Genetic improvement of cooking bananas for high levels of pro-vitamin A

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Biosafety Forum: June 21st -23rd, 2022

INTRODUCTION

- Vitamin A Deficiency (VAD) is a global public health threat
- Vitamin A deficiency puts 140 million children at risk of illness and death – UNICEF
- VAD impairs proper immune function and increases risk of mortality

VAD intervention strategies

-Supplementation

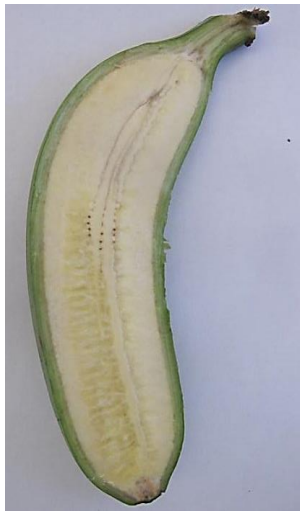
-Food fortification

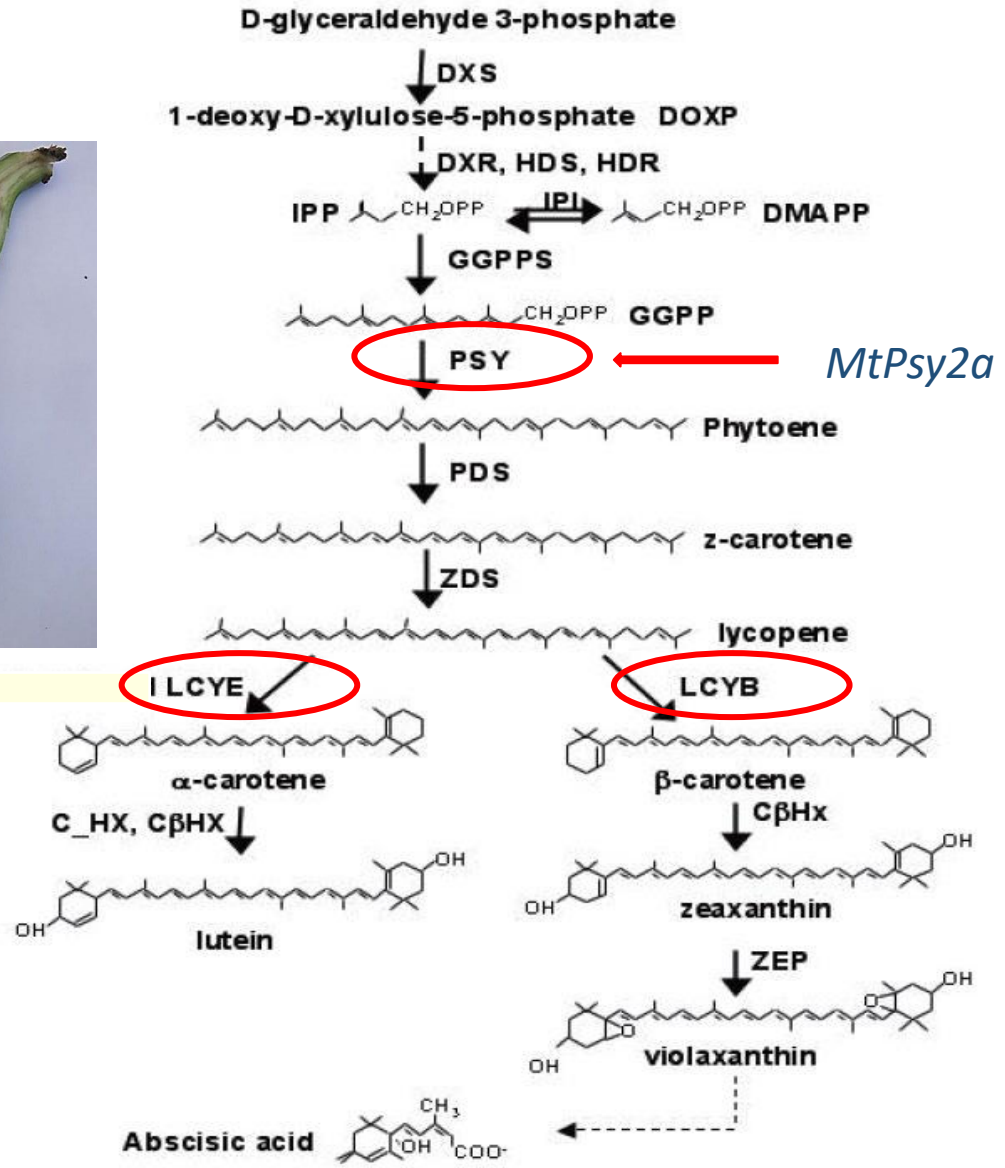
-Biofortification

Project goal

- The aim of this program is to alleviate vitamin A deficiency (VAD) in Uganda through biofortification of a major staple food crop, banana

