTGCATTAAGAG
AAATGATCCTGAATTGCATGGCCCAAGACTTGTCATGGAAGGGACCAGCCAGAATGTGGGAGAAAATGCTCCTGG
ACCTGGAAGTTACTGGCAGCGAACCTGGCACTGAAGGGGAGGAGATCGCTCCTCTTGCTAAGGAGAACGTCCCA
CGCCTTGA

B



C

gRNA name	Sequence 5'-3'+ PAM	Location
gRNA-GBSSI-5	TTGGGATACCTCTGTATCGGTGG	Exon 2
gRNA-GBSSI-4	GCGTGTTTGTAAATCCCCATAGG	Exon 6
gRNA-GBBSI-1	GGTAGATTAGAAGAGCAGAAGGG	Exon 9

Figure 17. (A) coding sequence of the GBSSI gene, with gRNAs highlighted in green and the PAM sequence in red. The cytosine (C), which corresponds to the allelic variant of the natural mutant AM 206-5, is highlighted in pink (Aiemnaka et al., 2012). (B) pHSE401 binary vector used as a destination vector for dicots. (C) detail of the gRNA sequences and their location within the cassava GBSS CDS.

The three gRNAs were tested through independent transformation essays. Gene editing was performed according to Taylor et al. (2012) with some modifications. A total of 163 plants were regenerated from transformations with three different gRNAs; all were transferred to the greenhouse (Figure 18A-D). Molecular analyses with PCR and southern blots detected 129 independent transgenic events (Table 4). An iodine staining test was run across all in vitro plants (stems) as a primary screening to search for waxy plants. As a positive control, in vitro stems of the waxy line AM206-5 were also stained with iodine. Approximately 95% of the independent transgenic lines showed shades of the characteristic brown-red coloration of waxy stems, just like AM206-5, while the control wild type variety 60444 stained dark blue. In total, 82% of the lines showed an unmistakable brown-red color in the stems, thus indicating changes in amylose content (Figure 18E).

Table 4. Number of transgenic plants and independent events generated for three gRNAs targeting the GBSSI gene in cassava variety 60444 .

gRNA target	Regenerated plants	Independent events by southern blot	In vitro Iodine screening test – waxy staining
gRNA-GBSS-5	24	14	14
gRNA-GBSS-4	63	52	52
gRNA-GBBS-1	76	63	57
TOTAL	**163**	**129**	**123**

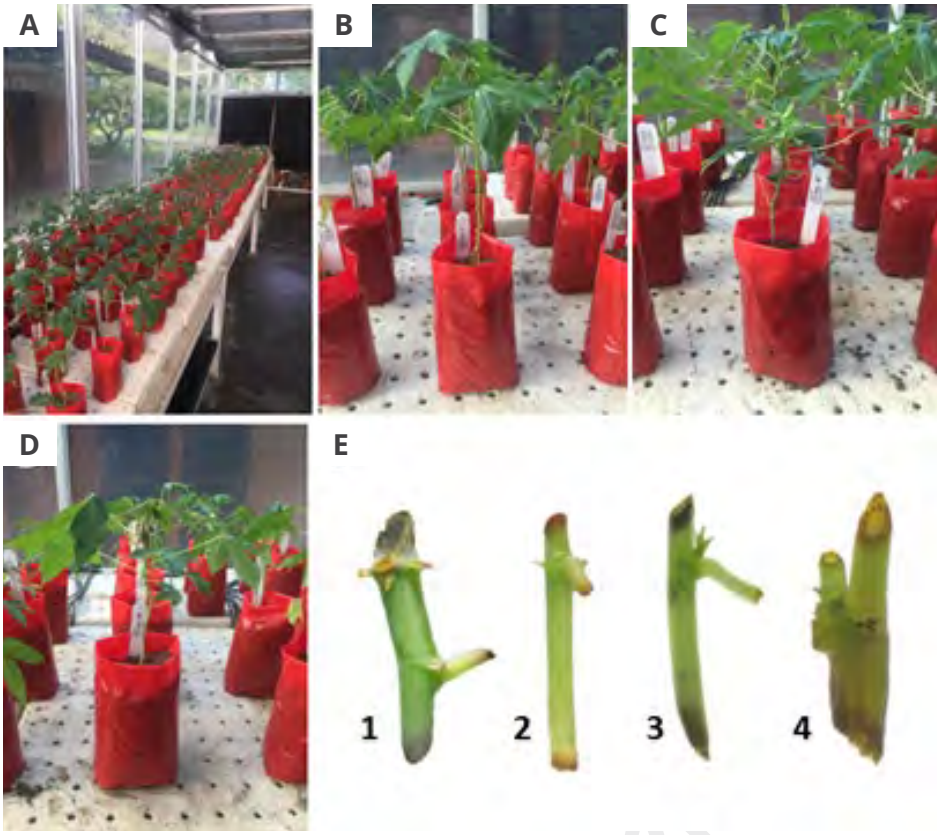


Figure 18. *GBSSI* knockout transgenic plants obtained using three constructs harboring a single gRNA each. (A) independent lines growing in the greenhouse, (B) plants of gRNA-*GBSSI*-4, (C) gRNA-*GBSSI*-5 and (D) gRNA-*GBSSI*-1. (E) Preliminary iodine test performed on stems of in vitro plants: (1) non-transformed 60444 showing the dark-blue color typical of higher amylose content; (2) waxy line AM 206-5; (3) regenerated, non-mutated line from GBSS-1 construct; and (4) transformed and mutated line showing the brown-red color typical of low amylose content from GBSS-4 construct.

Harvest of CRISPR-Cas9 edited TMS60444 from the field

Transgenic/edited TMS60444 lines for the *GBSSI* gene were harvested after 12 months in the field. An iodine test performed on the harvested lines showed 90% waxy lines (Figure 19D and Table 5).

Table 5. Iodine test on harvested transgenic/edited TMS60444 lines for *GBSSI* gene.

Construct	Lines evaluated	Waxy line
PHSE401GBSSI#1	4	4
PHSE401GBSSI#4	26	23
PHSE401GBSSI#5	9	8
TOTAL	**39**	**35**

In general, plants were over 3 m high (Figure 19A) with a dense root system (Figure 19B and 19C); the highest root quantity was 20 and the highest weight 21.5 kg.

Figure 19. (A, B, C) Aspects of harvesting transgenic/edited TMS60444 line PHSE401GBSSI#1-40. (D) Iodine test on freshly harvested roots turned brown for the edited line (left) or dark purple for the control.

Waxy transgenic/edited line analysis

Mutations in the GBSSI gene are expected to reduce amylose content, since this gene is responsible for amylose synthesis. Lines from the three constructs showed a drastic reduction of amylose content in starch (Figure 20), compared with control TMS60444. However, a few transgenic-confirmed lines had high amylose content, suggesting that *GBSSI* was not mutated or mutations did not affect gene function, i.e., lines #4-24, #4-27 & #4-62 in Figure 20, three heterozygous mutants that may still retain a wild type *GBSSI* allele (see Figure 23). Iodine test results were congruent with amylose content. PHSE401GBSSI#4-54 showed atypical behavior: The iodine test stained brown (waxy), but amylose content was slightly higher than expected (waxy <5%).

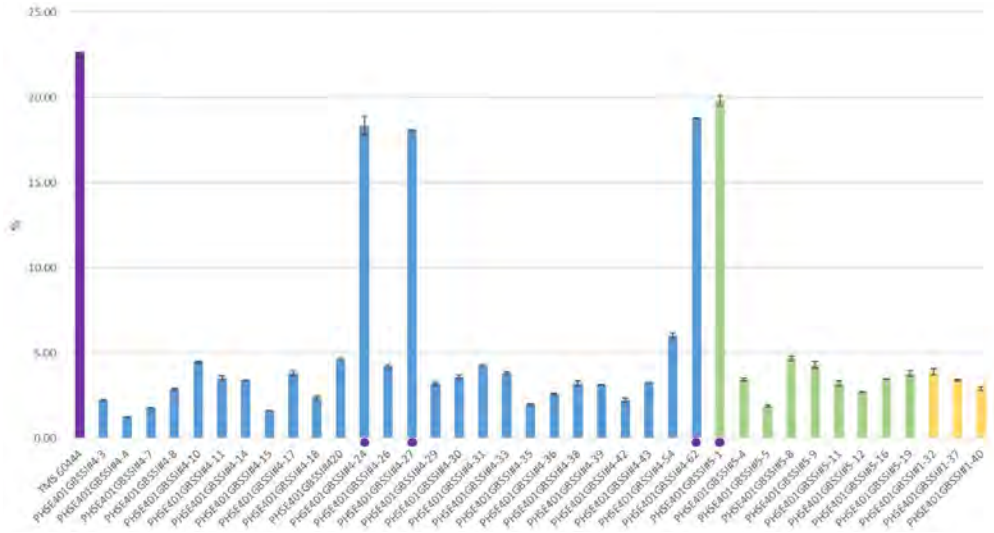


Figure 20. Amylose content in starch of cassava transgenic/edited lines with mutations in the GBSSI gene. Amylose content under 5% is considered waxy. TMS60444 included as non-waxy control. Lines marked with purple dots are non-waxy. PHSE401GBSSI#1-35 line not included. Note that the number of individuals analyzed for constructs PHSE401GBSSI#1 and PHSE401GBSSI#5 was substantially smaller than for construct PHSE401GBSSI#4.

Dry matter content showed differences depending on the construct used (Figure 21). In general, PHSE401GBSSI#4 lines had lower dry matter content in roots than TMS60444, with all below 30%. This construct targeted exon 6, the same exon mutated in AM 206-5. Meanwhile, PHSE401GBSSI#1 and pHSE401HBSSI#5 presented individuals with dry matter content over 30%. These two targeted exons 9 and 2, respectively, suggesting that mutations in these two regions may produce waxy cassava lines without affecting dry matter heavily. Nevertheless, a large-scale field trial is required to statistically confirm the tendency.

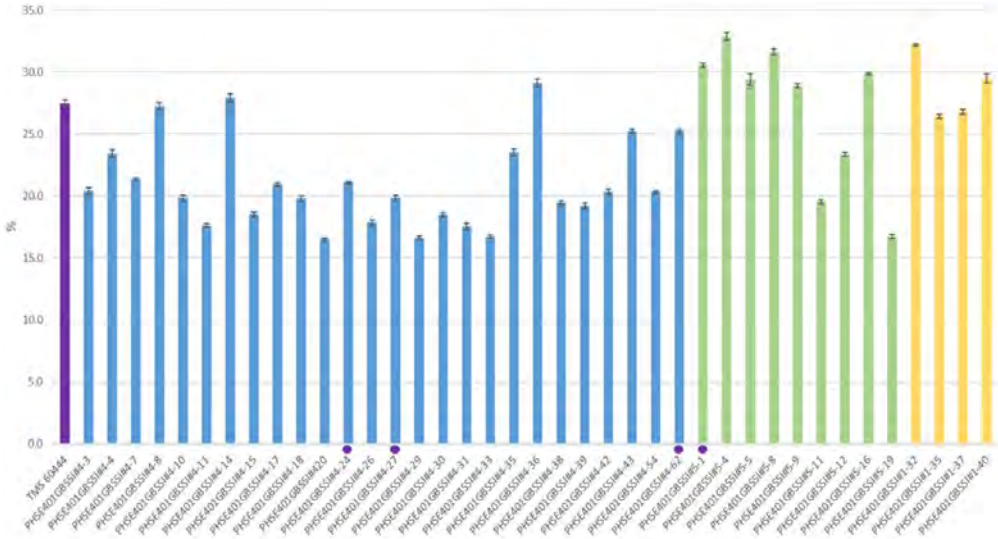


Figure 21. Dry matter content in roots (fresh weight) of cassava transgenic/edited lines with mutations in the *GBSSI* gene. TMS60444 included as non-waxy control. Lines marked with purple dots are non-waxy. Note that the number of individuals analyzed for constructs PHSE401GBSSI#1 and PHSE401GBSSI#5 was substantially smaller than for construct PHSE401GBSSI#4.

To test germination, 10 stakes per line were planted in individual bags and the germination score recorded after 20 days, with the results expressed as the percentage of germinated stakes. Transgenic/edited lines showed effects on their ability to germinate (Figure 22). However, waxy lines from the three constructs showed germination over 70%.

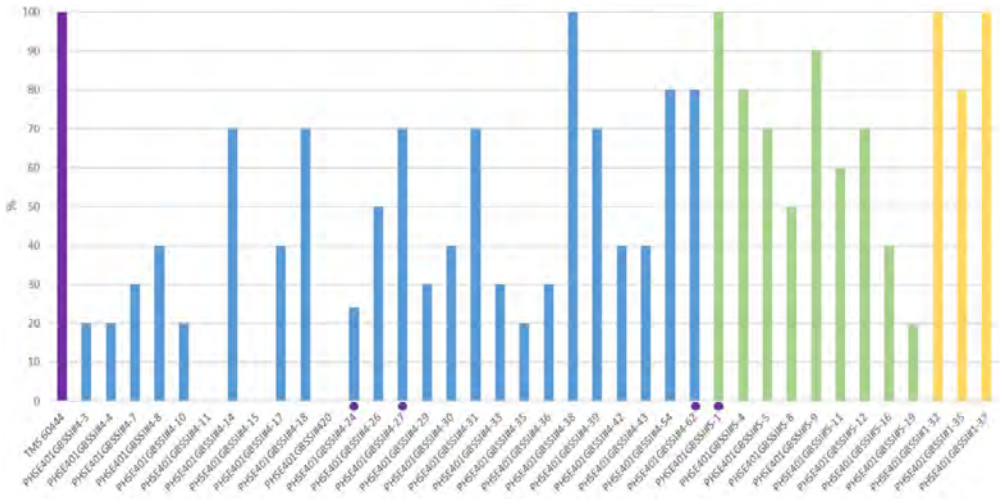


Figure 22. Germination of cassava transgenic/edited lines with mutations in the GBSSI gene. TMS60444 included as non-waxy control. Lines marked with purple dots are non-waxy. pHSE401GBSSI#1-40 lines not included. Note that the number of individuals analyzed for constructs PHSE401GBSSI#1 and PHSE401GBSSI#5 was substantially smaller than for construct PHSE401GBSSI#4.

