

# Biofortified East African Highland Bananas to Alleviate Vitamin A Deficiency in Uganda

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## BACKGROUND

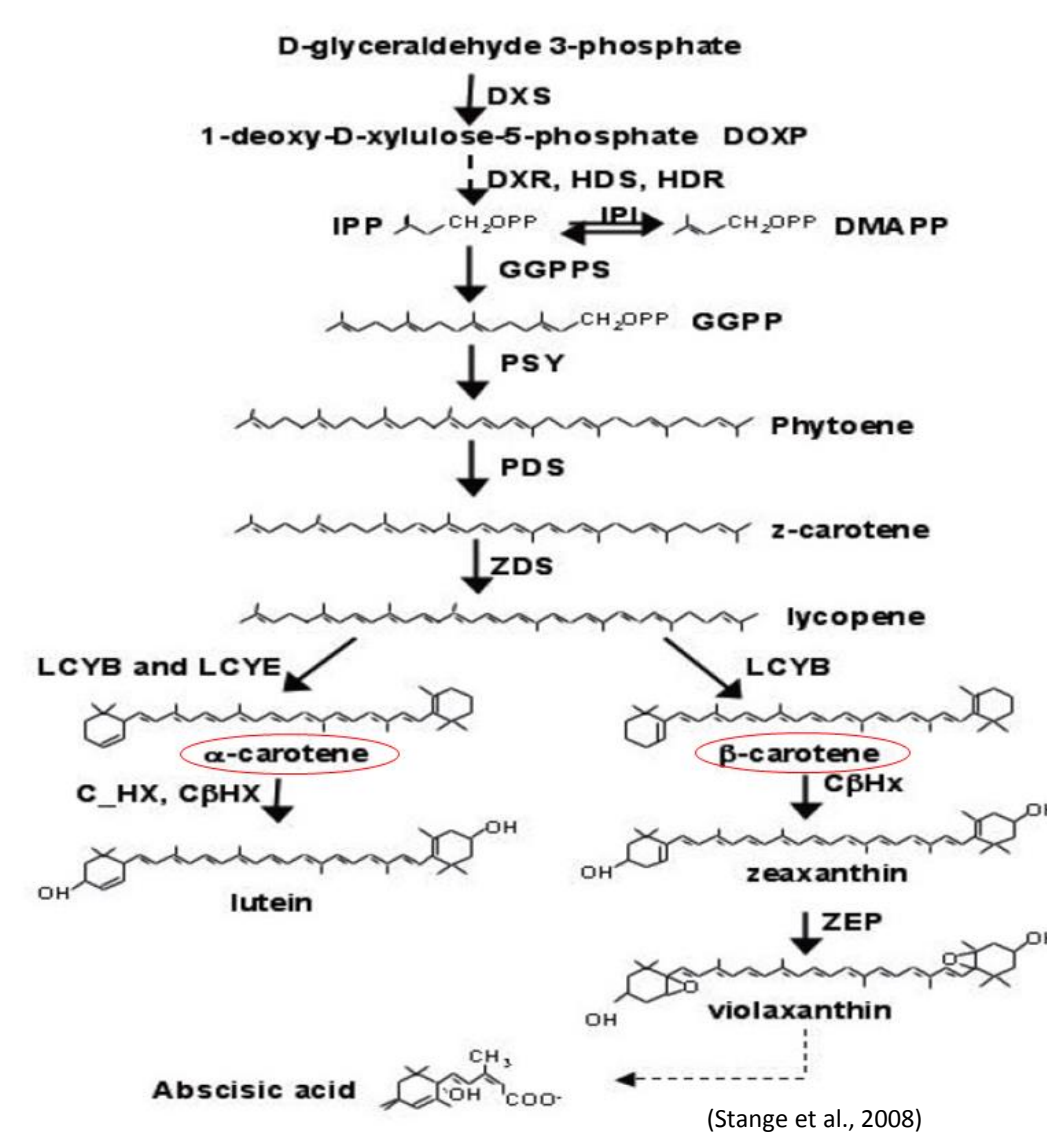
Micronutrient deficiency, also referred to as *hidden hunger*, is a global public health problem that affects over two billion people (Tulchinsky, 2010).