Project strategy





Asupina: a naturally high pVA banana from Papua New Guinea

Promoters:

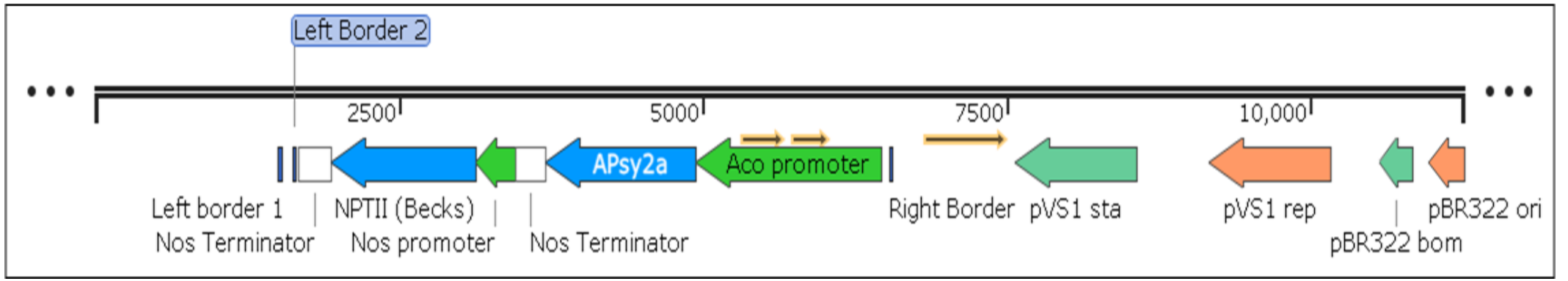
Aminocyclopropane-1-carboxylate oxidase (ACO): Banana Fruit preferred

Maize Ubiquitin (Ubi) : Constitutive

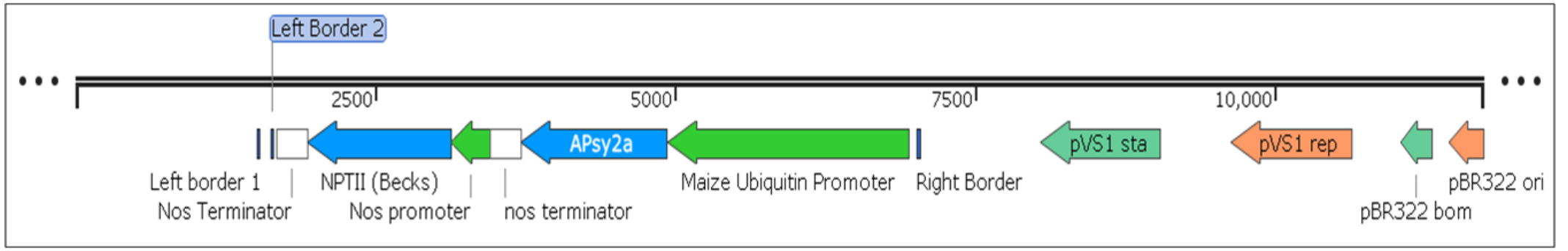
1. ACO-*MtPsy2a*
2. Ubi-*MtPsy2a*

Constructs used in product development

ACO-*MtPsy2a*



Ubi-*MtPsy2a*



Key milestones achieved

- Transformable and regenerable embryogenic cell suspensions established for both hybrid M9 and Nakitembe
- Efficient transformation protocols developed
- Transformants generated from both cultivars with both constructs
- Early event selection trial completed at NARL, Kawanda





