Molecular diagnostic tests to strengthen seed systems

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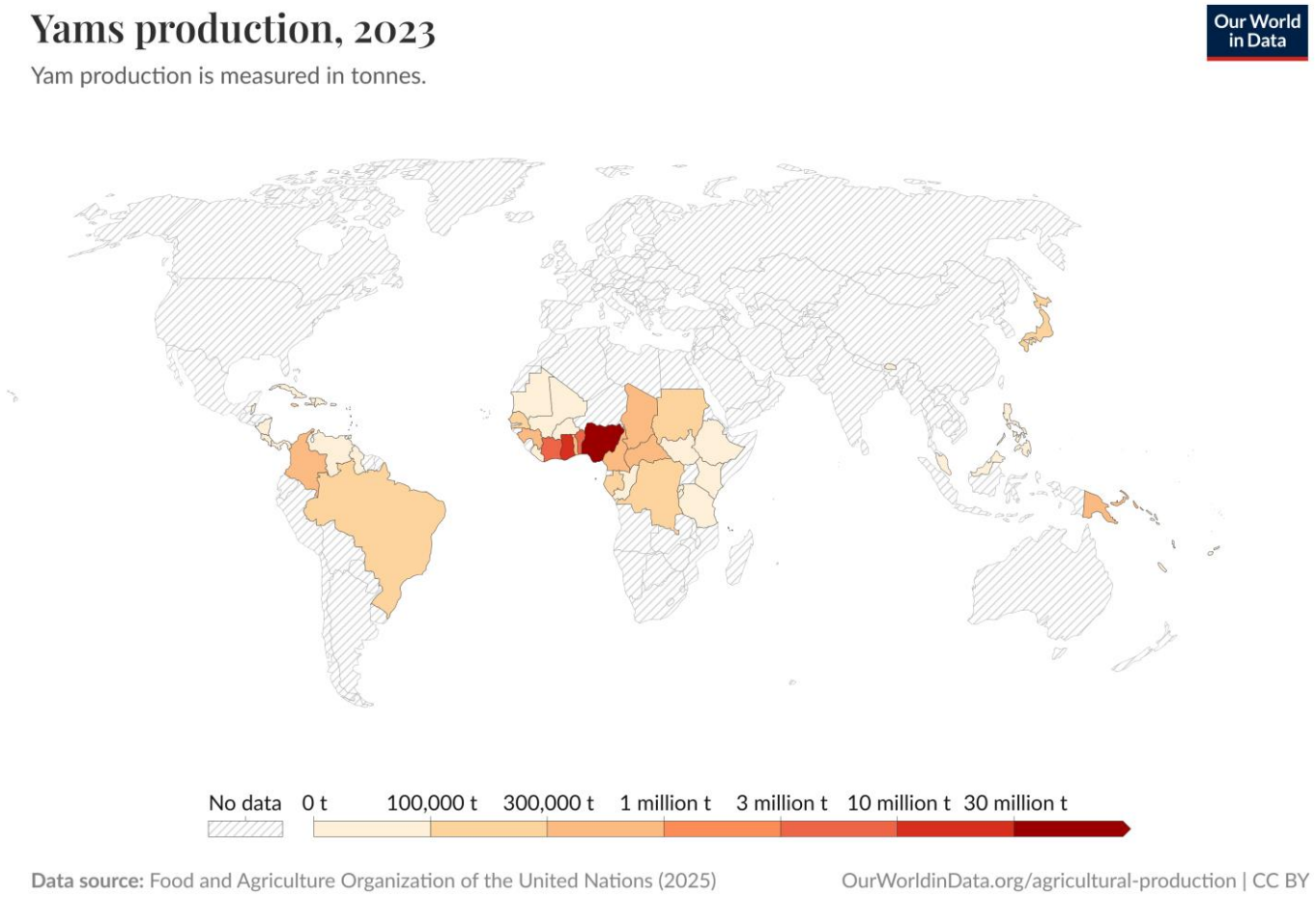
Yam (*Dioscorea* spp.)

- Major staple food worldwide, particularly for West Africa
- Important role in food security and for the livelihoods of >60 million people in West Africa



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Diagnostics needed for yam germplasm

- The **lack of a formal seed system** encourages farmers to use low-quality planting material from their own or neighbouring farms resulting in the **accumulation of diseases in yam germplasm.**

- **Sanitation methods** are critical for the rapid multiplication of virus-free planting materials but can lead to low virus titres below detection thresholds.

