Safe introduction and multiplication of cassava mother plants

The introduction of rapid multiplication system for disease-free planting materials production

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Introduction

Crops	Area (ha)	%
1. Rice	**3,552,738**	**66.83**
2. Horticultural Crops	**309,555**	**5.82**
Mango	152,073	2.86
Vegetables	66,433	1.25
Banana	43,593	0.82
Longan	12,837	0.24
Orange	7,497	0.14
Watermelon	6,268	0.12
Duren	5,452	0.10
Jackfruit	4,758	0.09
Pinaple	3,035	0.06
Other (12 crops)	7,609	0.14

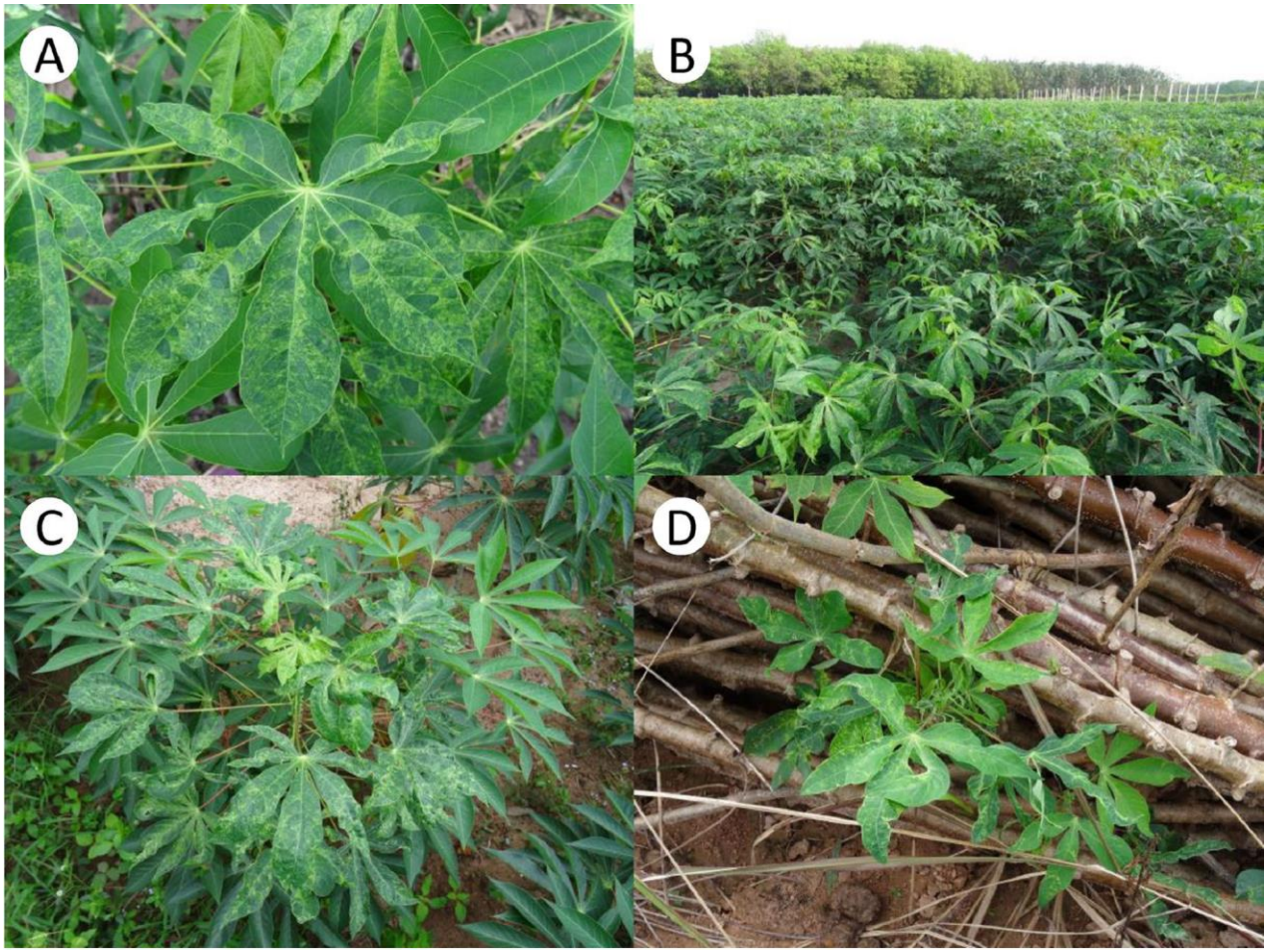
Crops	Area (ha)	%
3. Industrial Crops	**1,453,530**	**27.34**
Cassava	739,430	13.91
Cashewnut	405,991	7.64
Maize	144,207	2.71
Surgacane	46,923	0.88
Coconut	32,117	0.60
Mungbean	25,101	0.47
Soybean	16,218	0.31
Sesame	13,848	0.26
Peanut	8,263	0.16
Other (8 crops)	21,432	0.40
Total Area	**5,315,823**	**100**