	M9 CONTROL	M9 TRANSGENIC	NAKITEMBE CONTROL	NAKITEMBE TRANSGENIC
				
AVERAGE β-CAROTENE EQUIVALENTS	5 µg	22 µg	12 µg	35 µg

Stage 1: Early event selection

- **Selection based on:**
  - Fruit beta-carotene equivalent (β-CE) concentration
    - 20 µg/g (dry weight) or above
  - Minimum bunch weight of 20% of the respective control
    - Hybrid M9 - 16 kg
    - Nakitembe - 10 kg

Selection	Hybrid M9	Nakitembe
Total number of lines in the field	**356**	**162**
Number of lines harvested (at least one crop)	**314**	**121**
Number of lines analysed by HPLC (at least one crop)	**310**	**99**
Number of lines meeting PVA target (at least one crop)	**234**	**85**
Number of lines meeting yield target (in at least one crop)	**153**	**57**
Total No. of lines meeting both β-CE and yield targets (at least one crop)	**61**	**52**

Stage 2: Advanced molecular event selection

- **Detailed molecular and chemical analysis of the lines selected from Stage 1**
  - Number of T-DNA insertions (copy number)
  - Plasmid backbone integration into host genome
  - Identification of insertion sites: Illumina whole genome sequencing
  - Fruit compositional analysis

Elite M9 events selected for ML-CFTs

No.	Plant ID	Vector	Copy #	Plant crop		Ratoon crop 1		Ratoon crop 2	
				Bunch (kg)	β-CE (µg/g dw)	Bunch (kg)	β-CE (µg/g dw)	Bunch (kg)	β-CE (µg/g dw)
1	M9-12083	P	2	23	31.3	14	25.4	7.5	26.2
2	M9-12104	P	1	17	21.8	2	34.9	22	12.8
3	M9-12113	M	1	21	25.6	10	20.7	8	6
4	M9-12152	P	1	15.5	23.5	7.5	27.8	14.5	24.1
5	M9-12141	P	2	15	31.4	10	24.5	8	17.2