In Uganda, micronutrient deficiency is the third most important public health problem after HIV/AIDS and malaria.

Vitamin A deficiency (VAD) and Iron Deficiency Anaemia (IDA) are the major causes of anaemia in Uganda. VAD affects 20-35% of children below five years of age and the same proportion of women of child-bearing age.

The aim of the Banana 21 project is to alleviate vitamin A deficiency through biofortification of Uganda's major staple food, banana.

### Pathway for pro-vitamin A biosynthesis



## METHODS

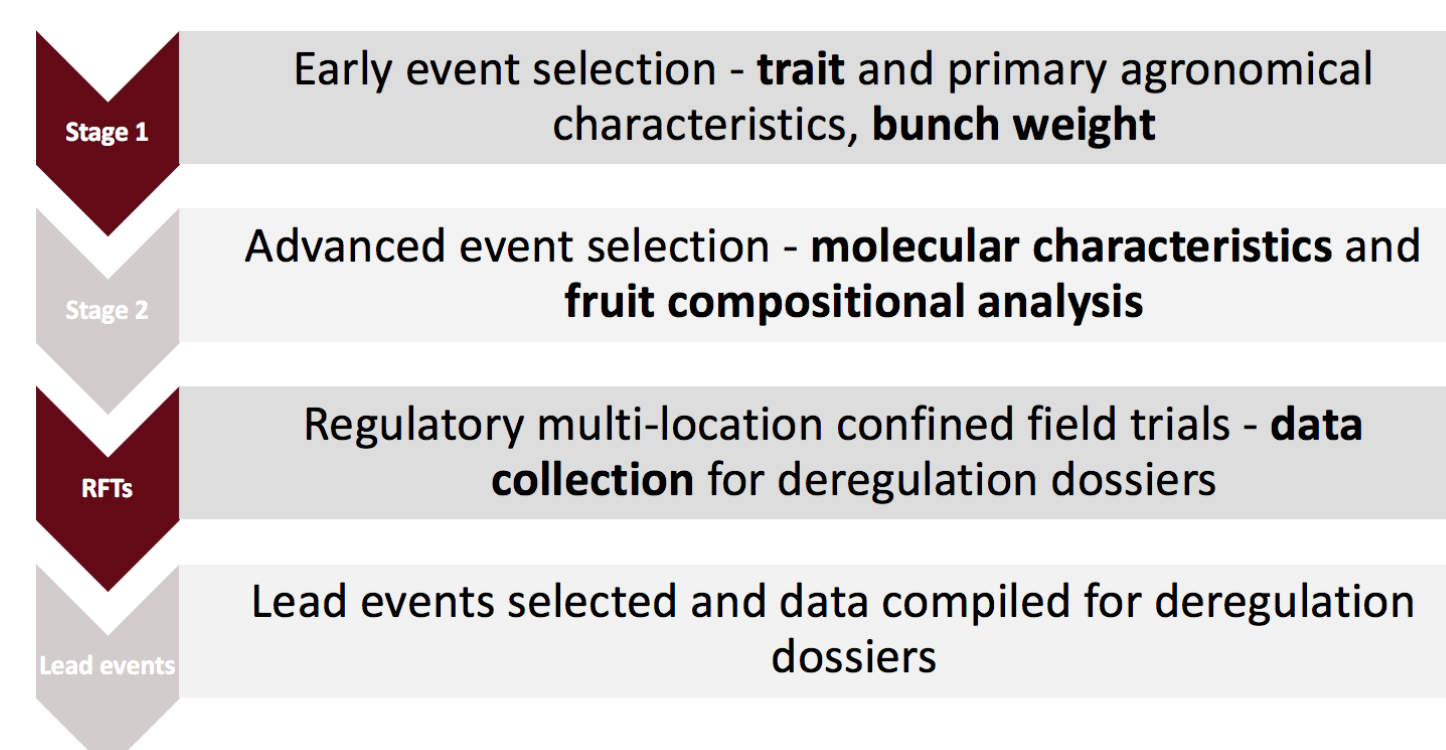
### Strategy for pro-vitamin A enhancement

Genetic modification of two popular local banana cultivars using *Agrobacterium*-mediated transformation

- Hybrid M9 - Tolerant to major biotic and abiotic stresses and suited for lowland areas where disease pressure is high.
- Nakitembe - Great culinary characteristics and suitable for highland areas where banana production is higher.