Introduction

- In 2020, Cambodia produced 12,680,987 tonnes of cassava with average yield of 20.55 t/ha (MAFF 2021).
- Cassava production in Cambodia is affected by cassava mosaic disease (CMD) (Wang et al. 2016). and cassava witches' broom (Graziosi 2016), present the most serious threats to country production.

CMD was first reported in Cambodia (Ratanakiri) in 2015, with its causal agent subsequently identified as Sri Lankan cassava mosaic virus (SLCMV) (Wang et al. 2016).

Planting resistant varieties has been shown to be the most effective strategy for controlling the disease



Source: Uke et al.,2019

Clean planting material and resistant varieties are ungently NEEDED

Introducing of cassava varieties for CMD resistance

- **AusAID-CAVAC:** Screenhouse establishment for cassava clean seed and rapid multiplication.
- **CIAT:** CMD resistant clones, rapid multiplication in screenhouse and on-field stake multiplication.

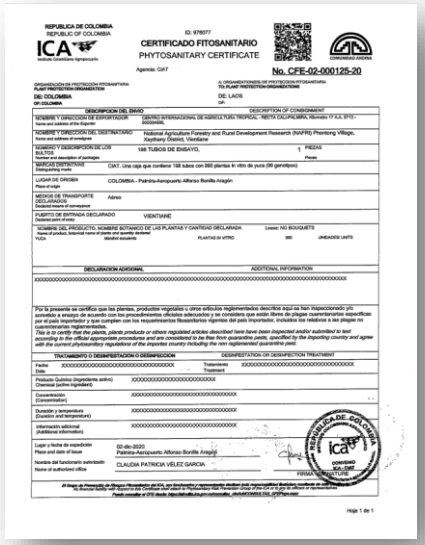
Materials from CIAT & AGI for clean seed multiplication in 2020

Genotype	Designation	No. of plantlets	Genotype	Designation	No. of plantlets
KU50	KU50	500	IITA-3	IITA-TMS-IBA972205	100
IITA-1	TMEB419	100	IITA-4	IITA-TMS-IBA980505	100
IITA-2	IITA-TMS-IBA920057	100	IITA-5	IITA-TMS-IBA980581	100

Materials from CIAT for clean seed multiplication in 2022 (based on previous assessment in Vietnam)

No.	Variety name	Origin	No. of plant (plant/tube)
1	AR35-1	Colombia	50
2	CR13-8	Colombia	50
3	CR27-20	Colombia	50
4	CR24-3	Colombia	50
5	CR24-16	Colombia	50
6	CR52A-4	Colombia	50

Official phytosanitary certificates & import permits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Nation Religion King
MINISTRY OF AGRICULTURE FORESTRY AND FISHERIES
GENERAL DIRECTORATE OF AGRICULTURE
PLANT PROTECTION SANTITARY AND PHYTOSANITARY DEPARTMENT
IMPORT CERTIFICATE FOR PLANT QUARANTINE MATERIAL No.000055/21