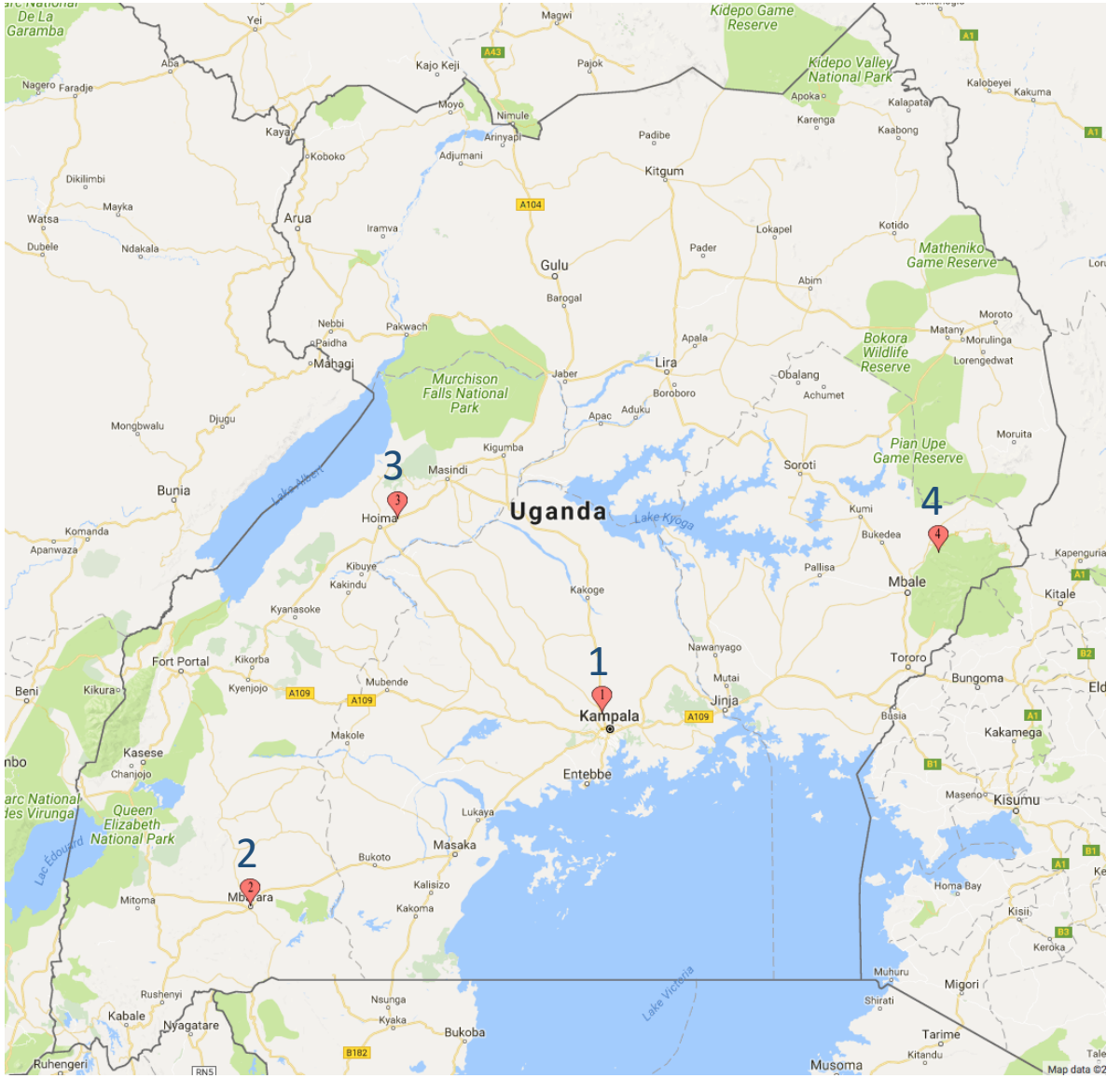
Elite Nakitembe events selected for ML-CFTs

No.	Plant ID	Vector	Copy #	Plant crop		Ratoon crop 1		Ratoon crop 2	
				Bunch (kg)	β-CE (µg/g dw)	Bunch (kg)	β-CE (µg/g dw)	Bunch (kg)	β-CE (µg/g dw)
1	NKT-12418	P	1	21	18.6	22	41.5	40	34.9
2	NKT-12431	P	1	15	19.7	16	57.9	28.5	44.7
3	NKT-12468	P	1	14	39.9	28	45.9		
4	NKT-12477	M	1	16	41	25	34.4		
5	NKT-12478	M	1	19	62.6	23.5	49.7		

Multi-location confined field trails

Approved ML-CFTs sites:

- 4 locations:
  - Kawanda (1)
  - Mbarara (2)
  - Bulindi (3)
  - Buginyanya (4)



Regulatory field trial design

- 4 locations: Kawanda (1), Mbarara (2), Bulindi (3) and Buginyanya (4)
- 4 x 5 randomized block design

Cultivar	Number of Lines	Number of copies	Total per site	Total for 4 sites
M9 Transgenics	5	20	100	400
M9 TC controls	1	20	20	80
M9 Cell Line Control	1	20	20	80
Sub-total (M9)			**140**	**560**
NKT Transgenics	7	20	140	560
NKT TC controls	1	20	20	80
NKT Cell Line Control	1	20	20	80
Sub-total (NKT)			**180**	**720**
Grand total (M9 and NKT)			**320**	**1280**