The CRISPR/Cas9-mediated gene induces different mutations by generating double-strand ruptures in the DNA; the cell repares them by introducing indels (insertions/deletions). Such indels can be easily detected by PCR to indicate the possible new alleles obtained. The number of PCR bands gives an estimate of the number of alleles generated after DNA repair (Figure 23). In the case of the waxy lines described here, we observed up to four different alleles in a single line. It must be noted that non-waxy transgenic-edited lines also displayed several bands, suggesting that mutations happened but did not impede amylose synthesis.

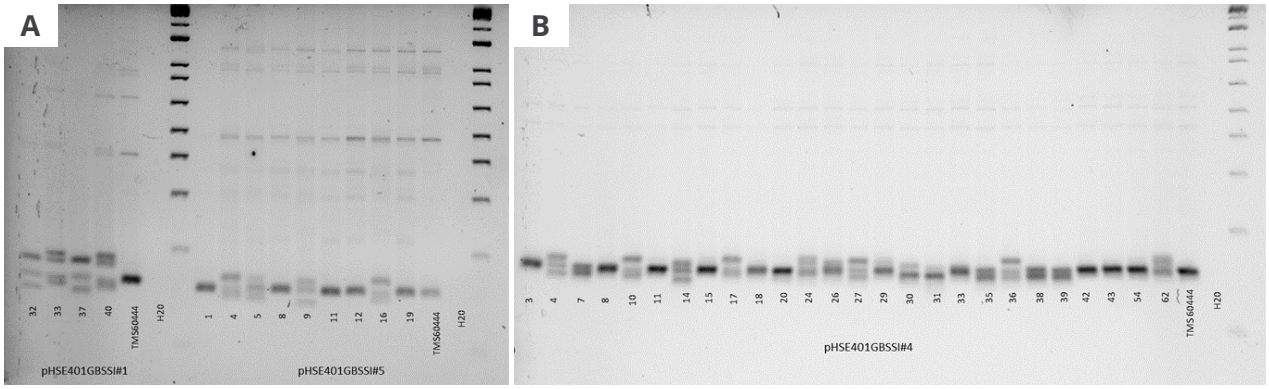


Figure 23a and 23b. Nested PCR in high-resolution MetaPhor™ agarose gel 3% of cassava transgenic/edited lines with mutations in the GBSSI gene. (A) pHSE401GBSSI#1 and pHSE401GBSSI#5 lines. (B) pHSE401GBSSI#4 lines, TMS60444 and H_2O included as PCR controls. Note that the number of individuals analyzed for constructs PHSE401GBSSI#1 and PHSE401GBSSI#5 was substantially smaller than for construct PHSE401GBSSI#4.

Based on previous information, the waxy lines PHSE401GBSSI#1-32, PHSE401GBSSI#4-54, PHSE401GBSSI#5-4, PHSE401GBSSI#5-5 and PHSE401GBSSI#5-8 were chosen for a large-scale field trial, to obtain statistically sound data on yield, dry matter, germination, and amylose content. PHSE401GBSSI#5-1 and TMS60444 will continue to be used as non-waxy controls.

The waxy lines chosen have between one and two T-DNA copies, and one and three alleles (Table 6). Segregation of T-DNA through self-pollination or crossing waxy lines is ongoing.

Table 6. Number of T-DNA copies determined by southern blot and number of *GBSSI* alleles determined by nested PCR in high-resolution MetaPhor™ agarose gel of chosen waxy lines for large-field trials (CW)

Line	T-DNA copies	Waxy line
PHSE401GBSSI#4-54	1	1
PHSE401GBSSI#5-4	2	3
PHSE401GBSSI#5-5	2	3
PHSE401GBSSI#5-8	1	1
PHSE401GBSSI#1-32	1	3
PHSE401GBSSI#5-1	1	1
TMS 60444	0	0

Root number and weight as well as stake germination are key traits related to yield. The results consolidated in Table 7 offer insight into what should be expected from the selected waxy lines. However, a large-scale field trial is required to statistically corroborate the data.

Table 7. Root number, root weight and germination of chosen waxy lines for large field trials (CW) .

Line	# roots	Root weight (kg)	Average root weight (kg)	Germination (%)
PHSE401GBSSI#4-54	5	11,5	0,4	80
PHSE401GBSSI#5-4	10	15	0,7	80
PHSE401GBSSI#5-5	11	9	1,2	70
PHSE401GBSSI#5-8	20	6,5	3,1	50
PHSE401GBSSI#1-32	6	9	0,7	100
PHSE401GBSSI#5-1	9	11	0,8	100
TMS 60444	8	17,5	2,19	100

Waxy candidate lines were also chosen based on the starch characterization shown in Table 8. All lines had a typical waxy starch profile, except for PHSE401GBSSI#4-54, with slightly higher amylose content, and PHSE401GBSSI#5-4 with high solubility. HCN content in all lines was within the accepted range (<100 µg HCN/g Cassava WB). PSHE401GBSSI#5 lines had the highest dry matter content in roots, starch in flour and flour in fresh roots, and are possibly the most suitable for high yields.

Table 8. Starch characterization of chosen waxy lines for large field trials (CW). db: dry basis, wb: wet basis.

Line	% root dry matter content	µg HCN/g (wb)	Starch in flour (%)	Flour in fresh roots (%)	Iodine test	Amylose (% db)	Solubility (% db)
PHSE401GBSSI#4-54	20,3	29,62	60,0	11,2	Wx+	6,02	7,14
PHSE401GBSSI#5-4	32,9	13,91	79,8	24,3	Wx+	3,45	13,65
PHSE401GBSSI#5-5	29,4	24,85	73,6	20,2	Wx+	1,89	8,20
PHSE401GBSSI#5-8	31,6	12,56	74,0	21,7	Wx+	4,69	8,65
PHSE401GBSSI#1-32	32,2	34,94	60,6	18,0	Wx+	3,90	7,49
PHSE401GBSSI#5-1	30,5	16,54	73,3	20,7	Wx-	19,84	13,97
TMS 60444	27,5	29,54	70,5	17,9	Wx-	22,66	18,46

To obtain non-transgenic waxy lines, T-DNA must be segregated through sexual reproduction. Segregation of T-DNA through self-pollination to obtain transgene-free edited progeny in cassava has been previously reported (Bull et al., 2018). For this purpose, several stakes per line (Table 9) were sown in the field to perform pollination. The plants have reached maturity (Figure 24A), and are producing both male and female flowers (Figure 24B). However, cassava flowering is asynchronous, with early aperture of female flowers, followed by the male flower one or several weeks later in the same plant (Halsey et al., 2008). This delays self-pollination of selected waxy lines. Thus, crossing between "sister" lines has been implemented, until self-pollinations can be performed regularly. The waxy phenotype is a recessive trait (Ceballos et al., 2007), meaning that non-waxy alleles must be absent in the selected waxy lines; otherwise, the non-waxy phenotype should appear (i.e., line PHSE401GBSSI#5-1). Therefore, the progeny of two different selected waxy lines should remain waxy but free of transgenes.

After non-transgenic waxy lines are obtained, a small-scale trial will be performed to collect data on agronomic traits to start the de-regulation process of gene-edited, non-transgenic waxy cassava lines in Colombia.

Table 9. Number of stakes sown in the field for selected transgenic/edited waxy lines.

Line	Stakes sown in field
PHSE401GBSSI#4-54	8
PHSE401GBSSI#5-4	8
PHSE401GBSSI#5-5	7
PHSE401GBSSI#5-8	4
PHSE401GBSSI#1-32	10
PHSE401GBSSI#5-1	10
TMS 60444	3

Figure 24. Transgenic and gene-edited waxy cassava lines that were established in the field at the end of 2020 for crosses and auto-pollinations (A), and started flowering in July-August of 2021 (B). Male and female flowers denoted by symbols.

Editing cassava sweet genes for resistance to *Xanthomonas*

Cassava is vulnerable to many diseases of bacterial, fungal and viral origin, which affect different organs and cause a variety of symptoms that harm the plant. One of the main bacterial diseases is cassava bacterial blight (CBB), which is caused by the phytopathogenic bacterium *Xanthomonas axonopodis pv. Manihotis (Xam)* and results in crop losses of 12-100% (Mora, 2017).

The *Xam* bacteria have pathogenic mechanisms that allow them to infect host cells and are known as proteins of the AvrBs3/PthA family or as TAL effectors (TALEs), for Transcriptional Activator-Like proteins, which modulate gene expression in host cells (Bart et al., 2012; Castiblanco et al., 2013). TALEs can activate susceptibility genes in the plant, which play an important role in the plant-pathogen interaction. On the other hand, TALEs can induce or repeat resistance genes that are activated by the plant to defend against infection. It has been found that one of the genes related to this disease is of the SWEET/Nodulin-3 gene family, known to be targeted by TALEs of Xoo, the causal agent of bacterial leaf blight in rice (Cohn et al., 2014). The sweet genes are transporters and provide a source of sucrose for plants; during infection by bacteria such as *Xanthomonas*, they are over-expressed, making them an important factor in the infection and colonization of this type of bacteria in plants such as rice and cassava, from the leaves to the roots (Chon et al., 2014; Mora., 2017).

SgRNA design for the MeSweet10a and 10b genes

The sequences of the MeSweet10a and MeSweet10b genes were obtained from the phytosome program (Version 12). The SgRNAs were designed with the help of the CRISPER-P version 2.0 program. For the specific case of the MeSweet10a gene, the design was directed to the EBE site (effector-binding element) that recognized the TAL20 of the strain *Xam668* (*Xanthomonas axonopodis pv. manihotis*), which is 11 and 92 base pairs from the start codon (Table 10 and Figure 25). For the MeSweet10b gene, the design of the SgRNAs was directed to the exon 1 and 3 of the gene, to generate a knockout of this gene (Table 10 and figure 26). The primers for the amplification of the desired region of the genes were designed using the primer design tool available at the Integrated DNA Technology (IDT) website.

Table 10. Information on the SgRNA site of the MeSweet10a and MeSweet10b gene (name, sequence, and location in the genome).

Name	Sequence	ATG gen Sweet10a
MeSweet 10- XamEBE-1	GGGCGAGAAGCGTTTATATA**GGG**	-115
MeSweet 10a -XamEBE-2	CTATGTTGTGCAATGATGGA**TGG**	-94
MeSweet10b Exon 1	TTCACAATGGCGTTTGCTTT**CGG**	-9
MeSweet10b Exon 3	AGTTTCCATCAAAAAGGTGG**CGG**	-492

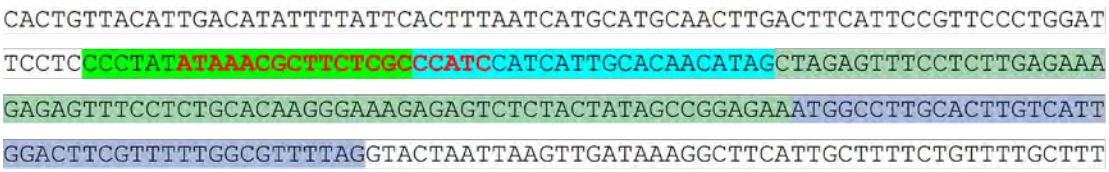


Figure 25. Sequence of the MeSweet10a gene. The region highlighted in red corresponds to the EBE site (*Xam 668*) sequence. The region highlighted in light green is the sequence of the SgRNA-XamEBE 1, and light blue is the sequence of the SgRNA-XamEBE 2.

Figure 26. Sequence of the MeSweet10b gene. Highlighted in yellow is the sequence of the SgRNA-exon 1 and in light blue the sequence of the SgRNA-exon 3.

SgRNA designs for the double target of MeSweet10a and 10b genes and MeSweet10a deletion

Two designs with double targets were developed. The first aimed to generate a complete knockout (deletion) of the MeSweet10a gene, with targets designed for the 5'UTR and 3'UTR regions (Figure 27A), while the second aimed to generate a double knockout for the MeSweet10a and 10b genes, with a SgRNA in exon 1 of the MeSweet10a gene and another in exon 2 of the MeSweet 10b gene (Figure 27B).

A)

B)

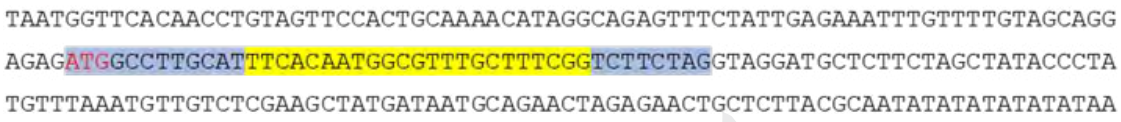


Figure 27. A) sequence of the MeSweet10a gene. Yellow highlighting shows where the first SgRNA was designed to 5' UTR region, and light blue shows where the second SgRNA was designed to 3'UTR region. B) sequence of the gene MeSweet 10b. Yellow highlighting shows where the SgRNA was designed to exon 1..

Bio-informatic analysis in phytosome 12

According to bio-informatics analysis that the phytosome program (version 12) carried out for the cassava genome (*Manihot esculenta* 6.1), the selected region of interest – the EBE site for the *Xam668* (TAL20) strain to activate the MeSweet10a gene – shows no genetic variation in 45 varieties of *Manihot esculenta*, indicating that it is a highly conserved area of the genome. In the case of the MeSweet10b gene, no variations were found (no SNPs or indels) in Exon 1, where the first target was designed. For Exon 3, a heterologous variation (SNP) was found in the variety TMS 60444; however, it is not in the sequence where the target was designed in exon 3 and so would not affect its efficiency.

Transformation of cassava plants variety TMS 60444

Plasmid PHSE401 (Figure 28) was used to transform competent *E. coli* strains (One-Shot TOP10) and later *Agrobacterium tumefaciens* (LBA4404). This plasmid together with the insertion of the oligos specific for the selected SgRNAs will be used to infect embryogenic callus and produce transgenic plants.