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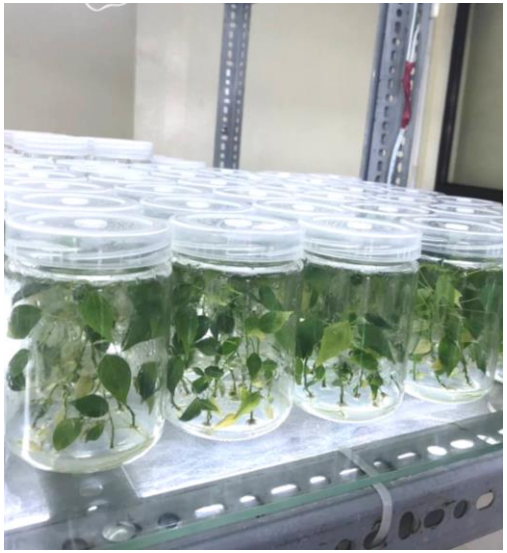
PLANT PROTECTION SANTITARY AND PHYTOSANITARY DEPARTMENT
ATTACHMENT TO PSC NO: 000055/21
LIST OF IMPORTED PLANTING MATERIALS
KER MONTHIVUTH, Ph.D.
Director
Department of Plant Protection
Sanitary and Phytosanitary

Clonal multiplication from In-vitro to field in 2021



Hardening

1 month

8-10 months

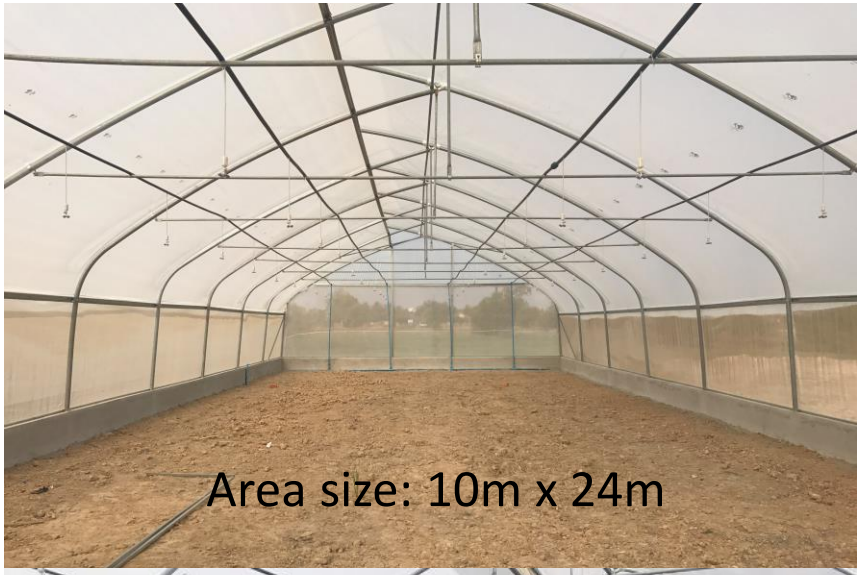
Number of plantlets produced by tissue culture

No.	Variety name	No. of plantlets/plant	
		In-vitro	screenhouse
1	IITA 1	749	128
2	IITA 2	410	186
3	IITA 3	531	349
4	IITA 4	590	40
5	IITA5	115	
6	KU50	480	206
7	AR35-1	1591	575
8	CR13-8	1188	334
9	CR27-20	950	62
10	CR24-3	1006	400
11	CR24-16	767	168
12	CR52A-4	1822	346

Application of rapid multiplication method using greenhouse system

Screenhouse establishment in CARDI

Growing bed construction for rapid multiplication



Rapid multiplication of KU50 disease-free in screenhouse

CMD-free (KU50) from Laos

Planting method: 1-2 node

2 months after planting

Cutting plantlets with 5 to 6 nodes

First harvesting of KU50 free CMD multiplied by rapid multiplication method in CARDI's screenhouse

Prepare the cutting in the plastic tray for incubation.