- Planted M9 in October 2019, and Nakitembe in February 2020

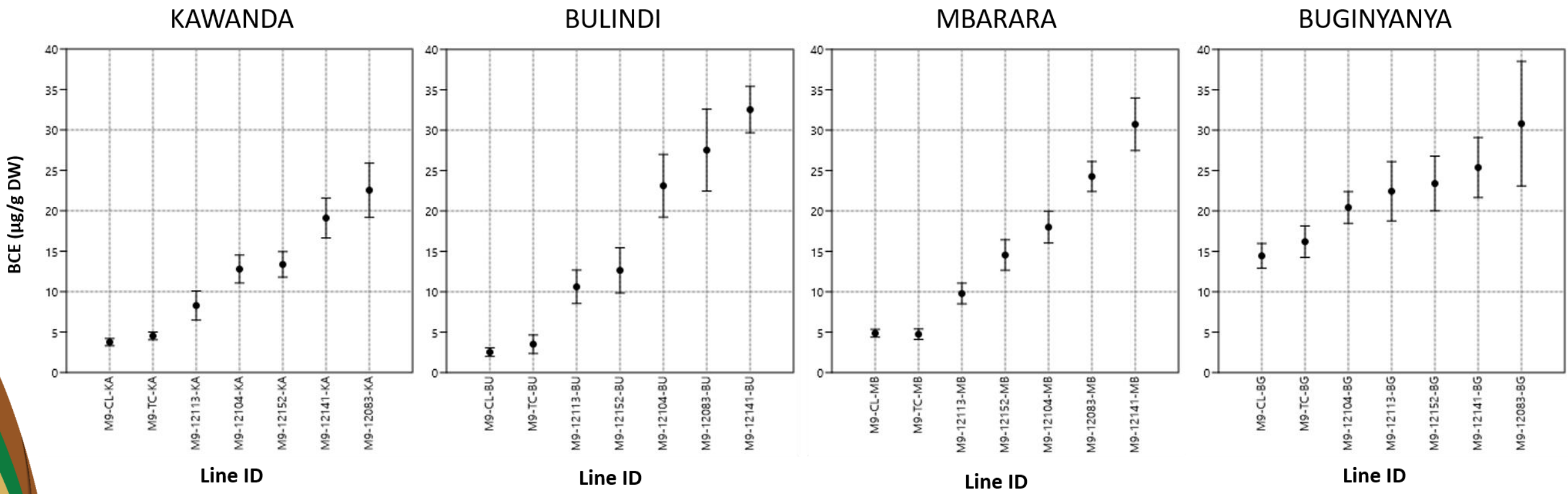
Regulatory field trials – Data collection

