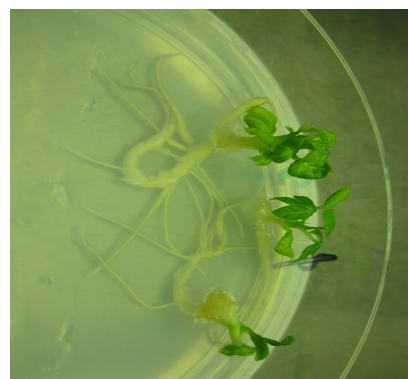
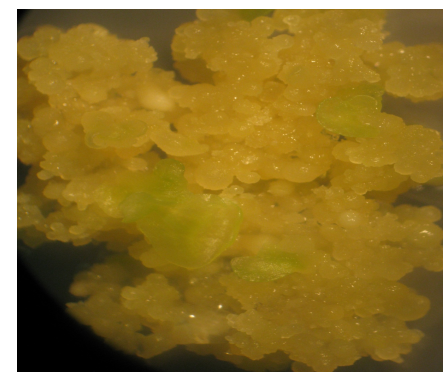
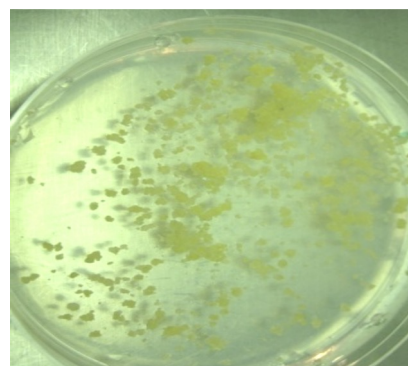
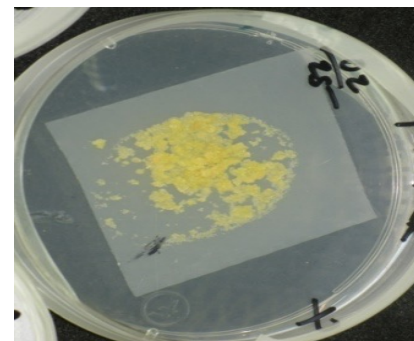
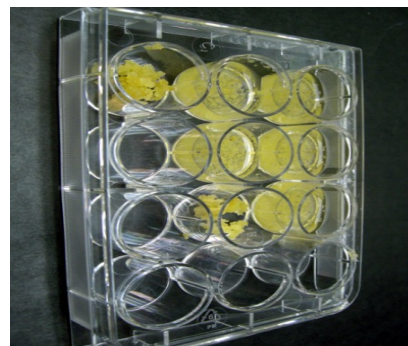




Transformation progress of farmer-preferred genotypes

NRCRI Umudike
Nigeria TEAM



NRCRI
Delivering the Roots of Prosperity



Key achievements

- Identification of Target cultivars
- Transformation with marker genes
- Enhancing FEC target tissue production from TMS 980505, CR 36-5, UMUCASS 36
- Transformation with CASS constructs
 - GGL2-PPase-HygR &
 - GGL2-PPase+GFP+NTT)
 - #2-8
 - #46