- **Diagnostics needed** to test sanitised planting material and support the production of high-quality “clean” seed yams.

Taxonomy		
Family	**Genus**	**Species**
Alphaflexiviridae	*Potexvirus*	Dioscorea latent virus (DLV)
		Yam virus X (YVX)
		Yam potexvirus 1
		Yam potexvirus 2
		Yam potexvirus 3
Betaflexiviridae	*Carlavirus*	*Yam latent virus* (YLV)
	Unassigned	Yam virus Y (YVY)
Bromoviridae	*Cucumovirus*	*Cucumber mosaic virus* (CMV)
Caulimoviridae	*Badnavirus*	*Dioscorea bacilliform ES virus* (DBESV) DBV1† (Kenyon) or DeBV-A†† (Bousalem) **episomal**
		DBV2 (Kenyon) † or DeBV-C†† (Bousalem) **unknown**
		Dioscorea bacilliform AL virus 2 (DBALV2) DBV3 † (Kenyon) or DeBV-B†† (Bousalem) **episomal**
		Dioscorea bacilliform SN virus (DBSNV) DBV4† (Kenyon) or DsBV† (Bousalem) **episomal**
		Dioscorea bacilliform RT virus 3 (DBRTV3) DBV5† (Kenyon) or DBV-C†† (Bousalem) **episomal and endogenous**
		DBV6† (Kenyon) or DeBV-D†† (Bousalem) **unknown**
		DBV7† (Kenyon) or DeBV-E†† (Bousalem) **unknown**
		Dioscorea bacilliform AL virus (DBALV) DBV8† (Kenyon) or DBV-A(A)/DaBV†† (Bousalem) **episomal and endogenous**

Taxonomy		
Family	**Genus**	**Species**
	Badnavirus	*Dioscorea bacilliform TR virus* (DBTRV) DBV9† (Kenyon) or DBV-B†† (Bousalem) **episomal and endogenous**
		DBV10† (Kenyon) or DpBV†† (Bousalem) **unknown**
		DBV11† (Kenyon) or DeBV-F†† (Bousalem) ***unknown**
		DBV12* (Umber) or DBV-A(B) †† (Bousalem) **endogenous and presumably episomal**
		Dioscorea bacilliform RT virus 1 (DBRTV1) DBV13 (Bömer) **episomal**
		Dioscorea bacilliform RT virus 2 (DBRTV2) DBV14 (Bömer) **episomal**
		DBV15 (Bömer) **presumably episomal**
	Dioscovirus	*Dioscorea nummularia associated virus* (DNUaV)
Closteroviridae	*Ampelovirus*	*Yam asymptomatic virus 1* (YaV1)
		Air potato virus 1 (AiPoV1)
	Velarivirus	*Cordyline virus 1 (CoV1)*
Geminiviridae	*Begomovirus*	Yam yellow spot mosaic virus **presumably endogenous**
		EGV1 and EGV2 **endogenous**
	Macluravirus	*Chinese yam necrotic mosaic virus* (CYNMV)
		Yam chlorotic mosaic virus (YCMV)
		Yam chlorotic necrosis virus (YCNV)
Potyviridae	*Potyvirus*	*Yam mosaic virus* (YMV)
		Yam mild mosaic virus (YMMV)
		Japanese yam mosaic virus (JYMV)
		Dioscorea mosaic virus (DMV)
		Yam potyvirus TGwadE2
Secoviridae	*Fabavirus*	*Broad bean wilt virus 2* (BBWV2)
	Sadwavirus	*Dioscorea mosaic-associated virus* (DMaV)
Tombusviridae	*Aureusvirus*	*Yam spherical virus* (YSV)

Silva et al., 2019; Diouf et al. 2022

Viruses infecting yam

At least 25 different viruses belonging to 12 genera and eight families

Current seed yam production process

- Over 7-8 months wait before PCR indexing!