Data being collected for the deregulation dossier

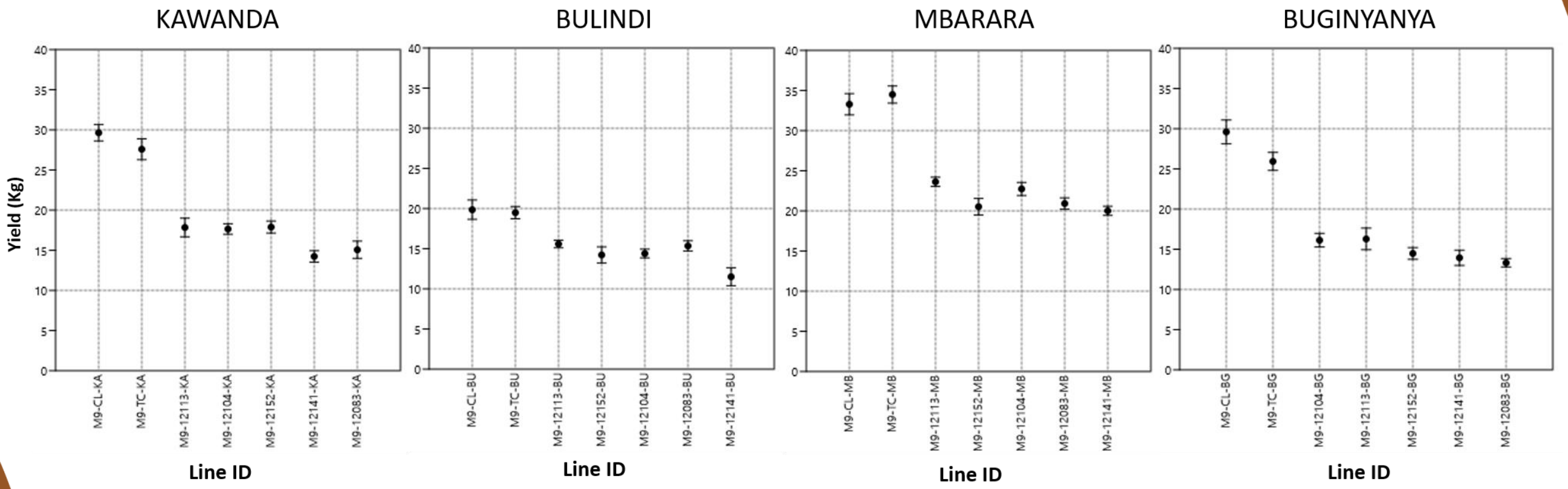
The following data for plant crop and ratoon 1 are being collected

- Agronomic data
- PVA levels in FG green fruits
- Fruit compositional analysis
- Transgene mRNA expression
- ELISA to measure MtPsy2a and NPTII proteins in green, ripe and cooked fruits

Mean BCE levels across sites – M9, PC



Mean yield levels across sites – M9, PC



Other ongoing/accomplished activities

- DUS data collection by MAAIF
- Communication - media and community engagement
- Environmental impact assessment

Community engagement

Dissemination

REGIONAL NEWS

rnal deaths
ck of ambulances

over an ambulance to Buseruka LC3 chairperson Rujumba Wakabi (second-left). Dr Joseph Ruyonga ise in Hoima recently. Photo by Lawrence Mulindwa

seruka Health Centre

referral hospital," he said.

Ambulances key

GMOs not cancerous, say experts

MBARARA

By Abdulkarim Ssengendo

16 | Orumuri November /Musenene 25 - 01 December/Muzimbeezi , 2019 Amakuru

Okurya kubi omu baana

Ebitookye ebirimu ekiriisa kya Vitamin A kurwanisa endwara ezirikureetwa okurya kubi

FRED TURYAKIRA

Bwanyima y'endwara ezirikureetwa okurya kubi kweyongyera kuta omu kabi amagara g'abaana hamwe n'abakazi b'enda omu ihanga hamwe n'omu nsi yoona, ekitongore kya Mbarara Zonal Agriculture and Research Development Institute (MBAZARDI) hamwe na NARO, bakozire ebika by'emitumba ebirimu ekiriisa kya Vitamin A ei barimu nibagyeyesa ahari MBAZARDI, Ishanyu, Kakiika, Mbarara Munisiparite.

Dr.Jimmy Tindamanyire omucondooza kuruga NARO, Kawanda, agizire ngu okucondooza okukozirwe aha mitumba erikumanywa nka banana 21, neeza kuhwera aha kurwanisa endwara ezirikureetwa okurya kubi.

Puroguraamu y'okukora emitumba n'ebitookye ebirimu Vitamin A erikumanywa nka Multi-location confined field trials of bananas with enhanced levels of pro-vitamin A neeshagikwa Bill& Melinda Gates Foundation.

Wiiki ehweire, aba NARO na MBAZARDI barikubugana nibeegyesa abahingisa, abeebembezi hamwe n'abahingi okuruga Kakiika oku ebitookye ebi bibarahwere aha kurwanisa endwara z'okurya kubi.

Tindamanyire agizire, ngu endwara ezirikureetwa okurya kubi, nizo zikizireyo okwita abaana abari abansi y'emyaka etaano.