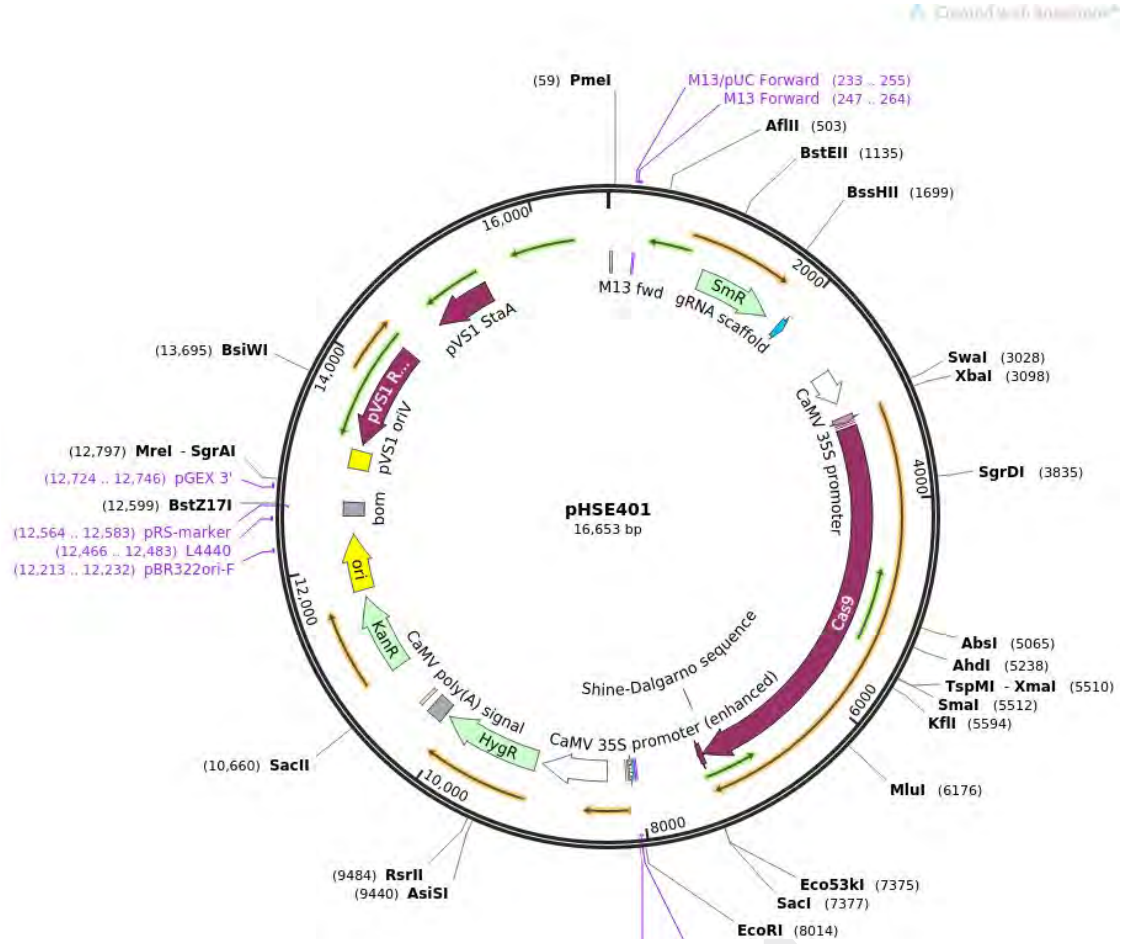


Figure 28. PHSE401 plasmid of the SnapGene. https://www.addgene.org/62201/

Ligation of plasmid pHSE401 with the oligonucleotides of MeSweet10a Xam-EBE1, Xam-EBE2, MeSweet10b Exon 1, Exon 3 and double targets

Ligation of the oligonucleotides designed for the EBE region of the MeSweet10a gene, Exons 1 and 3 of the MeSweet10b gene, and the double targets were ligated to the plasmid pHSE401 (Figure 29). Once the transformed colonies in *E. coli* (One-Shot Mach1-T1) were obtained, we selected a maximum of 10 colonies to perform the PCR colony and confirm that they are transformed with the plasmid, with the bands showing a molecular weight of 500 base pairs (Figure 29). Afterwards, we transformed our targets into Agrobacterium T (LBA4404). Once we obtained colonies, we repeated the process described before.

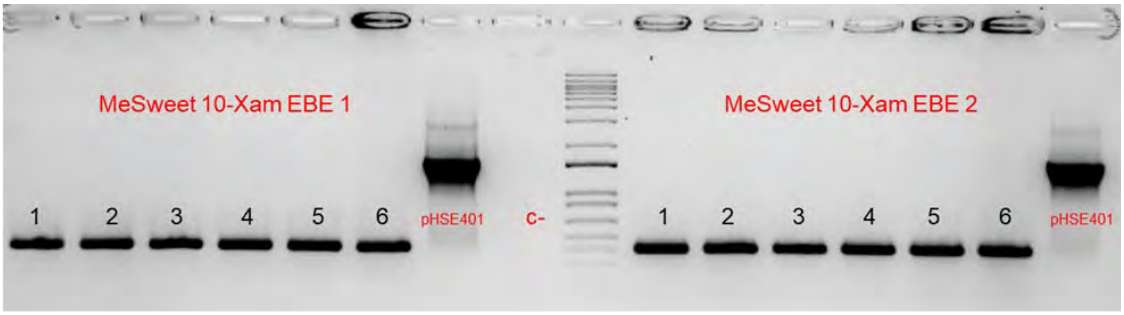
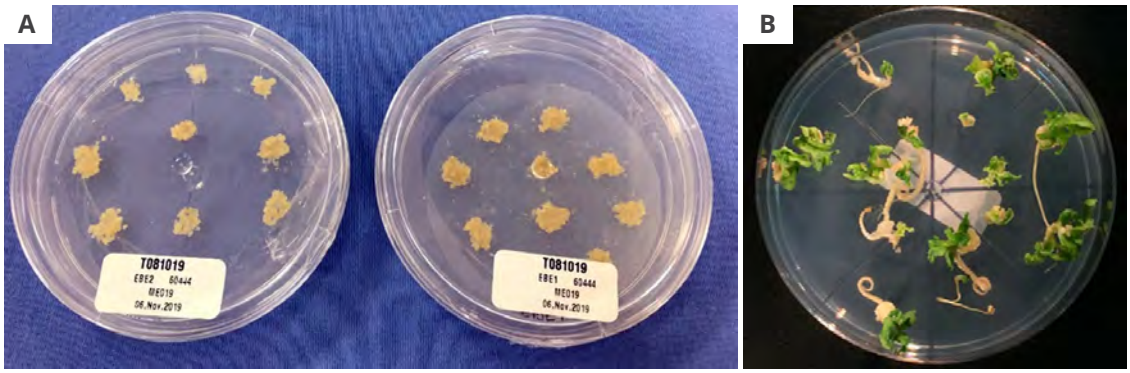


Figure 29. Example of PCR colonies of the SgRNAs MeSweet10a-XamEBE1 and XamEBE2. These colonies grew in the LB + Kan50 medium + *E. coli* One-Shot Mach1-T1.

Genetic transformation

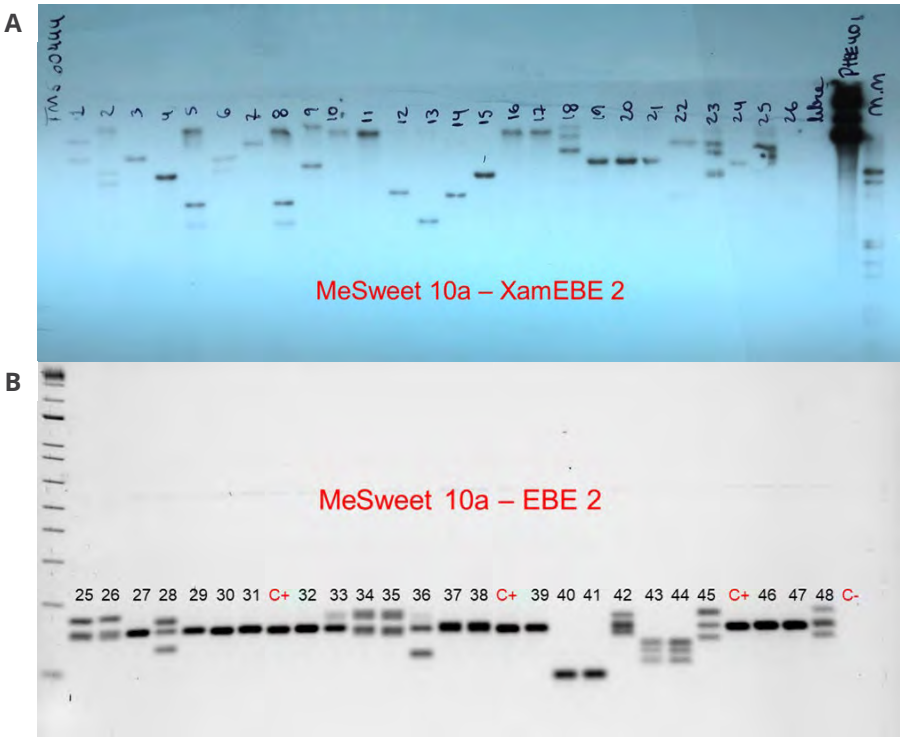
Using cassava variety TMS60444, transformation of targets XamEBE-1 and 2 for the MeSweet10a gene began on 10 August 2019. Transformation of the Exon 1 and 3 targets for the MeSweet10b gene began on 20 September 2019. Transformation of the double target for simultaneous knockout of the MeSweet10a and 10b was performed on 31 January 2020, while transformation to generate a complete knockout (deletion) of the MeSweet10a gene was performed on 27 February 2021. In all cases, we did three selections with hygromycin, which is a selection agent for transformation (Figures 30A and 30B). Our transformation control for all the transformations was the pHSE401 plasmid construct together with pCambia1305.2. This has a reporter gene, the GUS gene, which upon contact with a buffer casuses staining of the tissue that is transformed.



Figures 30A and 30B. A) Friable embryogenic callus (FEC) for genetic transformation of MeSweet10a-XamEBE 1 and 2 targets in ME019 medium (selection II). B) Embryos of the transformation MeSweet10a-EBE 2. The embryos were grown in a media supplemented with BAP + Hygro.

Molecular analysis

Molecular tests to determine editions generated in the lines obtained so far were carried out using specific primers that amplify the selection gene (hygromycin). For all lines obtained from the different constructs, the southern blot procedure was carried out to determine the number of copies of the transgene in each line (Figure 31a). In addition, a metaphor gel was made for each construct to observe the type of editing caused by Crispr/Cas 9 around the interest zone, involving deletions or insertions (Figure 31B).



Figures 31A and 31B. A) southern blot analysis of transgenic plants edited in XamEBE2 of the MeSweet10a gene. B) metaphor gel of lines for the construct MeSweet 10a-EBE2.

So far, a total of 182 lines of the six constructs have been made (Table 11). Molecular tests for the Xam-EBE1, Xam-EBE 2, MeSweet10b Exon1 and MeSweet Exon 3 constructs have been performed. The MeSweet Double T 10a and 10b and MeSweet 10a knockout constructs are in process.

Table 11. Summary of the current process for all constructs, including a description of each construct, the date of transformation, the number of lines per construct and molecular testing per line (southern blot and metaphor gels).

Gene	Targets	Date of transformation	Lines	Southern blot	# edit. lines
MeSweet10a	EBE 1	8 OCT 2019	21	11	9
	EBE 2		58	58	32
MeSweet10b	EXON 1	29 OCT 2019	19	5	5
	EXON 3		11	7	6
Double -T MeSweet10a and 10b	Double T Mesweet10a and b	31 JAN 2020	43	Process	16
Double -T Me Sweet10a O. K	Knockout Sweet10a, remove all the gen	27 FEB 2021	30	Process	Process
TOTAL	**6**	**-**	**182**	**81**	**68**

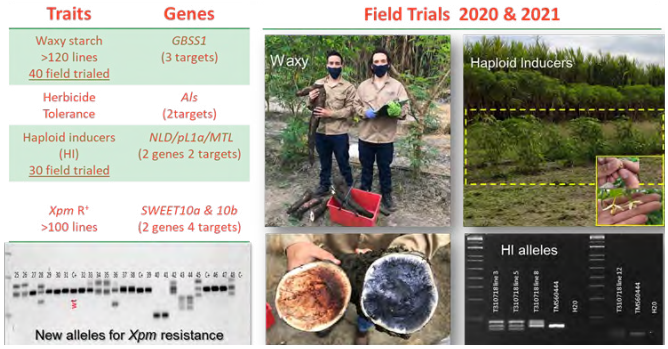
Summary

During the period 2020-2021, the Alliance's genome editing platform worked on four traits, all mediated by single gene knockouts using CRISPR-Cas9: waxy starch, haploid induction, herbicide tolerance (imidazolones), and *Xanthomonas* (*Xpm*) resistance. Several gene-edited lines for the first two traits were tested in the field, and trials are underway to produce transgene-free progeny for waxy cassava and to make crosses between potential haploid inducer lines (males) and receptor plants (females).

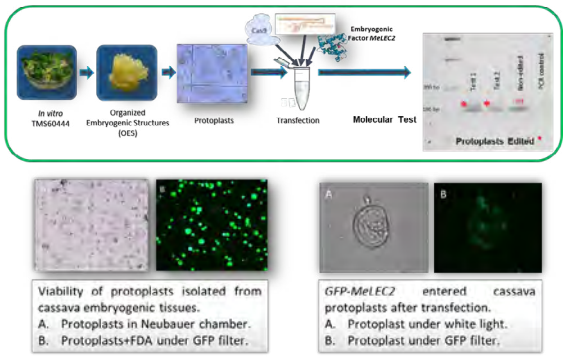
Over 100 edited lines for *Xpm* resistance were produced by targeting two genes involved in *Xpm* susceptibility. These lines will be challenged with *Xpm*, once funding for OneCGIAR initiatives has been approved.

Herbicide tolerance to imidazolones was the focus for producing transgene-free plants in vitro, not so much for commercial application, though this application has not been discarded. So far, we have cloned the sgRNA target sequence into plasmids to perform genetic transformation of embryogenic cells.

The basis for a **DNA-free gene editing protocol** in cassava was established using protoplasts as recipients of transfected *Cas9*-sgRNA or *GFP-MeLEC2* (embryogenic transcription factor). With *Cas9*+sgRNA, it was possible to detect editions. Similarly, *GFP-MeLEC2* was able to enter protoplasts to produce fluorescent signals. Figures 32 and 33 summarize the main achievements of gene editing in cassava.



Figures 32. Gene editing for cassava improvement.



Figures 33. DNA-free gene editing protocol for cassava protoplasts.