Sanitation / Tissue culture (TC) — 6-8 months

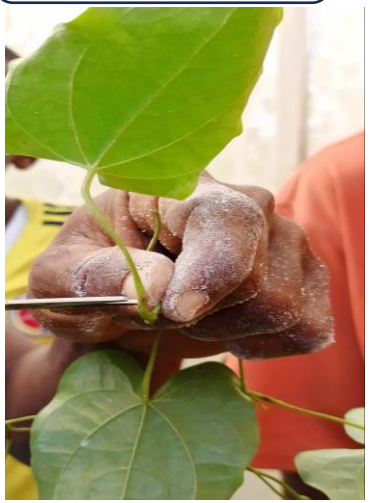
Hardening — 1-2 months

Virus indexing (PCR – YMV only)

Mass propagation in TC — 2 months

Hardening & establishment of mother plants — 3-4 months

Single node cuttings — 3 months



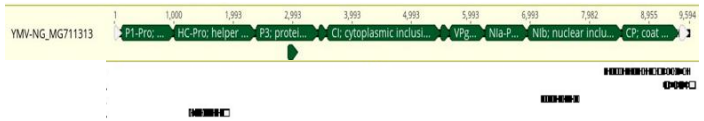
Yam plants in field — 6 months

≥20 months later...
Breeder seeds (virus-free)

Optimised MinION workflow for virus detection in yam

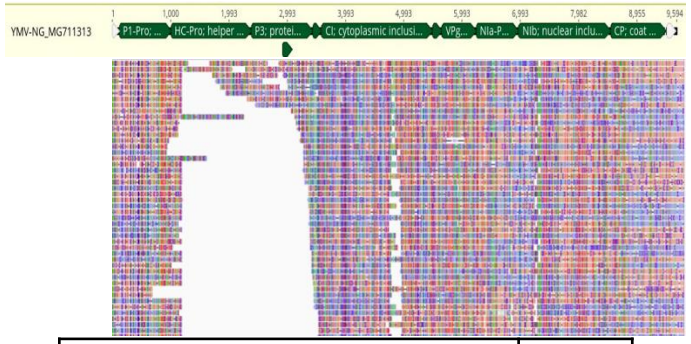
Sequencing strategy

Direct RNA sequencing



Yield/estimated bases (Gb)	1.5
Total number reads (M)	2.4

cDNA-PCR sequencing



Yield/estimated bases (Gb)	9.18
Total number reads (M)	23.5

RNA enrichment strategy

RNA Enrichment	Mapped reads (%)	Mean depth	Avg. read length (bp)
Total RNA	0.2–3%	64–110	~900
Poly(A)+	<2%	14–684	~630–890
rRNA-depleted	0.7–14%	226–4398	~470–580
rRNA-depleted + Poly(A)+	**0.7–13%**	**309–2085**	**~460–475**

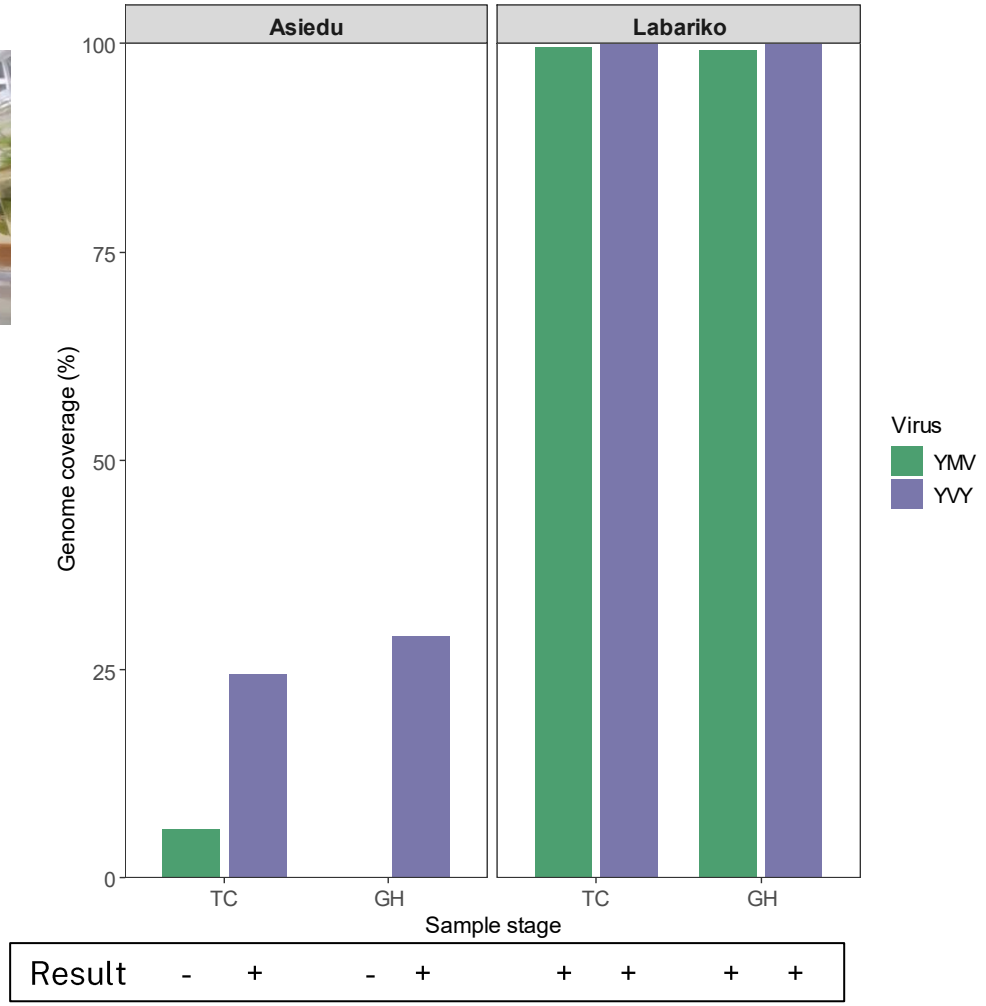
Optimised workflow: Combination of rRNA-depletion + poly(A)+ followed by PCR-cDNA sequencing