"Kimwe kya kabiri ky'abaana b'abansi y'emyaka 5 abarikufa omunsi yoona, nikireetwa endwara ezirikuga omu kurya kubi. N'ahabw'ekyo twine okutaho oburyo bw'okucondooza tukamanya oku abantu baine kuhinga eby'okurya ebiine ekiriisa, okukira munonga omu kyanga kya Burengyerwa-izooba ei ebitookye nibyo birikuingayo okwejunisibwa omu maka," Nikwe ashobooroire.

Endwara ezirikureetwa okurya kubi harimu okushinya/ okuguna, nari okuzingama omu magara g'omwana ekirikuteganisa okukura n'amagara marungi, okuteganisa obwonko ekikurigiiriramu yaakyerwa omu kushoma.

Minisita w'eby'esente, embariiriira n'entunguuka y'eihanga Matiya Kasaija, obu yasbaire nashoma bagyeti ya 2019/2020, akooreka ngu omu Uganda abaana obuweka 53 ahari 100 (53%) abari ahansi y'emyaka 5 nibo barikuteganisibwa endwara ezirikureetwa okurya kubi, n'obu Uganda eine ebyokurya bingi.

Eky'obusaasi, akongyera yaayoreka ngu obuweka 32 ahari 100 bw'abakazi abakirikuzaara, nibo barikuteganisibwa endwara ezirikureetwa okurya kubi.

Kurugiirira aha kucondooza okwakoziirwe 2016, aba Uganda Demographic and Health Surwey neeyoreka ngu abaana abarikuteganisibwa endwara ezirikuruga omu kurya kubi, n'abu banyina bo batashomireho n'akakye, nibahika obucweka 37 ahari 100 nibo bashinyiire wagyeragyeranisa omwana 1 ahari 10 (10%) abu ba nyinabo abaine obwegyese bw'aha rurengo rwa siniya.

Tindamanyire ayoorekire ngu abaana abarikukira kuteganisibwa endwara ezo, n'abu abazaire baabo barikutura omu byaro bakabura okutunga emibazi, n'inga ebiriisa bya Vitamin omu magara gaabo.

Ebitookye ebi byahamibwe nibiija okuhwera kihango aha kucendeeza ekizibu eki, kandi tibirikuteganisibwa oburwaire nibigumira n'embeera y'omushana.

Dr Halid Kirunda, Dayirekita wa MBAZARDI, agizire ngu abahingi barekye kutiina aha bihingwa ebirikuba byongyeirwemu ebiriisa, tibiine akabi aha magara gaabo hamwe n'ebindi bihingwa, ahabw'okuba nibiba bicondooziibwe kurungi.

"Abamwe nibagira ngu ebihingwa ebirikuba byongyeirwemu ebiriisa n'eby'akabi aha magara g'abantu, ebyo tibihikire ahabw'okuba nituba tubicondoorizibo kurungi, n'ahabw'ekyo abantu baine okubikunda, ahabw'okuba n'eby'omugasho aha magara, "Dr. Kirunda ahabwire.

Mukore ebitookye ebinuzire

Dorcas Twinsabaitu: Ndi ow'eby'amagara, nimkunda saiyansi ahabw'okuba ebintu nibiba bicondoozibweho kwonka ekizibu kiri ahari bimwe ebyakoziirwe nka Bogoya noogireeba omu maisho oreeba omuinwa gweyo gubonaire, kwonka waagiriye tenuzire. Ninshaba omu kucondooza kwabo baitemu ekintu ekirikubaasa kusikiriza abantu okukunda ekihingwa ekyo.

Samson Isingoma: Ndi omuhingi w'orufookye, emitumba bakagituheereeza neeteera ekifookye kihango, kwonka eine ekizibu ky'okureetuka kuhirikwa omujaga, omutumba nigwefenga enyomyo nying. zitwara omwanya muhango. Ninshaba baaza kiikore emitumba nk'egiyo, baareebe aha bizioli ebi erikuba eine ebitaraiteganise abahingi okwenda ngu bongyere okubikunda n'okubihinga.