PUBLICATIONS

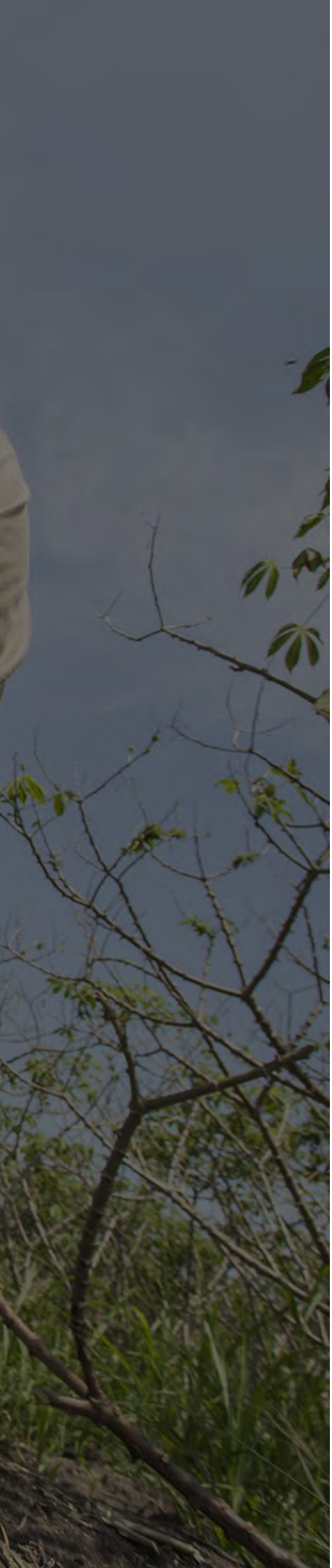
Fernando A; Selvaraj M; Chavarriaga P; Valdés S; Tohme J. 2021. A clearinghouse for genome-edited crops and field testing. Doi.org/10.1016/j.molp.2020.12.010

Monroe JG; Arciniegas JP; Moreno JL; Sánchez F; Sierra S; Valdés S; Torkamaneh D; Chavarriaga P. 2020. The lowest hanging fruit: Beneficial gene knockouts in past, present, and future crop evolution. Current Plant Biology. Doi.org/10.1016/j.cpb.2020.100185

RSA-2 RESEARCH AND SERVICE AREA-2:

Agronomy and Soil Management

Optimized fertility solutions



RSA-2 continued to target various problems that limit cassava yield and experiment with land-use practices that are highly productive, sustainable, economically viable, and environmentally safe in the LAC and SEA regions. In 2020, our research continued to focus on the need for fertilizer and site-specific nutrient management in SEA.

RSA-2 research results and the scientists contributing are detailed in the sections that follow.

Scientist contributing to RSA-2:

Imran Malik

Other contributing scientists and staff:

Lao Thao Yao Bee
Sophearith Sok
Thuy Cu Thi Le

IMRAN MALIK

Optimized fertility solutions

Collaborators: Laothao Youbee, Sophearith Sok and Thuy Cu Thi Le.

With the aim of developing improved cassava production technology for germplasm evaluation in Southeast Asia, trials on soil management and agronomic practices were conducted with smallholder farmers as well as government and industry representatives of four countries in the region: Laos, Cambodia, Vietnam and Indonesia. The idea is to expand the use of new technologies among growers receiving varying levels of support from private companies. Described below are results from the 2019-2020 season.

Result 1 – Development of alternative inputs within local farming and processing systems

An experiment on potassium balance was conducted, and the results were shared with farmers via on-farm demonstrations. A commercially available fertilizer mix, N:P2O5:K2O (14-5-35) and N:P2O5:K2O (15-7-18), was tested in Laos (Table 12) and Cambodia (Table 13).

Table 12. Fresh root yield and starch content in four districts of Laos during the 2019-2020 season. Values are the means for trials in each district (two trials in each district. n.s., non-significant).

District	Fresh root yield (t ha^{-1})			Starch content (%)		
Region	No Fertiliser	With Fertiliser N:P_2O_5:K_2O (14-5-35)	With Fertiliser N:P_2O_5:K_2O (15-7-18)	No Fertiliser	With Fertiliser N:P_2O_5:K_2O (14-5-35)	With Fertiliser N:P_2O_5:K_2O (15-7-18)
Kenethao	15.3 ± 8.03	25.2 ± 6.40	25.3 ± 6.69	28.0 ± 1.03	27.6 ± 0.64	27.8 ± 2.16
Paklai	13.0 ± 3.57	21.6 ± 1.53	20.1 ± 1.57	23.1 ± 1.43	26.7 ± 1.14	26.7 ± 1.88
Viengthong	18.4 ± 5.95	32.5 ± 2.78	28.7 ± 3.97	30.6 ± 0.58	33.5 ± 0.07	32.3 ± 0.34
Bolikan	24.1 ± 0.10	28.9 ± 3.68	36.7 ± 3.26	26.9 ± 3.13	29.9 ± 1.87	26.6 ± 1.32
Fertiliser	n.s.			n.s.		
Fertilize X Location	n.s.			n.s.		

Table 13. Fresh root yield and starch content of cassava variety KU50 in response to different fertilisers. NPK (14:7:35) fertiliser application showed no fresh root yield advantage over individual fertiliser application. Although all fertiliser applications increased yield by 2-3 t/ha relative to the control treatment without fertiliser, no significant effects were evident due to large variation. Starch content responded similarly to yield but was generally low (~20%). Prior to harvest in mid-May, frequent rainfall in the area after a long dry spell allowed the cassava crop to recover, producing significant numbers of new leaves. Increased availability of water in the soil triggered leaf emergence, presumably at the expense of storage energy.

Treatment	Freshroot yield (t ha^{-1})	Starch Content (%)
No fertliser	30.8 ± 2.0	18.9 ± 1.2
14:7:35=300 kg ha^{-1}	33.9 ± 2.1	19.2 ± 0.6
20:05:20	33.7 ± 2.8	20.2 ± 0.8
40:20:40	32.8 ± 2.5	20.5 ± 0.3

Result 2 – Identification of opportunities for high-impact genetic solutions to agronomic challenges

In Vietnam, an experiment was carried out to evaluate 21 new CIAT clones (i.e., elite lines), compared with the popular varieties KM419 and Km94. Wide variation was observed among the elite lines in terms of fresh root yield (Figure 34A). The highest yielding line, GM579-13, also had the highest starch content (30.1%), which varied from 25% to 30% (Figure 34B). The popular varieties Km419 and KM94 had 26.9% and 28.7% starch content, respectively.

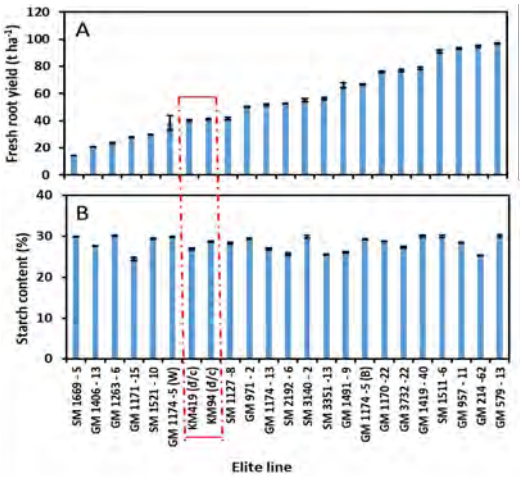


Figure 34A and 34B. Fresh root yield (A) and starch content (B) of 21 elite lines, compared with two popular varieties (between red dotted lines) after 8 months of growth. Planting density was 1.0 m X 0.8 m (i.e., 12,500 plants per hectare), and 90 kg N/ha, 60 kg P2O5/ha and 90 kg K2O/ha was applied as fertilizer. Values are means of three replicates with standard error bars. *w=white roots and B=brown roots.

Result 3 – Development of agronomic practices that support the transition to a broader farming system

Different soil management options were tested, including intercropping with different legumes, grass strips and use of cassava residues from the previous year. Intercropping with legumes was the preferred option for soil management (improving soil nutrient status) and has been scaled up; however, stakeholders have expressed concern about scarcity of farm labor. A 20-month long experiment conducted in Vietnam to study the potential for year-round cassava production demonstrated that fresh root yield increases with the duration of the crop (Figure 35A), although during the rainy season starch content goes down (Figure 35B).

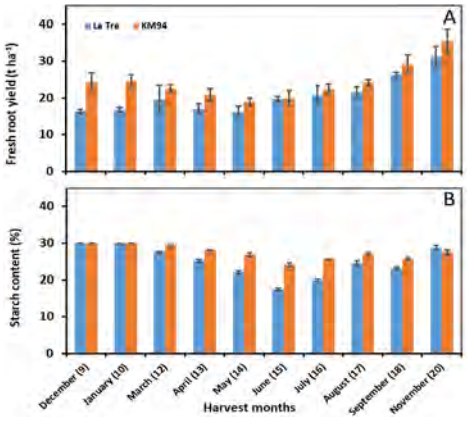


Figure 35A and 35B. Fresh root yield (A) and starch content (B) of cassava variety La Tre and KM94 at different harvest dates (crop duration in months). The crop was planted at the same time in April of the previous year (2018). Means are followed by standard errors (n = 3).

Partners: CRP-RTB, ACIAR, UWA, NAFRI, CARDI, NOMAFSI, TNU, UB, ILETRI, DOA-Myanmar

RSA-3 RESEARCH AND SERVICE AREA-3:

Crop Protection

Enhanced plant health and insect control

In 2020, RSA-3 continued developing, integrating and implementing an economically viable and environmentally sound approach for pest and disease surveillance and preemptive management that also helps protect human health in the LAC and SEA regions. To increase recognition of disease symptoms and specific pathogens, and to raise awareness of emerging diseases, we standardized molecular diagnostic protocols, monitored CBSD in Asia, optimized a method to identify cassava mites and developed a high-quality DNA isolation protocol using body segment tissue from fall armyworm (Spodoptera frugiperda). We also updated distribution maps for priority cassava diseases, initiated a multiplication scheme for resistance to CMD and cassava witches' broom disease (CWBD) in the in vitro genebank collection, and started collaboration with local universities on rapid genetic characterization of the SARS-CoV-2 virus in Cali, Colombia.

RSA-3 research results and the scientists contributing are detailed in the sections that follow.

Scientists contributing to RSA-3:

Wilmer Cuéllar
Imran Malik
Roosevelt Escobar

Other contributing scientists and staff:

Adriana Núñez
Ana María Leiva
Auradela Ríos
Erik Delaquis
Jenyfer J. Polo
Joe Tohme
Jonathan Newby
Juan Manuel Pardo
Lao Thao Yao Bee
Luis Augusto Becerra
María Isabel Gómez
Mónica Vélez
Peter Wenzl
Sophearith Sok
Xiaofei Zhang

WILMER CUÉLLAR

Management of pest and diseases to secure productivity gains

Collaborators: María Isabel Gómez, Juan Manuel Pardo, Jenyfer Jiménez, Ana María Leiva, Roosevelt Escobar, Adriana Bohorquez, Imran Malik, Sophearith Sok, Diana Katherine Castillo, Tatiana Melissa Ovalle and Luis Augusto Becerra.

Standardization of molecular diagnostic protocols

Pest and disease characterization form a key part of crop protection. To this end, we validate and implement robust protocols for pest and pathogen detection and identification (Figure 36), which are used to monitor pathogen distribution and evolution, screen for disease-tolerant genotypes and guarantee the pathogen-free status of cassava planting material. Detailed protocols, described in previous reports, are available and being implemented through our network of collaborators (Ovalle et al., 2020; Jiménez et al., 2021; Marín et al., 2021).

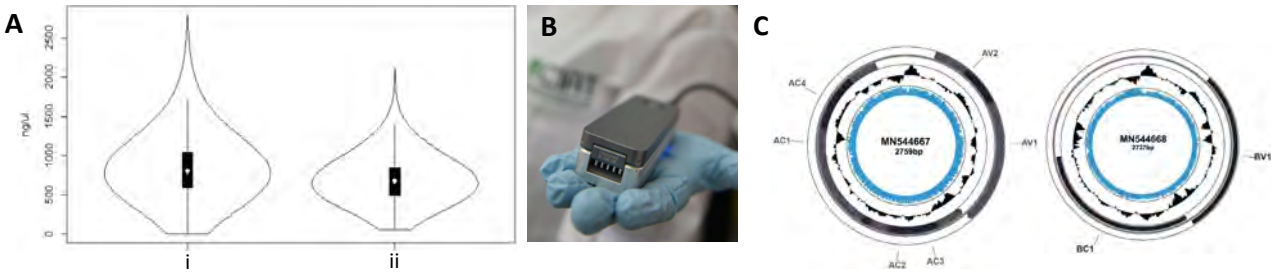


Figure 36. Standardization of protocols for pathogen characterization. A) distribution of nucleic acid concentrations (ng/µL) obtained before (i) and after (ii) DNase treatment; B) pathogen genome sequencing carried out in our laboratory using nanopore technology; and C) sequence assembly and coverage of pathogen genomes.

CMD monitoring in Southeast Asia

In collaboration with colleagues from the PPC in Laos, our team achieved early detection of CMD symptoms and confirmed the identity of the pathogen in the southern provinces of Attapeu and Champassack (Chittarath et al., 2021), as shown in Figures 37 and 38.

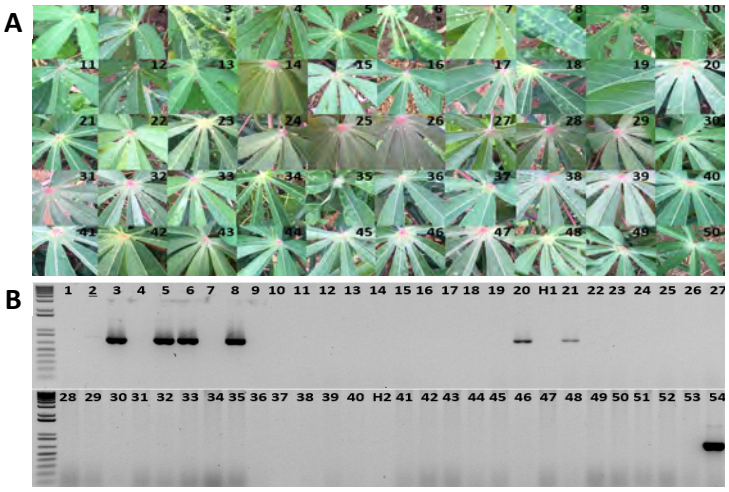


Figure 37. Results from CMD monitoring in southern Laos. The data is freely available through the PestDisPlace platform. A) plants collected from infected fields and B) results from molecular diagnostics, confirming the presence of SLCMV in symptomatic and asymptomatic plants.