Festus et al., *submitted*

MinION protocol reduces virus indexing timelines by ~12 weeks

Tissue culture (TC)
testing after 4 months

Hardening at glasshouse (GH)
testing after 7 months



Plant Stage	Total Reads	Reference virus	Mapped reads
Asiedu			
TC	5,082,266	YMV	1
		YVY	22
GH	2,427,095	YMV	0
		YVY	19
Labariko			
TC	3,039,879	YMV	577
		YVY	3,930
GH	1,754,636	YMV	1,278
		YVY	7,747

Festus et al., *submitted*

Opportunities & Challenges of adopting MinION in seed systems

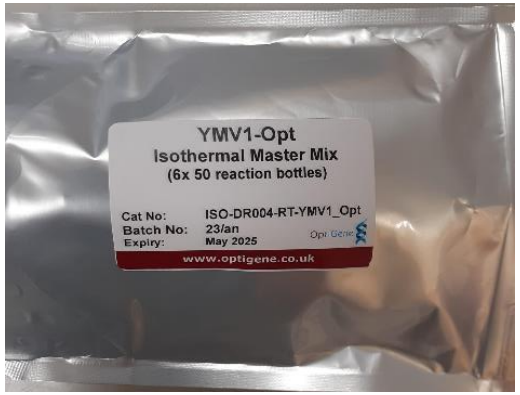
Confirm known viruses and identify new ones

YMV-NG (*Potyvirus*; 9.6 kb)

YVY-Mak (*Betaflexiviridae*; 7.5 kb)

DBRTV3 (*Badnavirus*; 7.4 kb)

Develop/Improve diagnostic tests



Ready to use YMV LAMP kit

- Cost constraints and strategic integration
- Equipment and infrastructure limitations
- Bioinformatics expertise
- Data storage

Optimised seed yam production workflow

- Saves time (~3–4 months) and resources.

Breeder seeds (virus-free)

MinION virus indexing

4 months

Sanitation / Tissue culture (TC)

2 months

Mass propagation in TC

Hardening & establishment of mother plants

3-4 months

PCR/LAMP testing

3 months

Single node cuttings

Yam plants in field

6 months

Opportunities & Challenges of adopting MinION in seed systems

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DBRTV3 (*Badnavirus*; 7.4 kb)

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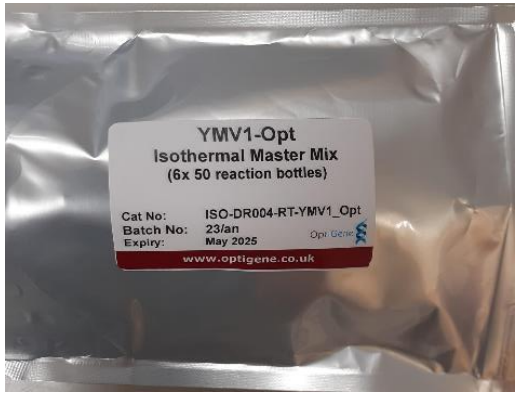

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Thank you!