A PSY gene isolated from a Fe'i banana cultivar, Asupina (*MtPSY2a*) was used under the control of either a fruit preferred (ACO) and a constitutive (Ubi) promoter.

### Selection criteria



# Bananas to Alleviate Vitamin A Deficiency in Uganda

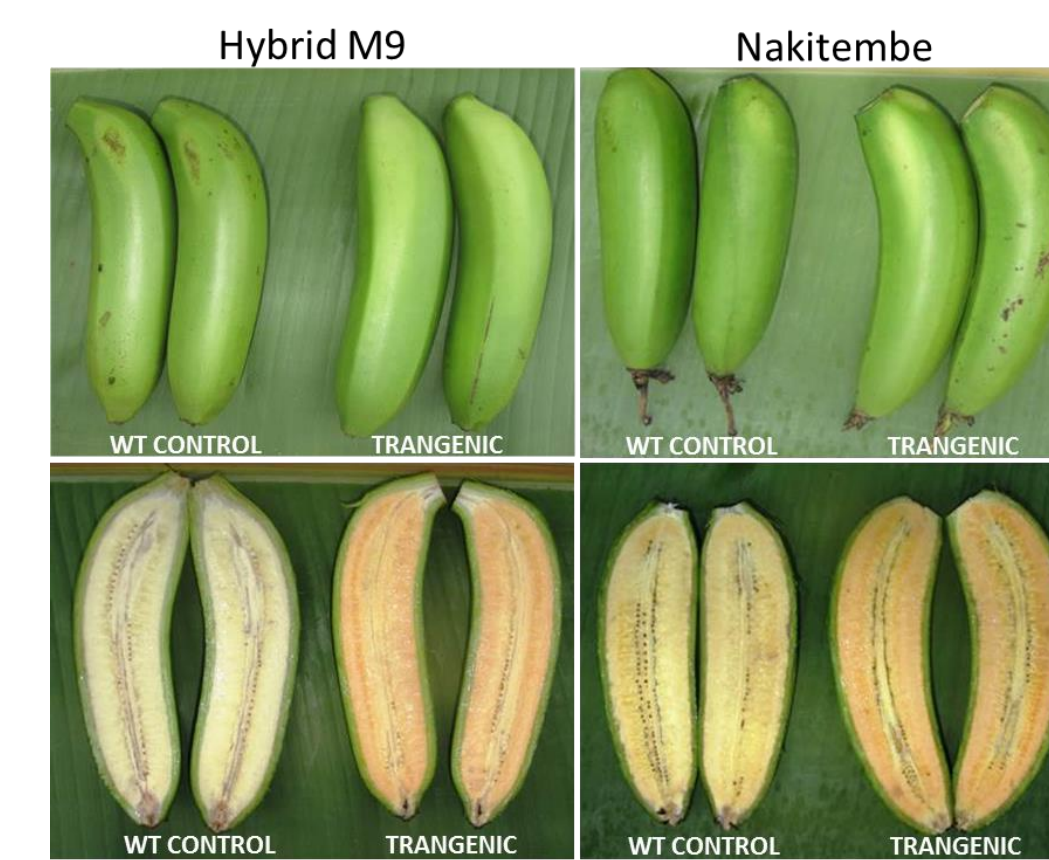
## RESULTS

### Generation of events for selection

Hybrid M9 was easier to transform than Nakitembe

Similar phenotype except fruit pulp of transgenics ranged from yellow to orange

Cultivar	Construct		Total
	ACO-MtPsy2a	Ubi-MtPsy2a	
Hybrid M9	153	203	356
Nakitembe	69	93	162
Total	222	296	518