Incubated in plastic tunnel for 3-5 days

Rapid Multiplication of IITA lines in greenhouse

Number of plantlets produced by rapid multiplication method

No	Variety name	No. of Plantlets			Total
		Cycle 1	Cycle 2	Cycle 3	
1	KU50	2239	534	657	3430
2	IITA1			44	44
3	IITA2		609	204	813
4	IITA3		290	494	784
5	IITA4			177	177
6	IITA5		931	318	1249
Total		2239	2364	1894	6497

A total of 1000 plantlets of KU50 were contribute to GDA (300 plants), farmer in Ratanakiri (200), famer in Kampong Cham (500).

Number of plants on the field

Name of varieties	Planting on 12 August &11 October, 2022		Planting on 26th January, 2023		Planting on 14th June, 2023		Planting on 9th August, 2023		Total number of plants on field
	Number of plantlets								
	Transfer to field	Remain on field	Transfer to field	Remain on field	Transfer to field	Remain on field	Transfer to field	Remain on field	
CR52A-4	96	171	43	40	84	58			269
AR35-1	113	151	42	62	84	71			284
CR24-3	102	118	43	49	84	69			236
CR13-8	84	98	43	32	57	44			174
CR27-20	36	61			42	20			81
CR24-16	69	148	41	41	42	19			208
KU50			42	91	273	198	354	284	573
IITA1	112	197	42	49	43	40			286
IITA2	111	155	117	147	100	54	65	48	404
IITA3	51	90	119	153	84	23	105	67	333
IITA4	49	38	39	53	126	18	118	99	208
IITA5	109	154	81	48	172	9	170	142	353

Evaluation of Starch Content among IITA lines in CARDI

No.	Variety name	Designation	Starch Content (%)
1	IITA1	TMEB419	20.62
2	IITA2	IITA-TMS-IBA920057	16.52
3	IITA3	IITA-TMS-IBA972205	16.34
4	IITA4	IITA-TMS-IBA980505	18.3
5	IITA5	IITA-TMS-IBA980581	20.65
6	KU50		24.49

Note: The low percentage of starch content maybe affected by wet soil because of heavy rain in the rainy season.

Clean material for multi-location evaluation throughout Cambodia

No.	Variety name	No. of plants
1	CR52A-4	130
2	AR35-1	142
3	CR24-3	118
4	CR13-8	87
5	CR27-20	45
6	CR24-16	104
7	KU50	600
8	IITA1	145
9	IITA2	300
10	IITA3	170
11	IITA4	105
12	IITA5	250
Total		2196

Fibercell production for rapid multiplication system

No. of Fibercell production for rapid multiplication

No.	No. of tray	No. of fiber cell	Other
1	1	96	Testing
2	2	192	Testing
3	15	1440	For CIAT
4	40	3840	For CIAT
5	24	2304	For PB
Total	82	7872	

Challenges and lesson learn

In-vitro

Challenge

- Unstable electricity cause damage of the lighting system and air conditioner.
- IITA-1 and CR27-20 are difficult to regenerate.

Lesson learned

- Medium for maintenance in-vitro collection have been modified.
- Proper sterilizing produce high survival rate

Rapid multiplication in tunnel

Challenge

- The misting system does not properly work due to high mineral of underground water, and it affects cassava's growth and needs more labor to take care of.
- High temperature in the greenhouse gave a low rate of survival of cassava plantlets

Rapid multiplication in tunnel

Lesson learned

- The hardening period should be taken more than one month before planting to get high survival rate and weed competitiveness in the field.
- Incubate the cutting for 3-5 days in a small plastic tunnel produce high survival rate in compare to open space condition.

In field

Challenge

- Limitation of land for on-field multiplication
- Labor shortage in weed control.
- Low soil fertility

Lesson learned

- Drip irrigation and fertigation system must be applied for in-field multiplication
- In rainy season, heavy rain could affect on survival rate of Cassava crop in compare to dry season.
- Soil structure affects on Cassava growth.