Murekye kutega amaisho Abakurisitaayo-Bugingo

Bishopu Sheldon n'abandi bahurikiriize Col. Bugingo (buryo)

AMON NATWETA

Col. Dick Bugingo owa IGDI Teaimu kiiruga Kyakabunga, Nyakashashara, Kiruhuura, aheebwire abeebembezi b'edini ababaire barimu ba Bishopi, hamwe n'abo abahuumwire n'abakazi baabo okiiruga omu kyanga kya Burengyerwa-izooba, kureka okuhsinga abakurisitaayo amaisho, kureka bakorese amafaika g'ekanisa okwihamu enfaasiya.

"Murekye kuhanga amaisho abakurisitaayo omu bikwatereine n'entunguuka y'ekanisa. Ekanisa Zine amafaika mugakoreraho eminimo y'obuhingi n'oburiisa okubeheere2e entaasiya," nikwe abahaizwire.

Abamjadidini abo baboire bataiyasyiire faaimu ye okushoma aha kuhinga okw'omuiindo wiiki ehweire.

Ayongiyeire yaabahebura okiifa amaani omu kwegyesa abakazi hamwe n'eminyeeto okwejumbira omu by'omu by'obuhingi n'oburiisa, baaba nibende kugiimya abantu omii kenisa. "Mwine kwegyesa abakazi n'eminjeeto, kiiari ekyo nibaZe kubaZorokaiho. Edimi etarikubaasa kuhindura eb!eekvaleeko by'abakurakisi bwayo, egyo neeba etari ya Ruhanga.

Bishopu Nathan Ahimbisibwe owa South Ankole, agizire ngu obireeberezi bwa South Ankole baatandikireho faaimu, beiiu neaza kwongyera amaani omu kubiiuhfira engiri y'okurwanisa obwono omu minyeeto n'abakaZi, kurabira omu buhingi n'obuniisa.

Dr. George Tibesigwa, Bishopu wa Ankole owaahiiumwire, agizire ngu kibaire kirungi okuteebakanisa omushomo gw'okwegyesa abeebembezi b'edini ebirwatiraine n'okwega okwebeisaho omu by'entunguka.

"Twine amafaika maingi garikufa butha, n'ahaiw'eiryo twagakoresa kurungi nigaza kuiuhwera kureetaho entaasiya y'ekanisa, haZa kihwere kukyendeeza omugiigu ogu ekanisa erikita aha bakiiriifaayo kiirabira omu kuha ebicwero," nikwe agizire.

Abanyadidini abandi abahikire aha mishomo barimu Dr. Sheldon Mwesigwa, Bishopu w'Ankole dayosiisi, Bishopu George Bagamuhiinda owa Kigezi n'abandi baingi.

Amashomero gahabwirwe kuta amaani omu buhingi

Francis Turyaheebwa Kaganda, Headmaastta wa Rwentaiga High School (ajweire okooti) naarombuza bamwe aha begyenyi orutookye ne'eishomero. Agizire ngu amashomero againe amataka baahemereine kugahinga okwogywa aha ntaaega y'eishomero n'oby'okuryа by'abaana. Buri kwezi nibatema ebitookye 60 ebi barikugaza akakiiko k'eishomero oby'okutsekyera abaana. Twiraho n'ente z'okukamwa, bamwe n'omkoko oby'abeegi barikwegyeraho.

Quality control and assurance mechanisms

- Constant update and backup the BananaTracker and MusaBase, physically and iCloud-based
- Developed and applied line specific PCRs in the greenhouse and field early after planting
- Developed and applied a barcode-based electronic data collection system
- Developed/updated SOPs for all lab and field procedures and processes
- Continuous training of all staff in biotech stewardship, risk assessment and regulation

Acknowledgements

Wilberforce Tushemereirwe

NARL-KAWANDA
BULINDI-ZARDI
BUGINYANYA ZARDI
MBARARA ZARDI

James Dale

National Biosafety Committee

Thank you for listening