115 lines passed early event selection based on PVA levels and bunch weight

Selection	Hybrid M9	Nakitembe
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
Number of lines meeting $\beta$ -CE and yield targets (at least one crop)	66	49

Lines with 1 to 3 integrated inserts

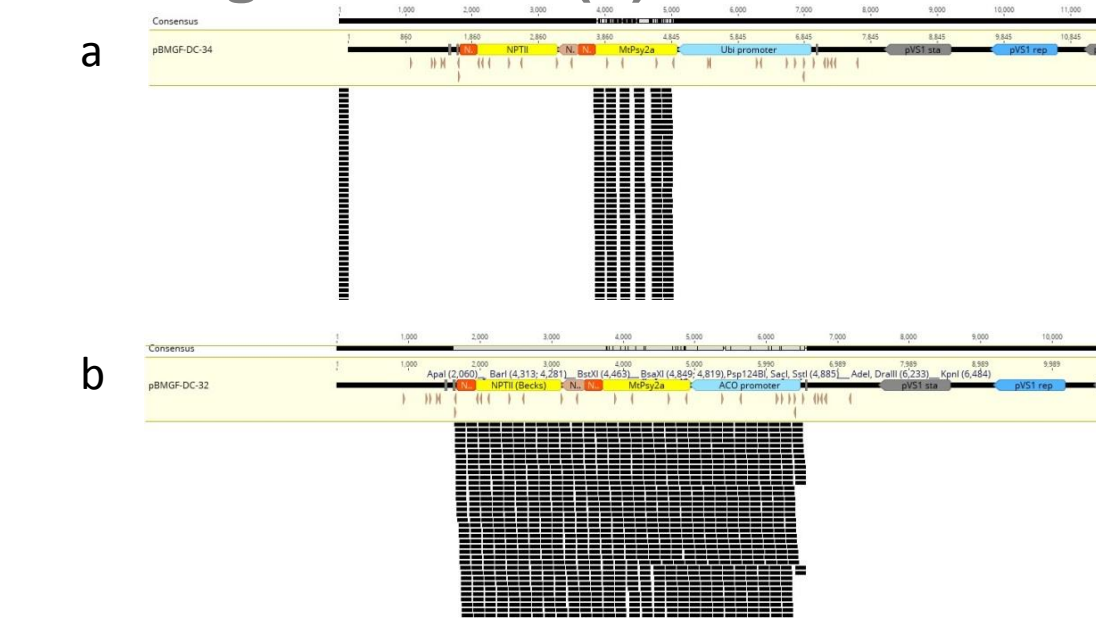
Cultivar	Hybrid M9	Nakitembe
Lines tested	66	49
1 copy	23	26
2 copies	23	15
3 copies	8	4
> 3 copies	12	4

Lines with no vector backbone

Cultivar	Hybrid M9	Nakitembe
Lines tested	55	45
1 copy	11/23	19/26
2 copies	12/23	6/15
3 copies	2/8	1/4
> 3 copies	1/1	0/0

### Advanced molecular characterisation of events

Whole genome Illumina sequencing and mapping against the plasmids revealed endogenous PSY in WT (a) and clean T-DNA insertion in transgenic lines (b)