■ Screening for potential candidates



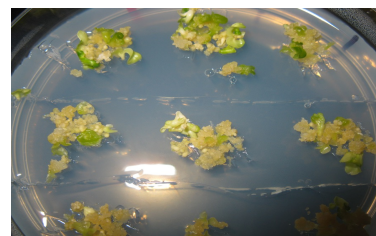
FEC Production

Quality embryos

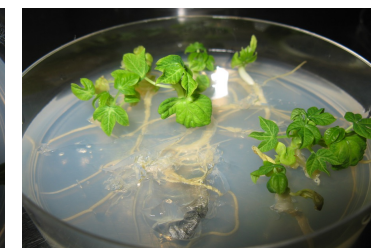
Cotyledon regeneration

Quality cassava plantlets

BAD



GOOD



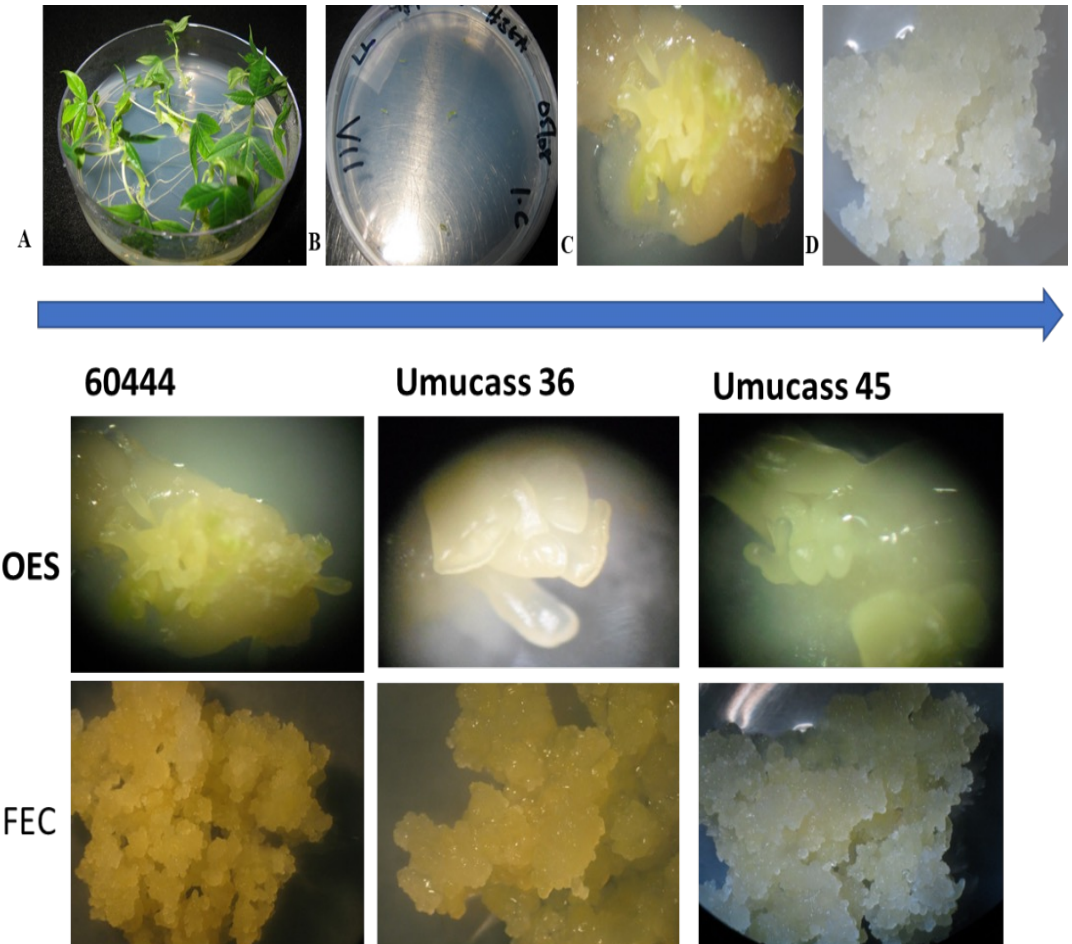
○ Optimizing Callus induction

MS2 SOP

- 2.416g/L picloram concentration (made as 10mM stock solution) for OES/FEC induction in 60444
- 0.04g/L picloram concentration optimized for OES induction for yellow root cassava and other farmer preferred cassava

GD2 SOP

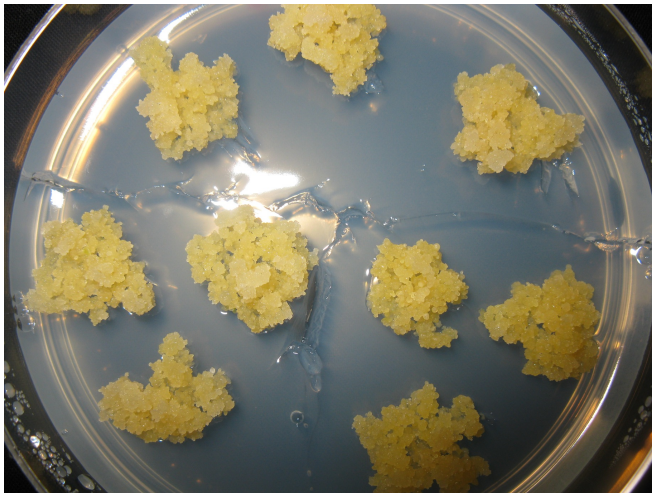
- 2.416g/L picloram concentration used for FEC induction of farmer preferred cultivars