Figure 38. Monitoring CMD in Southeast Asia. A) and B) the team from PPC in Laos collecting the first images of CMD in the country; C) and D) colleagues from the GDA in Cambodia and PPRI in Vietnam monitoring the spread of CMD; and E) the team from Kasetsart University in Thailand performing nucleic acid extractions.

The report and complete genome sequence of Laos isolates of SLCMV are publicly available:
https://bit.ly/3pxIfK4

More details on RSA-3 activities in Southeast Asia can be found here:
https://bit.ly/3IuSyY5

Improved identification of cassava pests

The RSA-3 team has optimized a method for identifying cassava mites at the species level, using morphological and molecular data (Figure 39). Permitting simple, accurate identification of *Mononychellus caribbeanae, M. tanajoa, M. mcgregori* and *Tetranychus urticae*, the method is intended to facilitate surveillance and monitoring of mite pests in cassava by crop protection programs in Africa, Asia and Latin America (Ovalle et al., 2020).

Figure 39. Characteristic symptoms of cassava frogskin disease in genotypes Valencia (A), BRA383 (B) and CM6740-7 (C). This disease affects cassava in the Americas, reducing yield and root quality.

Spodoptera frugiperda biotype characterization

An optimized high-quality DNA isolation protocol was developed using body segment tissue from fall armyworm (FAW). This facilitates study of the genetic variability of biotypes and monitoring of their distribution and population dynamics (Figure 40). The quality of the DNA produced using this protocol is suitable for subsequent molecular applications, such as (i) next-generation whole genome sequencing, (ii) conventional polymerase chain reaction for genotyping, (iii) barcodes and (iv) gene cloning. Validation of this protocol as part of a rapid diagnostic tool for invasive lepidopteran larval stages is underway (Figure 41).

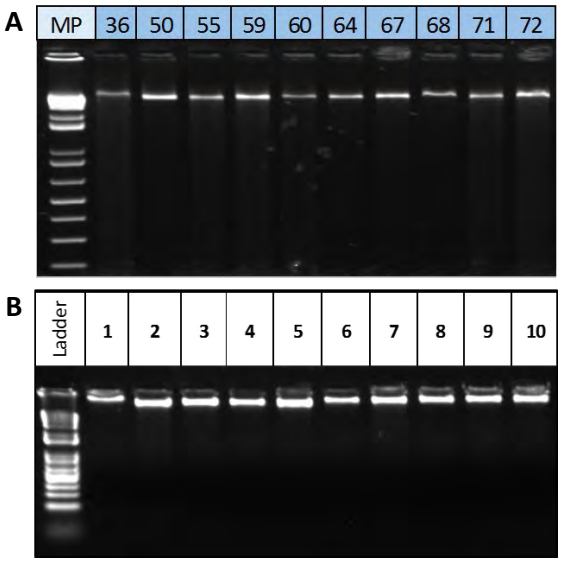


Figure 40. Quality gel of DNA isolated using modifications to the CTAB-based protocol proposed here. (A) DNA from FAW samples collected in the field and stored in 70% ethanol. (B) DNA from hind legs of FAW adults subjected to long-term storage at 4 °C.

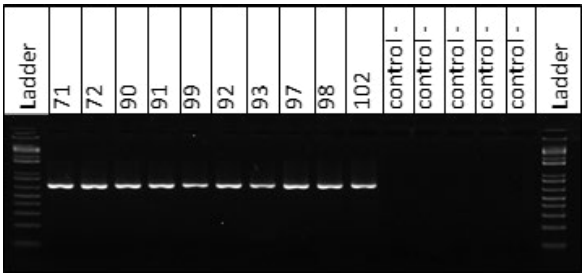


Figure 41. Amplification of the COI* region of FAW DNA isolated by application of the DNA extraction protocol modified and proposed here. (*) Using as a template 10 ng/µL isolated with the modified protocol implemented in this study. The negative controls correspond to control without the template to rule out the presence of cross contamination.

Updated distribution maps for priority cassava diseases

Information about emerging pests and diseases is rapidly growing and being made available through public databases. RSA-3 routinely examines early detection and genetic analysis of pathogen occurrence in the context of historical and newly published scientific information, which is collected, curated and communicated through the PestDisPlace platform. Results and maps have now been updated for cassava frogskin disease in the Americas, CBSD in Africa and CMD in Southeast Asia, and are available to all registered project collaborators.

Maps:

CFSD in the Americas: https://pestdisplace.org//embed/news/map/disease/5

CBSD in Africa: https://pestdisplace.org//embed/news/map/disease/6

CMD in SEA: https://pestdisplace.org//embed/news/map/pathogen/3

Supporting the Colombian National Genomic Surveillance Network of SARS-Cov-2 (COVID-19)

COVID-19 continues to have an enormous impact on livelihoods and economies in developing countries. When such a damaging pathogen emerges, we need to monitor its spread and genetics so as to quickly identify disease hotspots and new pathogen variants, and implement rapid responses. For this purpose, new technologies must be implemented on a large scale, and, most importantly, skilled personnel must be available. Based on the experience of CIAT's virology team with molecular diagnostics of cassava pathogens using the latest sequencing technologies, we started collaborating with local universities on rapid genetic characterization of the SARS-CoV-2 virus in Cali (López-Álvarez et al., 2020). At the same time, the cassava virology team offered training for young scientists in the latest technology available to characterize the genetics of SARS-CoV-2. Currently, our laboratory is part of the National SARS-Cov-2 Genomic Surveillance Network coordinated by the National Institute of Health of Colombia.

https://www.ins.gov.co/Noticias/Paginas/coronavirus-genoma.aspx

https://bit.ly/3DBjLEW

PUBLICATIONS

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- Cuellar WJ; Mwanzia L; Lourido D; Martínez AF; Rodríguez R; García C. 2020. Monitoring the distribution of pests and diseases. PestDisPlace: Version 3.0. International Center for Tropical Agriculture (CIAT).
- López-Álvarez D; Parra B; Cuellar WJ. 2020. Genome sequence of SARS-CoV-2 isolate Cali-01 from Colombia, obtained using Oxford Nanopore Technology. Microbiology Resource Announcements. Doi.org/10.1128/MRA.00573-20

Partners: PPC, PPRI, GDA, INIA, SENASA, Univalle, INS

IMRAN MALIK COLLABORATOR UNDER RSA-3

In an experiment on CMD management in Cambodia, KU50 consistently performed better than other tested varieties for the second year, with less infection and higher yields (Figure 42). The best options based on these results were shared with farmers through on-farm demonstrations (using clean planting material of variety KU50).

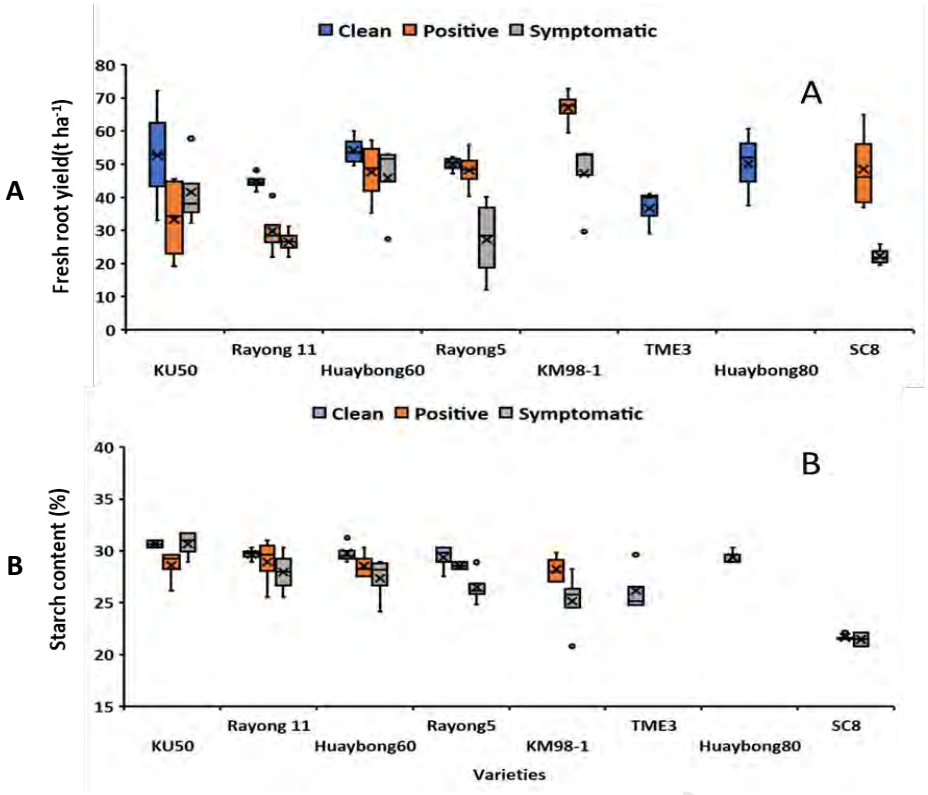


Figure 42. A) fresh root yield (t/ha) and B) starch content (%) of six popular cassava varieties in Southeast Asia, using disease-free stakes (clean), positive selected stakes from diseased fields (positive selection) and stakes selected from symptomatic plants (symptomatic) during the 2019-2020 season. Twelve plants were harvested from each plot. TME3 and Huaybong80 were planted with clean planting material due to the scarcity of clean planting material of SC8 and KM98-1(6). Values are the means (n=3) (X).

ROOSEVELT ESCOBAR

Identifying sources of resistance in regional collaboration and information sharing

Collaborators: Adriana Núñez, Auradela Ríos, Mónica Vélez, Lao Thao, Imran Malik, Erik Delakis, Peter Wenzl Xiaofei Zhang, Wilmer Cuellar, Jonathan Newby, Luis-Augusto Becerra and Joe Tohme.

The genebank in-vitro collection was used to start a multiplication scheme with a cassava core collection (CCC). Materials were transferred to Laos and Vietnam to be tested for CMD and CWBD. Of the 625 clones that make up the CCC, 79.84% are available, meaning that they are clean or pathogen free and thus can be sent to end-users.

The first batch of the CCC, with 160 in-vitro clones (10 plants/clone/recipient), was sent to the National Agriculture and Forestry Research Institute (NAFRI) in Laos and Agricultural Genetics Institute (AGI) in Vietnam. These materials were propagated on MS medium (Murashige and Skoog, 1962), supplemented with 3% sucrose, B5 vitamins (Gamborg et al., 1968), pH 5.7 and solidified with 0.22% Gelrite.

Cassava clone KU50 was also prepared for shipment to Laos to start a clean seed production program. For this purpose, a batch of KU50 with 500 plants was prepared in 50 mL Falcon plastic test tubes, and sent using the MS medium (Murashige and Skoog, 1962).

During the COVID-19 pandemic, was not possible to travel for technical support of the cassava seed system, tissue culture and hardening steps. However, researchers in Laos and Cambodia have received training virtually on the propagation and hardening process.

After the material is received, it is important to reestablish light and temperature for at least 2-3 days, and then start the propagation phase on 4E medium (Roca et al., 1984). The medium composition is as follows: MS full, 3% sucrose, 0.04 mg/L BAP, 0.05 mg/L GA3, 0.02 mg/L NAA, 1 mg/L thiamine-HCl, 100 mg/L m-Inositol, Duchefa Agar 0.45% and pH 5.7-5.8. This should be sterilized in an autoclave for 15 minutes. The rooting phase requires the use of 17 N medium and involves growth for 6-7 weeks.

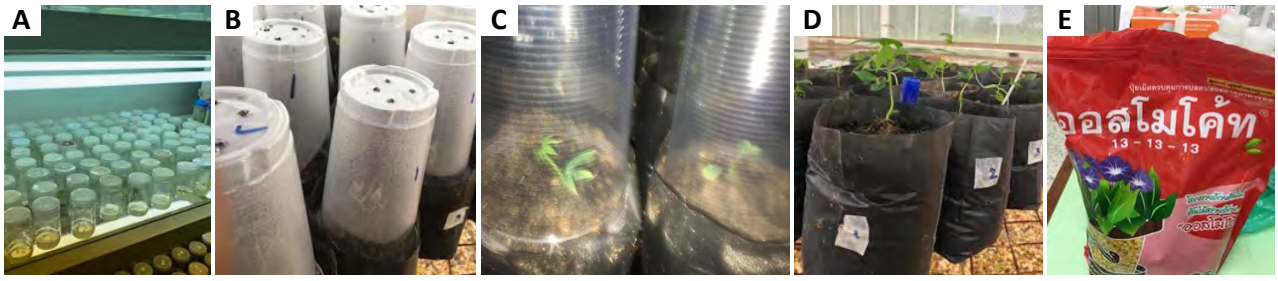


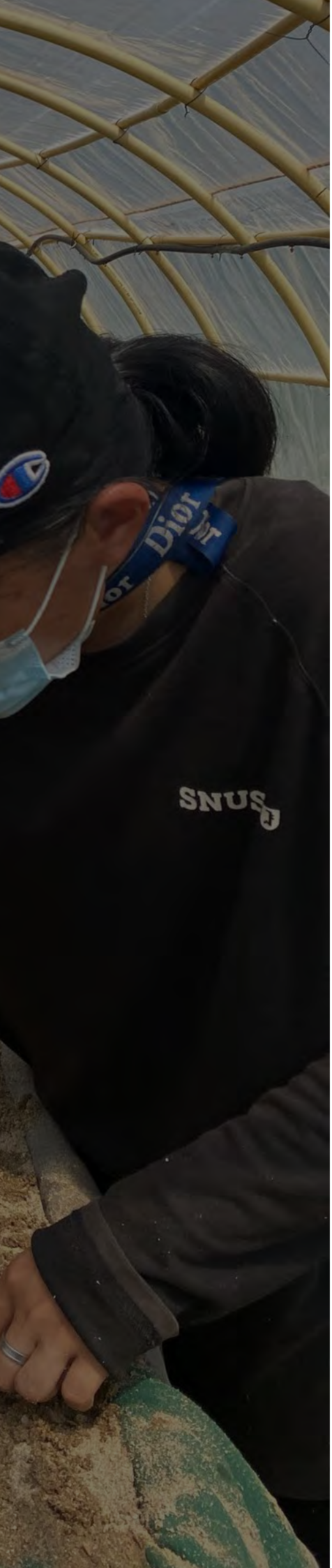
Figure 43. A) in vitro materials subcultured in NAFPRI´s lab. B, C, and D) hardening and transfer to ex-vitro conditions. B and C) use of clear cups to control humidity and avoid dessication of plants during the hardening phase. E) local formulation of fertilizers for greenhouse management.