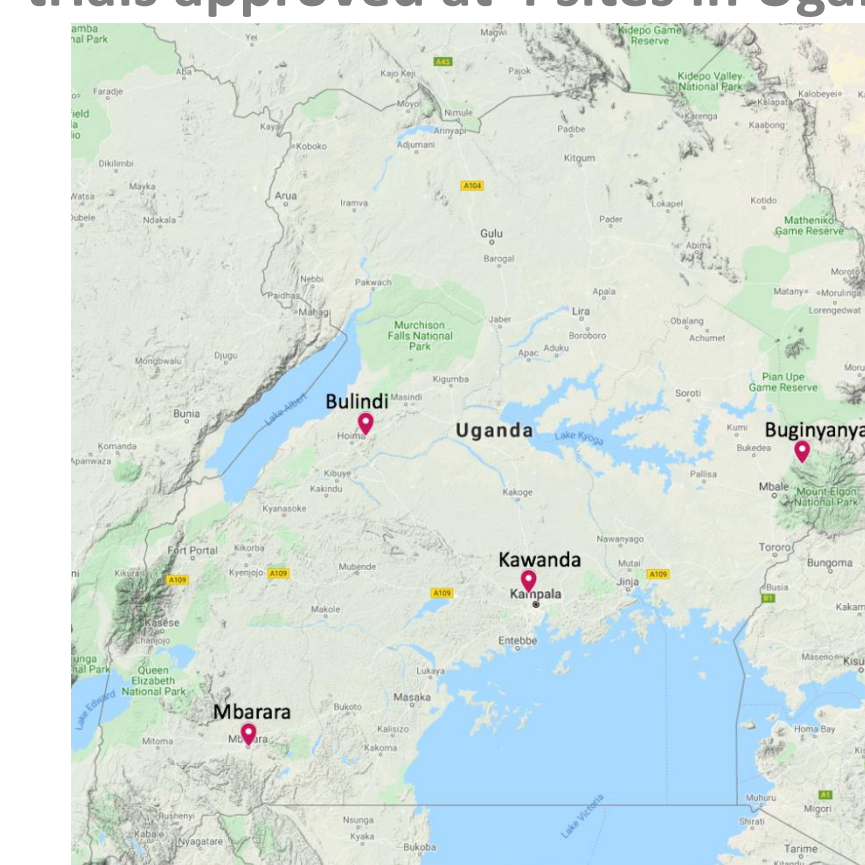


Twelve lines selected for regulatory multi-location confined field trials

Plant ID	Promoter:gene	Copy #	Plant crop		Ratoon 1 crop		Ratoon 2 crop	
			$\beta$ -CE (ug/g dw)	Bunch weight (kg)	$\beta$ -CE (ug/g dw)	Bunch weight (kg)	$\beta$ -CE (ug/g dw)	Bunch weight (kg)
M9-12083	Ubi-MtPsy2a	2	31.3	23.0	25.4	14.0	26.2	7.5
M9-12104	Ubi-MtPsy2a	1	21.8	17.0	34.9	2.0	12.8	22.0
M9-12113	ACO-MtPsy2a	1	25.6	21.0	20.7	10.0	6.0	8.0
M9-12141	Ubi-MtPsy2a	2	31.4	15.0	24.5	10.0	17.2	8.0
M9-12152	Ubi-MtPsy2a	1	23.5	15.5	27.8	7.5	24.1	14.5
NKT-12357	Ubi-MtPsy2a	1	18.8	13.0	29.2	37.0	27.0	57.0
NKT-12418	Ubi-MtPsy2a	1	18.6	21.0	41.5	22.0	34.9	40.0
NKT-12431	Ubi-MtPsy2a	1	19.7	15.0	57.9	16.0	44.7	28.5
NKT-12461	Ubi-MtPsy2a	1	74.9	20.0	44.3	24.5	12.06	16.5
NKT-12468	Ubi-MtPsy2a	1	39.9	14.0	45.9	28.0	56.09	6.5
NKT-12477	ACO-MtPsy2a	1	41.0	16.0	34.4	25.0	32.6	25.0
NKT-12478	ACO-MtPsy2a	1	62.6	19.0	49.7	23.6	65.8	31.5

### Roadmap to deregulation and general release by 2022

Regulatory multi-location confined field trials approved at 4 sites in Uganda



Data will be collected for both deregulation and variety release requirements

- Experimental setup**
- 4 x 5 randomized block design
  - 5 M9 and 7 Nakitembe elite lines (12 total) planned
  - 20 plants/line/location (240 transgenic plants)
  - Cell line and non-cell line controls
  - Planting of M9 RFTs started on September 20, 2019

- Data collection**
- Agronomic data
  - PVA levels in green and ripe fruits
  - Fruit compositional analysis
  - Transgenes mRNA expression
  - MtPsy2a and NPTII protein expression in green, ripe and cooked fruit