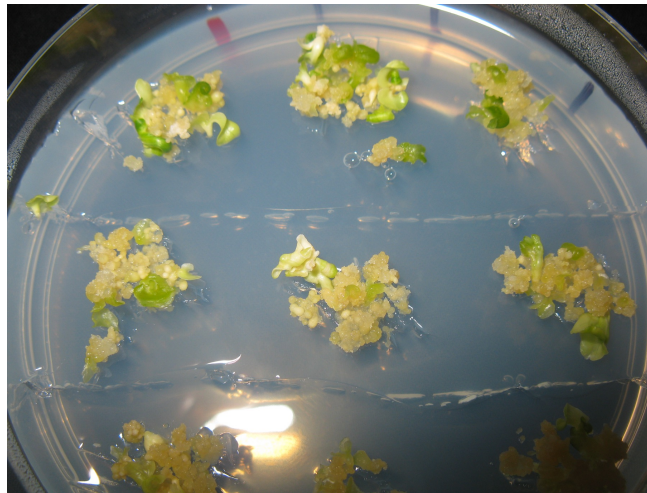


○ Embryo Regeneration regimen

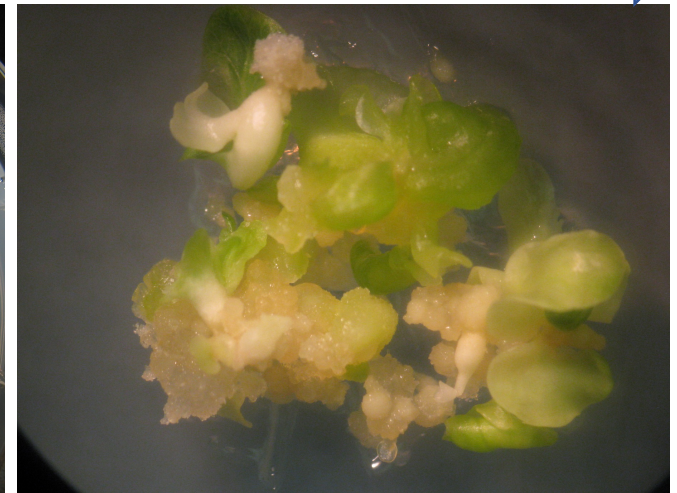
- Stage 1 (MS2/5 μ M NAA + 250 mg/L Carbenicillin/45 μ M Paramomycin)
- Stage 2 (MS2/0.5 μ M NAA/45 μ M Par)
- Stage 3 (MS2/0.05 μ M NAA + 45 μ M Par)



Stage 1



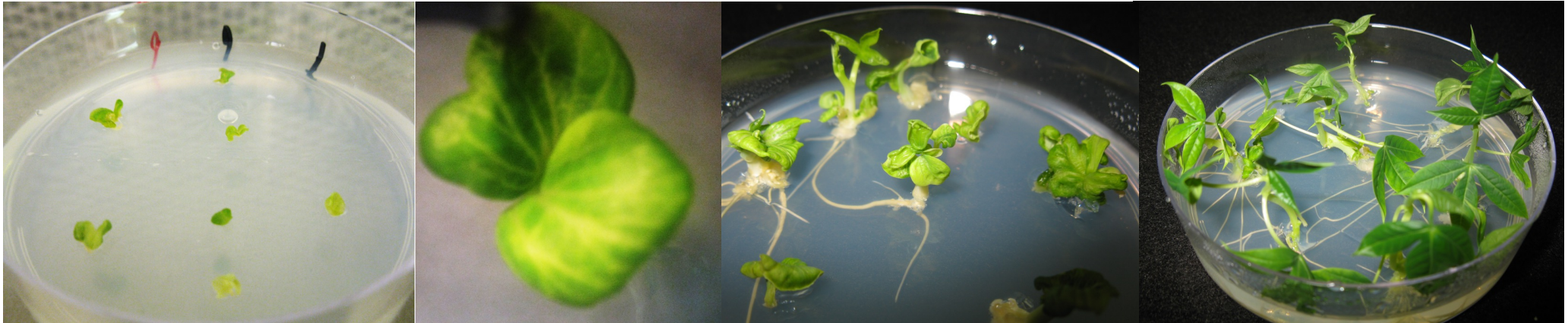
Stage 2/3



Matured embryos

○ Whole Plant Regeneration

- Embryo germination (MS2/2 μm BAP)
- Rooting (MS2 Agar)
- Multiplication (MS2 Agar)