In each country, a protocol for propagation, root induction, hardening and management has been adapted to local conditions, permitting the production of enough planting material for testing. Putting aside a set of tubes as a backup is important for ensuring that a stock of this germplasm is available for future research.

RSA-4 RESEARCH AND SERVICE AREA-4:

Seed Systems and Harvesting

Increased access to clean seed material



In 2020, RSA-4 continued developing healthy seed from more productive cassava varieties, leading to the adoption of new varieties and improved productivity in farmers' fields. Through improved access to clean seed material together with seed system characterizacion and modeling, we are helping maintain prosperous cassava-based cropping systems in Southeast Asia and Latin America.

RSA-4 research results and the scientists contributing are detailed in the sections that follow.

Scientists contributing to RSA-4:

Imran Malik
Erik Delaquis

Other contributing scientists and staff:

Lao Thao Yao Bee
Sophearith Sok

IMRAN MALIK

Increased access to clean seed material

Collaborators: Erik Delaquis, Roosevelt Escobar and Laothao Youbee.

ERIK DELAQUIS COLLABORATOR UNDER RSA-3

Seed system characterization and modeling

Seed system research continued to produce important applied and published results in 2020. In Southeast Asia, our work on the development of clean seed supply systems to deal with the expanding SLCMV epidemic led to the launch of the Future Stems facility in collaboration with NAFRI in Laos. The Lao government has also included a target for clean cassava stem multiplication in their draft agricultural strategy to 2030; once approved by the prime minister, the final strategy will mark a first for any country in the region. To scale out multiplication activities, tunnel-based multiplication sites have been established in cooperation with private sector actors in the south and north of the country, and a video was produced in the Lao language explaining how to build the tunnels (https://www.youtube.com/watch?v=VQ9OPzHBTv8).

Experimental auctions were begun in 2020, with all 20 village sites planning to complete the activity in early 2021. Following protocols developed by Cassava Program staff for implementation with root and tuber crops, this research centers on the CRP-RTB "toolbox for working with root, tuber and banana seed systems," which includes 11 distinct tools as well as a glossary for practitioners. A user guide for the toolbox has been published (Andrade-Piedra et al., 2020), with Cassava Program staff contributing importantly to several tools and guides.

Result 1

Completion of the "toolbox for working with root, tuber and banana seed systems," resulting from a global effort to synthesize tools and methods for vegetatively propagated crops like cassava. In preparation for a public launch in 2021, the toolbox content was finalized and a website created (https://tools4seedsystems.org/), along with a user guide that provides an overview of concepts, explains step-by-step tool usage, and describes individual tools and features (Andrade-Piedra et al., 2020). Cassava Program staff contributed significantly to this work, including in the design of the user guide for the experimental auction tool to be published along with the official website in the first quarter of 2021 (Delaquis et al., 2021).

Result 2

Publication of a short article in the journal Food Chain describing application of the concept of social differentiation in adapting seed systems to the impacts of COVID-19 (Delaquis and Almekinders, 2020).

Result 3

The installation and official launch of the Future Stems cassava clean seed production facility in Vientiane (a first in Lao PDR) as well as establishment of multiplication tunnels with private sector actors in southern Laos (Khounsub Ltd., Champassak Province). These activities were complemented by the release of several capacity building videos on CMD and establishment of clean seed multiplication systems in Lao PDR.

Result 4

Sustainable rapid multiplication of disease-free cassava planting material established in Lao PDR. The tunnel-based system devised for this purpose accelerates the multiplication rate from mother plants by 6-10x compared to traditional field multiplication and by 100-125x over the course of a season. This greatly lowers the cost of distributing planting stems to farmers and thus facilitates variety dissemination (Table 14).

Result 5

Ongoing multiplication of available exotic germplasm. The tissue culture laboratory at NAFRI's Rice Research Centre in Vientiane, Laos, and CARDI in Phnom Penh received five IITA varieties – TMEB419, IITA-TMS-IBA980581, IITA-TMS-IBA980505, IITA-TMS-IBA972205, and IITA-TMS-IBA920057 – as in vitro plantlets. Upon arrival, the plantlets underwent in vitro multiplication, with capacity building support from the Cassava Program, followed by hardening in screenhouse facilities. Some plants are already in the field and growing well in both countries (Figure 44). These plants will be used for rapid multiplication through the Cassava Program's tunnel system during the 2021 season to obtain an adequate number of plants for testing in multilocation trials during the 2022-2023 season.

Result 6

Quantification of water input for cassava varieties during different growth stages. For this purpose, cassava varieties are being evaluated for optimum yield under different water availability regimes during the drier period of the growth cycle in both Laos and Vietnam (Figure 45).

Table 14. Number of plants produced from tunnel systems – an example of tunnel multiplication in 1 year from two tunnels (each tunnel was cut 6 times).

Variety	# of seedlings per season per tunnel	# of viable sprout in each cutting	# of days to get new plantlets	# of days to transplant to field (from Tunnel)	# of plants in the field	Transplantation field success rate (%)
KU50	3,840	768 ± 74	[a]50 ± 4.6	[b]96 ± 15	*2,690	100
Rayong11	5,040	840 ± 123	[a]49 ± 3.0	[b]95 ± 4	4,210	100

* Lost one batch to mealybugs a= delayed by 7 day due to unavailability of substrate, b= delayed by 10 to 15 days due to delayed in irrigation system set up.

Figure 44. IITA in vitro plants transplanted to the field in Cambodia (above) and Laos (below).

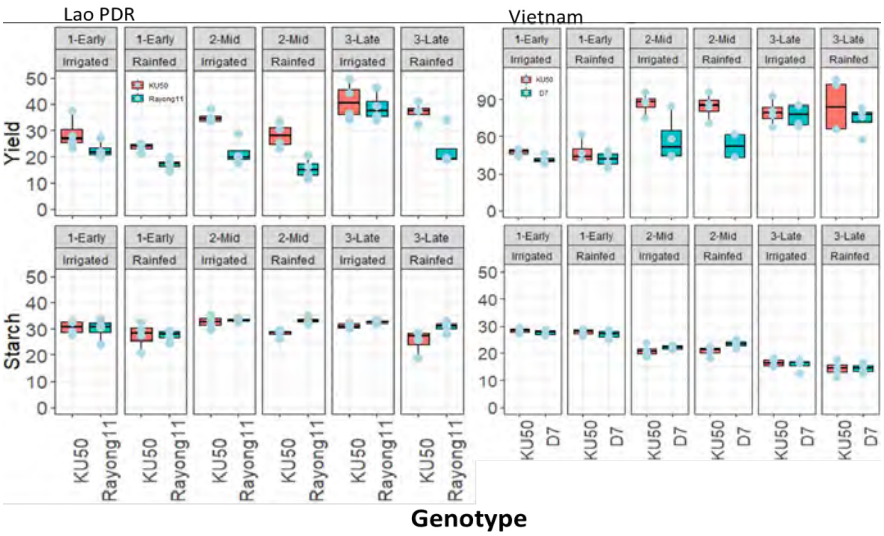


Figure 45. Fresh root yield (t/ha) and starch content (%) of the varieties KU50 and Rayong11 (Lao PDR) as well as KU50 and D7 (Vietnam) at the early, mid-, and late harvest periods in irrigated and rainfed (non-irrigated) plots. Early harvest took place at 6 months (Lao PDR) and 8 months (Vietnam); mid-harvest at 8.5 months (Laos) and 10 months (Vietnam); and late harvest at 10.5 months (Lao PDR) and 13 months (Vietnam). Irrigation treatments positively impacted fresh root yield in Laos but had little impact in Vietnam. Due to problems with the water supply, the sprinkler irrigation system became operational only at the end of February 2020 and continued operating until early April, when regular rainfall eliminated the need for irrigation in Vietnam.

PUBLICATIONS

- Andrade-Piedra JL; Almekinders CJM; McEwan MA; Kilwinger FBM; Mayanja S; Delaquis E; Garrett KA; Omondi AB; Rajendran S; Kumar PL; Thiele G; 2020. User guide to the toolbox for working with root, tuber and banana seed systems. RTB User guide (RTB User Guide No. 2020-1). CGIAR Research Program on Roots, Tubers and Bananas (RTB), Lima, Peru. Doi.org/10.4160/9789290605577 https://tools4seedsystems.org/
- Delaquis E; Almekinders CJM. 2020. COVID-19, seed security and social differentiation: When it rains, it pours. Food Chain 9:103–106. Doi.org/10.3362/2046-1887.20-00003
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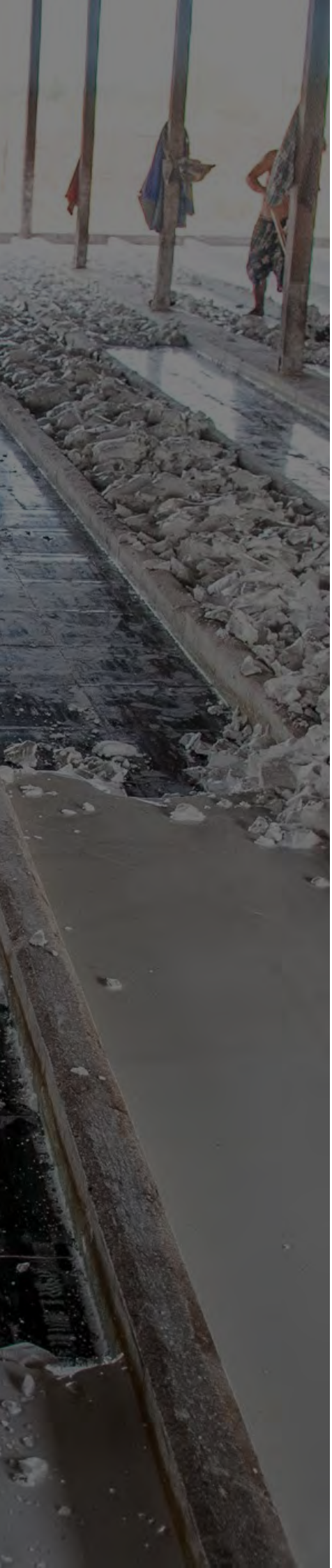
Partners: CRP-RTB, ACIAR, UQ, NAFRI, GDA, CAVAC, CARDI, AGI, CATAS

RSA-5 RESEARCH AND SERVICE AREA-5:

Post-harvest and Enhanced Nutrition

Better nutrition and income



RSA-5 is developing technologies and strategies to diminish root losses and increase cassava value through specialized starch uses and increased micronutrient potential. We address these challenges through the development of faster protocols for phenotyping the postharvest quality traits of cassava products, classification of cassava genotypes by means of near-infrared spectroscopy (NIRS), creation of a new protocol for faster waxy identification and implementation of a harmonized coding system in collaboration with RSA-1.

RSA-5 research results and the scientists contributing are detailed in the sections that follow.

Scientist contributing to RSA-5:

Thierry Tran

Other contributing scientists and staff:

Andrés Escobar
Cristian Duarte
Jhon L. Moreno
John Belalcázar
Jorge L. Luna
Maël Clergue
María A. Ospina
Matthieu Vergnol
Xiaofei Zhang

THIERRY TRAN

Better nutrition and income

Collaborators: John Belalcazar, Jorge Luis Luna, María Alejandra Ospina, Jhon Larry Moreno, Andrés Escobar, Cristian Duarte, Maël Clergue, Matthieu VergnolXiaofei Zhang

In 2020, RSA-5 continued to develop faster protocols for phenotyping postharvest quality traits of cassava products, with the objective of integrating quality criteria assessments early in the selection of improved clones. A new water absorption method (WAB) reduced by 50% the time necessary to assess the cooking quality of boiled cassava, requiring 30 minutes per sample instead of 60 minutes with the conventional method. The new method was implemented in several projects. Specifically, the RTBfoods progenitors collection and two progeny collections (30, 293 and 353 genotypes, respectively) were comprehensively phenotyped, using water absorption and additional characteritics, such as texture (Figures 46 and 47). For the first time, RSA-5 and RSA-1 were also able to screen water absorption in a large number of clones (3,196) from F1C1 biofortified cassava trials, because of the faster phenotyping methods, and to thus integrate cooking quality among the selection criteria. This effort resulted in the selection of 389 high-potential biofortified clones for advanced trials (CET).

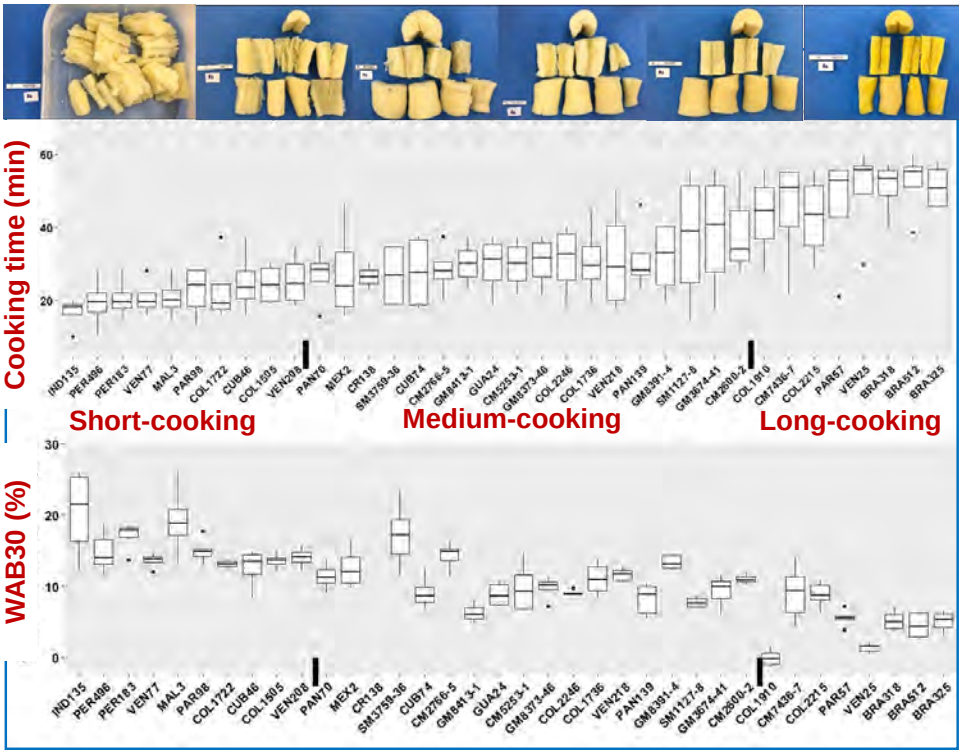


Figure 46. Diversity of cooking time and water absorption (WAB30) among cassava genotypes.