Soil test analysis

Description parameter		B1 Planting on 12th August 2022 & 11th October 2022	B2 Planting on 26th January 2023
Total Nitrogen, N%		0.25	0.22
Total Phosphorus, P%		0.069	0.072
Exchangable Cation(meq/100g, soil),	Potassium K	0.38	0.45
	Magnesium Mg	1.38	1.98
	Calcium Ca	7.71	5.53
	Sodium Na	0.57	0.61
pH		6.32	6.78

Source: Soil and water division

ព្រះរាជាណាចក្រកម្ពុជា

ជាតិ សាសនា ព្រះមហាក្សត្រ

សេវាកម្មវិភាគដី និងទឹក

លេខ: ២១១៨៨៥-០៨/២៣

Ref: ១៣/២៣ ម.ជ.ក

ថ្ងៃច័ន្ទ ៨កើត ខែស្រាពណ៍ ឆ្នាំថោះ បញ្ចស័ក ព.ស.២៥៦៧

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លទ្ធផលវិភាគដី (Soil Analysis Results)

ឈ្មោះក្រុមហ៊ុន (Company Name): Miss Vochly

អាសយដ្ឋាន (Address): Plant Breeding Office

ទូរស័ព្ទ (Tel): 855 17 724 435

ថ្ងៃទទួលសំណាក (Receive Date): ១៦/០៨/២៣

Lab ID: 11/23	**Lab No.:**	**1**		**2**	
Description parameter	**Field ID:**	**B1**		**B2**	
កាបូន Total Carbon (Black & Walkey Method), C %		2.14	Very High	1.95	High
អាសូតសរុប Total Nitrogen (Kjedal Sulfuric Method), N %		0.25	Medium	0.22	Medium
អនុបាត C/N Ratio (Unit)		9	Good Decompost	9	Good Decompost
សារធាតុសរីរាង្គ Organic Matter (OM) %		3.69	High	3.35	High
ផូស្វ័រសរុប Total Phosphorus (Nitric Digetion) P %		0.069	Very high	0.072	Very high
កាចុងដោះដូរ Exchangable Cation (meq/100g soil), (Method, 1M Ammonium Aceteat pH=7)	កាល់ស្យូម Calcium Ca	7.71	Medium	5.53	Medium
	ម៉ាញេស្យូម Magnesium Mg	1.38	Medium	1.98	Medium
	សូដ្យូម Sodium Na	0.57	Medium	0.61	Medium
	ប៉ូតាស្យូម Potasium K	0.38	Low	0.45	Low
កាចុងដោះដូរសរុប Total Exchangable Base (meq./100g soil)		10.04	Medium	8.57	Medium
បញ្ជូនអ.នីមើលជាតិប្រៃដី Electrode Conductivity µS/cm (1:5 soil: water)		67.30	Non-saline	64.80	Non-saline
ប៉េហាសទឹក pH H2O (1:5 soil: water)		6.32	Slightly Acid	6.78	Neutral
ប៉េហាសអំបិល pH KCl (1:5 soil: 1N KCl)		4.95		5.39	

ចំណាំ៖ លទ្ធផលនៃការវិភាគមានតម្លៃតែលើសំណាកដែលបានបញ្ជូនមកវិភាគតែប៉ុណ្ណោះ

Note: Analysis result refers to the sending sample only.

Prepared by:

Mr. Lim Vanndy, Soil technician

Future plan

- Maintain the 11 CMD resistant clones in *in-vitro*
- Maintain and multiply the six new arrival clones both in *in-vitro* and field for evaluation
- Supply mother plant for disease free planting material production in other provinces.
- Evaluate IITA and AR-CR clone for yield, starch content, disease resistance and durability against CMD.
- On-farm trial of promising genotypes

Constraints

- Lack of labor (skill and none-skill)
- Underground water cause many problem on soil medium (increase pH)
- Lack of capacity in tissue culture especially in meristem micropropagation
- Financial support for cassava varietal improvement is very limited.

Thank you very much