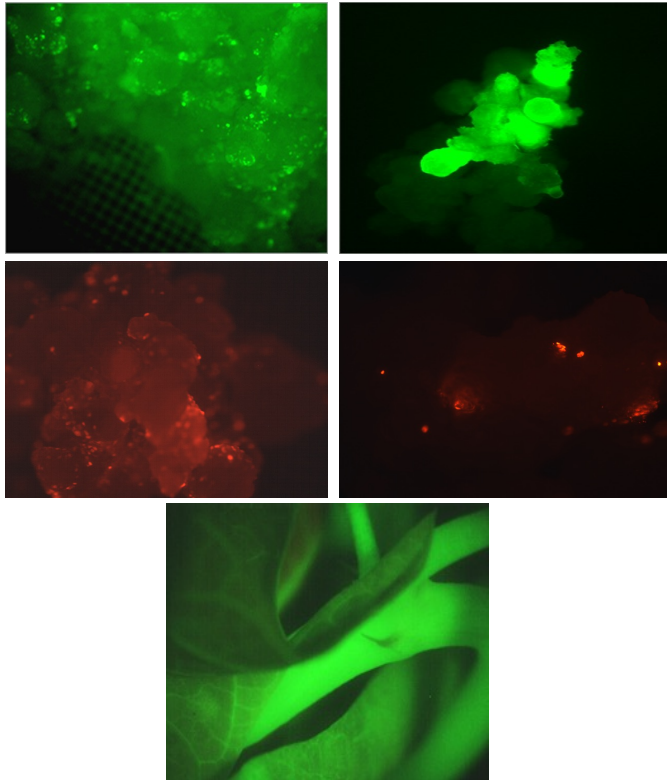


■ Identification of target cultivars



S/N	Cultivars evaluated	Status	Comment
1	CR 41-10	Good callus formation	not advanced
2	CR 36-5	Great callus induction & regeneration	Transformation
3	TMS 980505	Excellent callus induction & regeneration	Transformation
4	TMS 9102324	Good callus induction & regeneration	Transformation
5	TMS 011368(Umucass 36)	Good callus induction & regeneration	Transformation
6	TMS 01/1412	Poor callus induction	Optimizing callus induction
7	TMS011371	Poor callus induction	Not advanced
8	TMS 070220 (Umucass 44)	Good callus induction & regeneration	Not advanced (high viral load)
9	TMS 070593 (Umucass 45)	Excellent callus induction & regeneration	Transformation
10	TMS 070539 (Umucass 46)	Excellent callus induction & regeneration	Transformation
11	TMS 13F1160P004	Good callus induction	Optimizing regeneration
12	TMS 13F1343P002	Good callus induction	Optimizing regeneration
13	TMS13F1343P0044	Evaluating for callus induction	Optimizing callus induction
14	IITA IBA00070	Evaluating for callus induction	Optimizing callus induction
15	TMS 13F1307P0016	Evaluating for callus induction	Optimizing callus induction
16	NR130124	Evaluating for callus induction	Optimizing callus induction

■ Transformation with marker genes



Cultivars	No. of lines transformed	No. Embryo recovered	No of cotyledon plants derived	No. of whole plant regenerated	No. of lines evaluated	No. expressing GFP
TMS 980505	3	15	7	5	5	3
		7	4	2		
		9	3	2		
TMS 9102324	1	5	2	2	3	2
CR36-5	2	5	2	2	2	1
		3	2	2		

Fluorescent microscopy

■ Enhancing target tissue production from selected candidates



CR 36-5

TMS 980505

TMS 011368(Umucass 36)

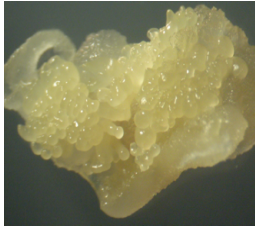


- Re-introduction of TMS 980505, CR 36-5 & Umucass 36 in culture every 6 months to avoid soma clonal variation
- Micropropagation of in-vitro mother plantlet every 6 to 8 weeks to ensure the plantlets are young and optimal
- Established on OES induction medium every alternative week to enhance FEC production
- Systematic transfer of somatic embryos to FEC induction medium every 18 to 21 days

■ Transformation with CASS constructs



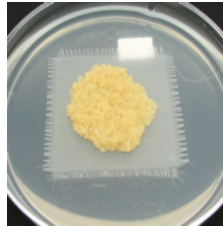
leaf
explant



OES



FEC



Agrobacterium
transformation



Antibiotic selection
regeneration



Transgenic
plant



■ Transformation with CASS constructs



S/n	Construct	Cultivars	No of transformation	No of lines recovered
1.	GGL2_Ppase_HygR	TMS 98/0505	6	12
		CR 36-5	2	5
		Umucass 36	1	In progress
2	#2-8	TMS 98/0505	4	In progress
		CR 36-5	1	In progress
		Umucass 36	1	In progress
3	#46	TMS 98/0505	Yet to commence	
		CR 36-5	Yet to commence	
		Umucass 36	Yet to commence	



- **Summary & Future plan**

- ✓ Initiated transformation in three cultivars with CASS constructs
- ✓ Increased capacity for FEC production to enhance transformation volume
- ✓ Improved transformation capacity in the three selected cultivars





**Thanks
For
Listening!**