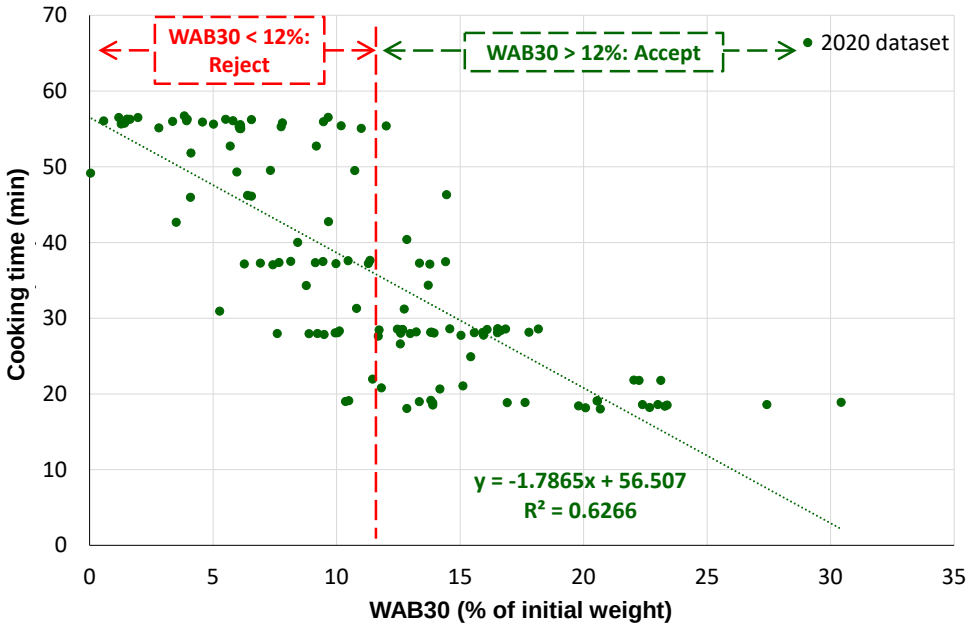
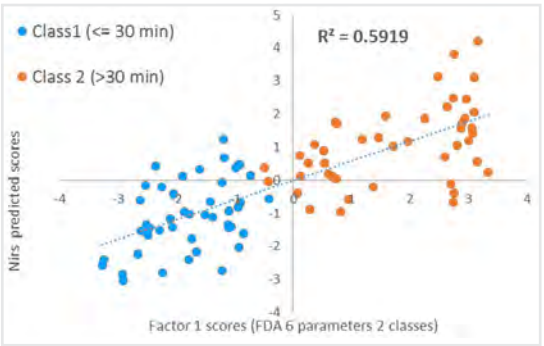
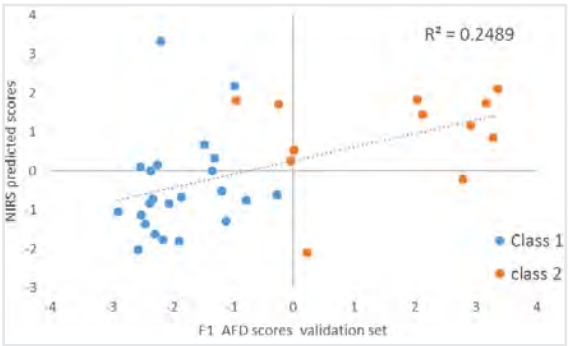


Figure 47. Significant correlation between water absorption at 30' (WAB30) and cooking time ($R^2 = 0.63$) and selection criteria.

We also made notable progress in predicting postharvest quality traits by means of NIRS, with the first demonstration of correct classification of cassava genotypes into two groups: short- and long-cooking (below and above 30 minutes, respectively). This was the first time a quality trait was predicted by NIRS in cassava; previously, it was possible to predict only compositional data, such as dry matter or beta-carotene content. The prediction was 80% correct (Figure 48), which is sufficient for early screening to select short-cooking clones and reject long-cooking ones. Taking only 5 minutes per sample, NIRS analysis promises to be a game-changer in screening quality traits for breeding, which allows true high-throughput phenotyping (HTPP) and selection of best candidate clones both for agronomic performance and postharvest quality.



F1 scores vs predicted scores for the **learning set**



F1 scores vs predicted scores for the **validation set**

Overall 80% correct prediction:

From \ To	C1	C2	Total	% correct
C1	19	4	23	82.6%
C2	3	9	12	75.0%
TOTAL	**24**	**11**	**35**	**80.0%**

Figure 48. Classification by NIRS of cassava genotypes into two classes: ≤ 30 min (C1) and > 30 min (C2).

Research aimed at detecting the waxy trait faster in newly planted clones led to a new protocol for analyzing starch in young leaves (Figure 49). The protocol involves forcing leaves to over-produce starch by illuminating them continuously for 24 hours, followed by chlorophyll extraction and iodine staining of the remaining leaves to classify them as waxy (brown color) or non-waxy (purple color). The waxy trait was thus successfully detected in leaves 3 months after planting. By eliminating the need to wait for the formation of roots 6-8 months after planting, the new protocol saves 3-5 months before screening and selection. Faster phenotyping protocols are key for increasing capacity to screen for postharvest quality traits and for accelerating the development of improved cassava varieties that match users' expectations and preferences.

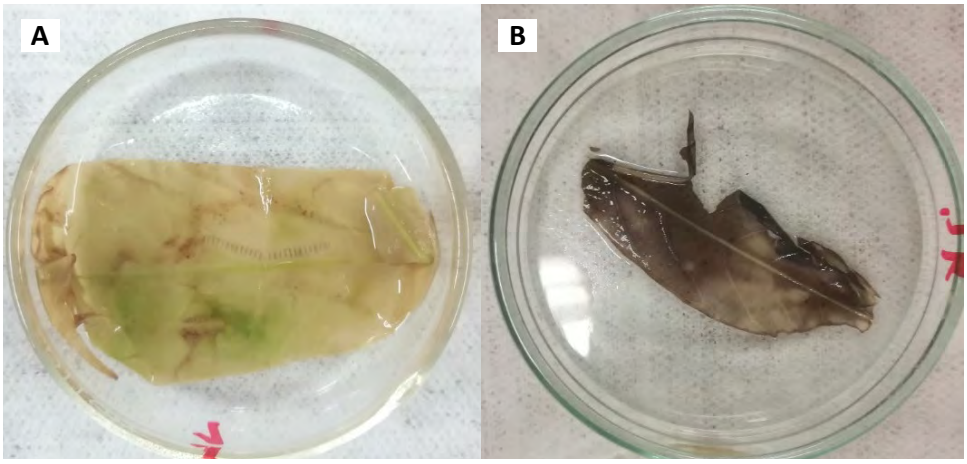


Figure 49. Cassava leaves (3 months after planting) colored with 2% iodine solution after chlorophyll extraction. A) waxy genotype and B) normal starch genotype.

To ensure data traceability, RSA-1 and RSA-5 implemented a harmonized coding system for field trials and phenotyping of postharvest quality traits. The codes are based on 16 digits and are compatible with the Cassavabase breeding database (Figure 50). The system uses QR codes to link each sample to the Fieldbook data collection app. This has streamlined the collection and uploading of datasets to Cassavabase, further reducing delays in making quality traits data available to RSA-1.

Trial Name	2019111CQQU1_ciat
Breeding Program	CIAT
Trial Location	CIAT. Valle, Colombia (Colombia)
Year	2019
Stock Type Being Evaluated in This Trial	accession
Number of Stocks in This Trial	28
Trial Type	Specialty Trial
Planting Date	2019-March-20
Harvest Date	2020-January-03
Description	2019111CQQU1_ciat, Quality, RTBfoods trial
Folder	CIAT_2019

2019111CQQU1_ciat SGN8152

Plot Width (m)	6
Plot Length (m)	8
Field Size (ha)	[No Field Size]
Trial Will Be Genotyped	no
Trial Will Be In Crosses	no

Figure 50. Example of CIAT dataset uploaded to Cassavabase (RTBFoods).

During the COVID-19 pandemic, careful planning and social distancing measures have made it possible to keep most laboratory activities on schedule and to avoid extended lockdowns. The activities most impacted were cassava processing optimization, for which only desk-based work and virtual training were possible.

PUBLICATIONS

- Chirinda N; Trujillo C; Loaiza S; Salazar S; Luna J; Tong Encinas LA; Becerra-López Lavalle LA; Tran T. 2021. Nitrous oxide emissions from cassava fields amended with organic and inorganic fertilizers. Soil Use and Management. Doi: 10.1111/sum.12696
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Partners: CRP-RTB, RTBFoods, CIRAD

RSA-6 RESEARCH AND SERVICE AREA-6:

Value Chain, Markets and Policy

Unlocking new market growth (in conjunction with all RSAs)

RSA-6 examines the production process and costs related to each element of the production chain in search of ways to add value to cassava products in the LAC and SEA regions. In 2020, we continued to provide the Cassava Program with cross-cutting support, based on a thorough understanding of Asian cassava markets, the value chains of smallholder cassava producers and partnerships. In addition, by collecting valuable data on cassava farming households through different surveys implemented in SEA, we generated evidence of the program's outputs and impacts.

RSA-6 research results and the scientists contributing are detailed in the sections that follow.

Scientists contributing to RSA-6:

Jonathan Newby
Vanya Slavchevska

Other contributing scientists and staff:

Erik Delaquis
Imran Malik
Lao Thao Yao Bee
Sophearith Sok
Thuy Cu Thi Le
Wilmer Cuéllar

VANYA SLAVCHEVSKA AND JONATHAN NEWBY

Unlocking new market growth

Collaborators: Imran Malik, Erik Delaquis and Wilmer Cuéllar.

Jonathan Newby:

Asian cassava market update (cassava-market-update-jan2021.pdf (wordpress.com))

Cassava markets gave mixed results in 2020, varying according to the geography and products, especially in the context of COVID-19. However, many other global factors impact these and related commodity markets – particularly in Asia, which remains the focus of the cassava market. Since the outbreak of COVID-19, commodity flows have been altered by a range of trade policy shifts together with outbreaks of animal disease (African swine fever) as well as crop pests (FAW) and diseases (CMD). Many market and trade developments have occurred in China, which remains the main destination for cassava from SEA. China's overall import growth has been one of the main contributors to trade resilience during the pandemic and to strong market prices for cassava in SEA. As the Food and Agriculture Organization of the United Nations (FAO) reported in 2020, unabated import growth was not caused by the outbreak of COVID-19 in China but took place in spite of it and despite the ensuing global health crisis (FAO, 2020).

Trade in cassava products includes cross-border trade in fresh roots, cross-border and regional trade in dried cassava chips, and global trade in cassava starch. While the global cassava trade remains a multi-billion-dollar industry, the aggregate value of traded roots, chips and starch declined by around 0.5 billion USD during 2018-2019. This was largely driven by lower demand for cassava chips in China. Global trade in cassava products remains dominated by Asia as both the major source and destination. In 2020. there was some recovery in the volume (27%) and value (33%) of cassava chips exported from Thailand into China. This trend is expected to continue into 2021, as the derived demand for cassava chips increased due to increasing maize prices in China. Starch exports declined slightly (-2%), reducing export value by around 6.5%. Higher starch prices have caused exports to Indonesia to decline significantly, as processors seek alternative feedstocks for applications that are easier to substitute between starch types – i.e., toward maize.

Elevated root prices are a factor of both supply shocks and trends coupled with a significant increase in the derived demand due to the maize situation in China. The supply of cassava is likely to increase in the coming season, with farmers changing from other annual crops to cassava, involving in some cases the conversion of perennial crops. The frontier is also likely to come under pressure, with expansion into forested areas where regulations are not enforced. Increased production is also likely to influence the flow of planting stems and the spread of disease into new frontier regions.

ACIAR Value Chain and Livelihood Program concludes

The production and marketing of cassava by smallholder farmers is part of a complex global value chain influenced by many factors outside the control of farmers or other actors within these countries. However, despite price fluctuations, the sector contributes significantly to the livelihoods of smallholder farmers engaged in the industry, leads to economic development in rural communities and contributes importantly to national economies.

The ACIAR Value Chain and Livelihood Program posed the question of whether the productivity and sustainability of smallholder cassava production could be enhanced by strengthening market linkages and thus accelerating the spread of improved technologies. In search of answers, the program used cases study sites in Vietnam, Indonesia, Laos, Cambodia and Myanmar. The results indicate that in reality the potential for scaling out varies significantly between technologies and in the different production and value chain contexts.

The evidence further indicates the high likelihood of generating better practices for new varieties, while underlining the importance of new models and partners to generate changed behaviour with respect to fertiliser as well as the need to work with farmers for redesigning technologies aimed at minimising land degradation to ensure that these match farmers' priorities and preferences. In some cases, the constraints that need to be addressed are not directly related to the technology itself. In other cases, there is a clear need to continue to invest in technology development and refinement with farmers and other stakeholders.

Regardless of the technology or value chain context, there was an evident need for the creation of partnerships between public and private sector actors, and also for better coordination between these actors, ministries and development partners. The requirements for partnering with the private sector are summarised below. The "key conditions" listed can be regarded as provisional generalisations arising from the cross-case analysis and are not intended as a simple recipe for knowledge partnerships. As we have emphasised, there are many case-specific factors that restrict our ability to make such firm generalisations. Nevertheless, these key conditions can serve to delimit situations where private-sector partnerships are more likely to succeed (Table 15).

Tabla 15. Key conditions for effective knowledge partnerships with private-sector actors, based on results of cassava case studies .

A fund of adoptable technologies (i.e., with moderate to high relative advantage and learnability) requiring no more than local adaptation
A commercially oriented farming population, experienced in repeat-dealing with stable agribusinesses
An articulated value chain that establishes strong, enduring links between farmers, traders and processors
A market structure OR industry regulation that assures agribusiness actors of capturing the benefits of investing in improved farm productivity
Absence of policy constraints, such as distortions in fertiliser pricing or sudden changes in cross-border trade restrictions
Involvement of a knowledge broker to catalyse and support the partnership (e.g., a public agency, a university, a development project or an NGO)
Individual actors with the interest and capabilities to pursue these partnerships

Vanya Slavchyevska:

Continuing to collect evidence of impact

The team registered several noteworthy achievements in 2020, despite the challenges posed by COVID-19, with interruptions or delays in some activities.

The team collected a nationally representative panel survey of cassava farmers in Vietnam with funding from CRP-RTB.

The survey, which was applied to the same cassava farming households interviewed by CIAT and Vietnamese partners in 2015 (Le et al., 2019), collected information on a wide range of key topics around cassava production and value chains, including agricultural practices and technologies, productivity, disease pressures, livelihoods, experiences of shocks, impacts of COVID-19, gender and youth entrepreneurial issues. DNA fingerprinting and disease surveillance were also carried out. The

survey data will be used to analyze the issues listed as follows, which are pertinent to most RSAs, including RSA-1: Enhancement of Genetic Resources, RSA-3: Crop Protection, RSA-4: Seed Systems and Harvesting, and RSA-6: Value Chains, Market and Policy. The data analyses and publications will be completed in 2021.

Global value chains, such as that of cassava, are improving incomes and enablilng many smallholder farmers in developing countries to escape poverty. However, they are often criticized for exposing farmers with limited access to insurance or credit to significant income shocks. To increase our understanding of cassava as a pathway to prosperity, it is critical to also understand how smallholders are impacted by global shocks, such as COVID-19. In 2020, the team was awarded a competitive grant from CRP-PIM to carry out an assessment of the impacts of COVID-19 on cassava value chains and cassava farmers' income and food security in Cambodia and Vietnam. The study, to be published in 2021, found that cassava value chains were minimally impacted by COVID-19, but its impacts on cassava farmers differed in the two countries. There were larger negative impacts on cassava farmers in Cambodia than in Vietnam, which could be attributed in part to Cambodian farmers' higher livelihood vulnerability, a scarcity of domestic starch processors and ad-hoc cross-border restrictions, which led to distress early selling of cassava to cope with the crisis. Vietnamese cassava farmers experienced small negative effects from COVID-19 because of support from processing facilities located within the country.

PUBLICATIONS

- Andrade-Piedra JL; Almekinders CJM; McEwan MA; Kilwinger FBM; Mayanja S; Delaquis E; Garrett KA; Omondi AB; Rajendran S; Kumar PL; Thiele G. 2020. User guide to the toolbox for working with root, tuber and banana seed systems. RTB User guide (RTB User Guide No. 2020-1). CGIAR Research Program on Roots, Tubers and Bananas (RTB), Lima, Peru. Doi.org/10.4160/9789290605577
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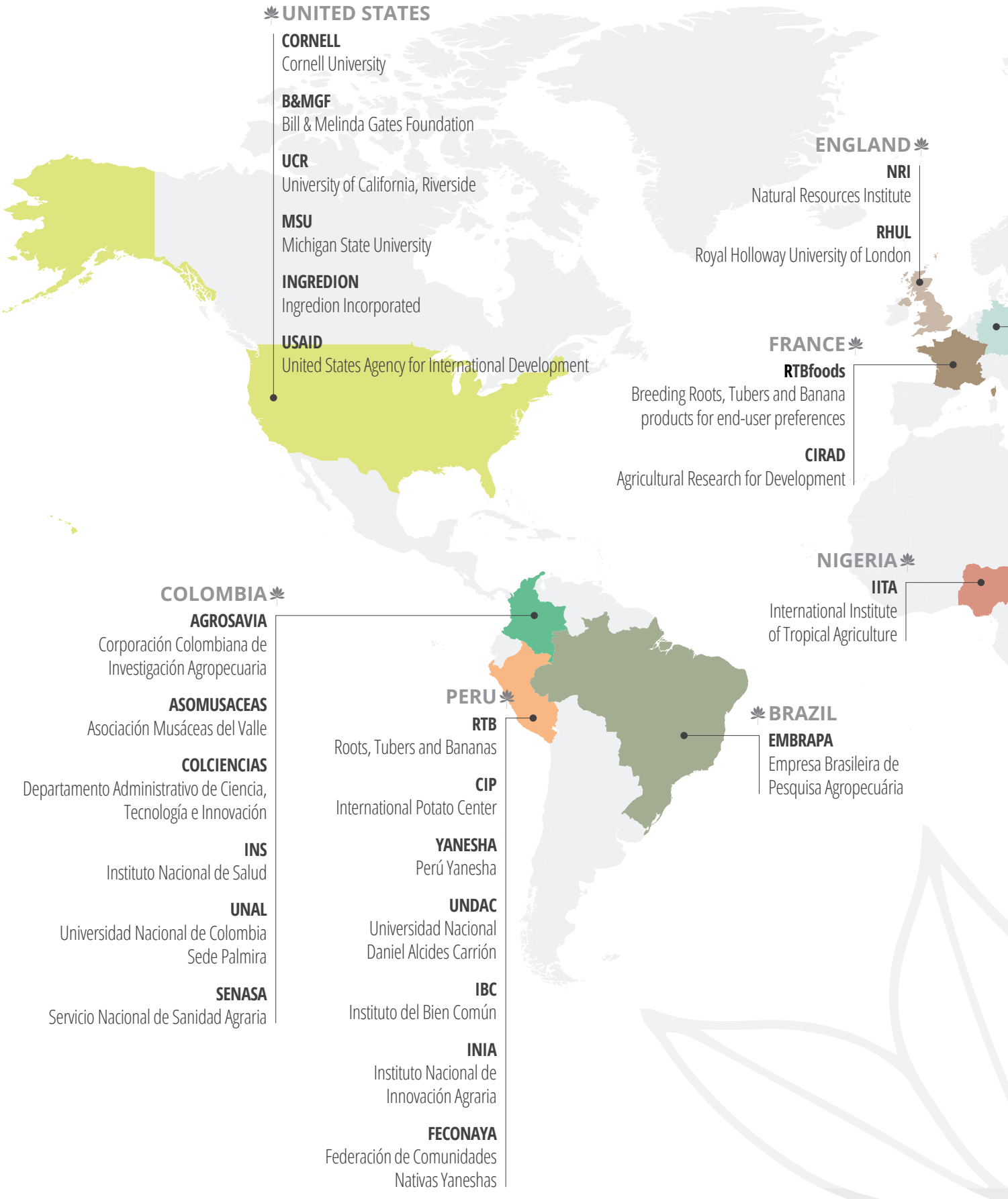
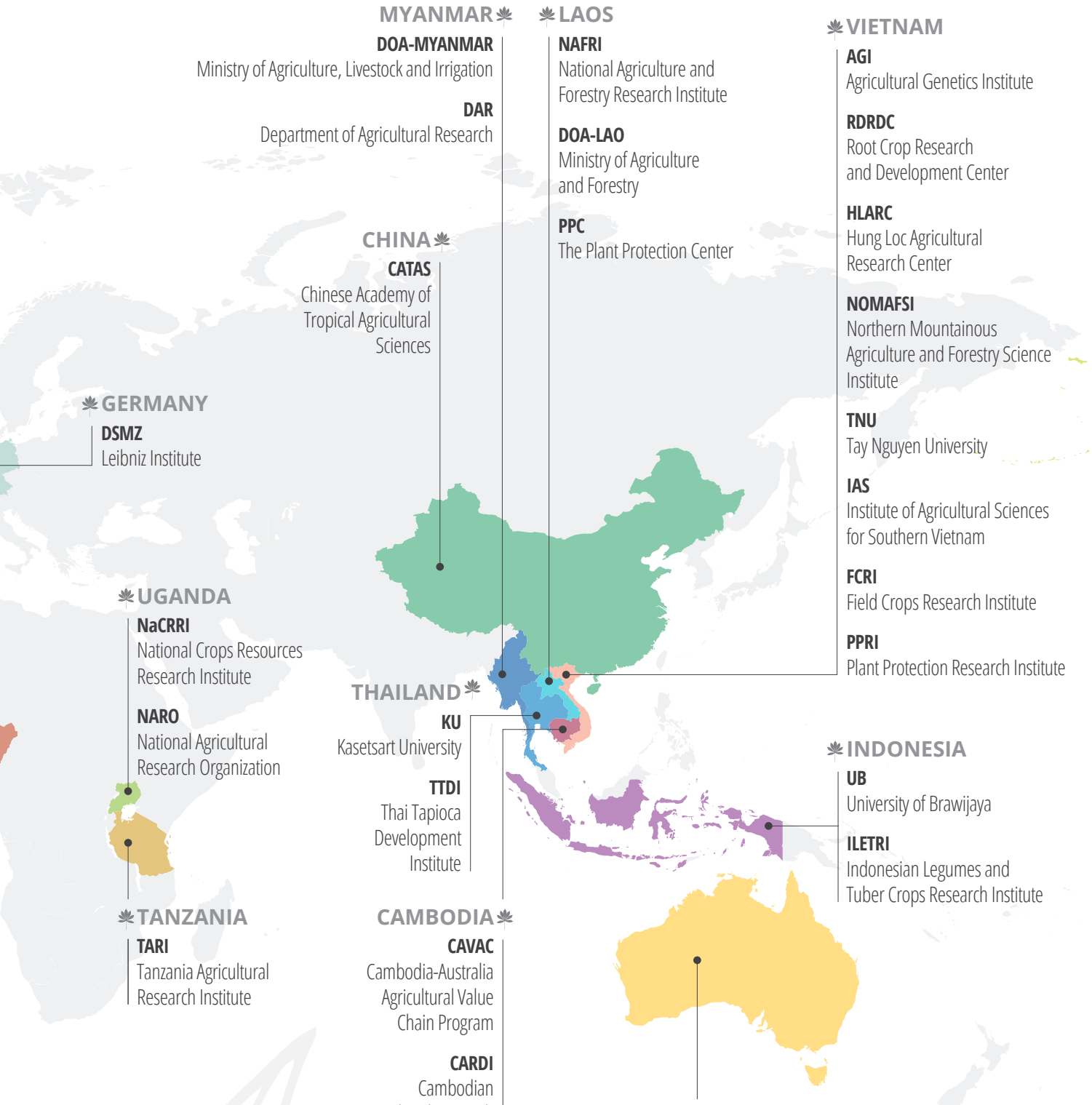
Partners: ACIAR, UQ, AGI, IAS, FCRI, NOMAFSI, TNU, MSU, KU, DOA, NAFRI, DOA-LAO, PPC, CARDI, GDA, ILETRI, UB, DOA-Myanmar, DAR, UNAL, ASOMUSACEAS, IIRR for the COVID-19 survey in Cambodia

Increased commercialization and globalization of cassava value chains in SEA have important social implications, including on local norms, gender relations and opportunities for women and men to participate in and benefit from globalized value chains. To promote shared benefits and gender equality, it is critical to identify constraints and opportunities for women and men in the globalized cassava value chains, specifically around access to inputs. This is the focus of a new 18-month project with CIP, co-funded by CRP-PIM and CRP-RTB, which started in 2020. Most data has been collected and is currently being analyzed, and the findings will be published in 2021.

With the aim of helping build the capacities needed to develop gender-inclusive value chains and interventions in value chains, the team contributed to the development of several publications on gender-responsive pest and disease management, which offer guidance in making technological innovation tools more relevant for diverse actors. The publications included a guide, a perspective article and a blog on gender issues in pest and disease management with case studies from cassava, other roots and tubers as well as bananas. In addition, guidelines were developed for mainstreaming gender in the use of willingness-to-pay (e.g., for planting material of different qualities) tools. Gender and social inclusion issues were also mainstreamed in a user guide to experimental auctions of vegetatively propagated seed.

Global Cassava Partners acronyms





MYANMAR

DOA-MYANMAR
Ministry of Agriculture, Livestock and Irrigation

DAR
Department of Agricultural Research

LAOS

NAFRI
National Agriculture and Forestry Research Institute

DOA-LAO
Ministry of Agriculture and Forestry

PPC
The Plant Protection Center

VIETNAM

AGI
Agricultural Genetics Institute

RDRDC
Root Crop Research and Development Center

HLARC
Hung Loc Agricultural Research Center

NOMAFSI
Northern Mountainous Agriculture and Forestry Science Institute

TNU
Tay Nguyen University

IAS
Institute of Agricultural Sciences for Southern Vietnam

FCRI
Field Crops Research Institute

PPRI
Plant Protection Research Institute

CHINA

CATAS
Chinese Academy of Tropical Agricultural Sciences

GERMANY

DSMZ
Leibniz Institute

UGANDA

NaCRRI
National Crops Resources Research Institute

NARO
National Agricultural Research Organization

THAILAND

KU
Kasetsart University

TTDI
Thai Tapioca Development Institute

INDONESIA

UB
University of Brawijaya

ILETRI
Indonesian Legumes and Tuber Crops Research Institute

TANZANIA

TARI
Tanzania Agricultural Research Institute

CAMBODIA

CAVAC
Cambodia-Australia Agricultural Value Chain Program

CARDI
Cambodian Agricultural Research and Development Institute

GDA
General Directorate of Agriculture

AUSTRALIA

ACIAR
Australian Center for International Agricultural Research

UWA
The University of Western Australia

USQ
University of Queensland

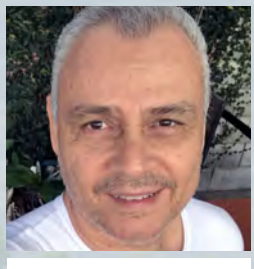
Contributing scientists

Luis Augusto Becerra
Cassava Program Leader

Xiaofei Zhang
Cassava Breeder

Thierry Tran
Head Cassava Processing Lab

Jonathan Newby
Cassava Program Coordinator Asia



Paul Chavarriaga
Head Genetic Transformation Lab

Adriana Bohórquez
Research Associate

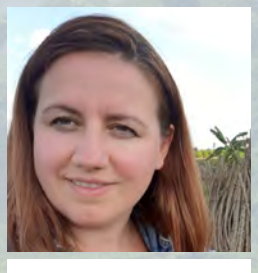
Wilmer Cuéllar
Head Virology Lab

Roosevelt Escobar
Research Associate

María Isabel Gómez
Research Associate

Katherine Castillo
Research Analyst

Tatiana Ovalle
Research Analyst



Vanya Slavchevska
Gender Specialist

Imran Malik
Cassava Production Systems Specialist

Juan Manuel Pardo
Research Associate

Ana María Leiva
Research Associate

Alliance

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Americas Hub

Km 17 Recta Cali-Palmira CP 763537
Apartado Aéreo 6713
Cali, Colombia

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