Indexing and elimination of viruses infecting yams (*Dioscorea* spp.) for the safe movement of germplasm

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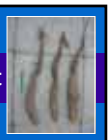
State of art

- Yams are vegetatively propagated crops
- No or limited sexual reproduction
- Strong susceptibility to anthracnose, nematods and virus diseases
- Changes in agricultural practices
- Very limited exchanges of genetic resources throughout the world

→ Strong genetic erosion

Development of a yam quarantine unit



- A quarantine unit for the production and movement of healthy yam germplasm
- Located out of production areas
- Initiated by CIRAD in 2002

Main functions

The main functions of the quarantine is to:

- study the health state of germplasms to be transferred
- clean the germplasm
- allow the distribution of healthy germplasm
- determine or improve appropriate diagnostic tools
- detect new diseases...

Origin of material

- **Benin** (INRAB): 1 *D. alata,* 1 *D. praehensilis*, 25 *D. rotundata*
- **Vanuatu** (VARTC): 55 *D. alata*, 7 *D. nummularia*, 1 *D. trifida*

  → **SPYN collection**
- **CIRAD**: 1 *D. alata*, 1 *D. rotundata,* 2 *D. trifida*

FAO-IBPGR Guidelines

- Nematodes
- Scale insects, mealybugs
- Tuber beetles
- Anthracnose

Insecticide + fungicide + *in vitro* nodal culture

- Viruses:
  - YMV
  - YMMV (= DaV)

Heat treatment + *in vitro* meristem culture

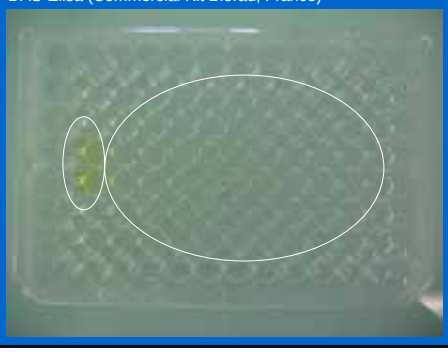
  - DLV
  - DBV
  - CMV

?????

Some viral symptoms in yams

ELISA test for CMV detection

DAS-Elisa (Commercial Kit Biorad, France)



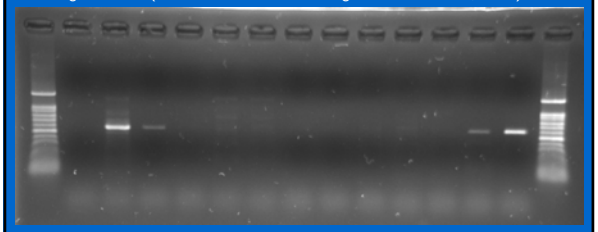
PCR test for Badnaviruses detection

Coating-PCR (modified from Yang *et al.*, 2003) (DBV)

PCR test for Potexviruses detection

(DLV)

Coating-RT-PCR (modified from van der Vlugt and Berendsen, 2002)



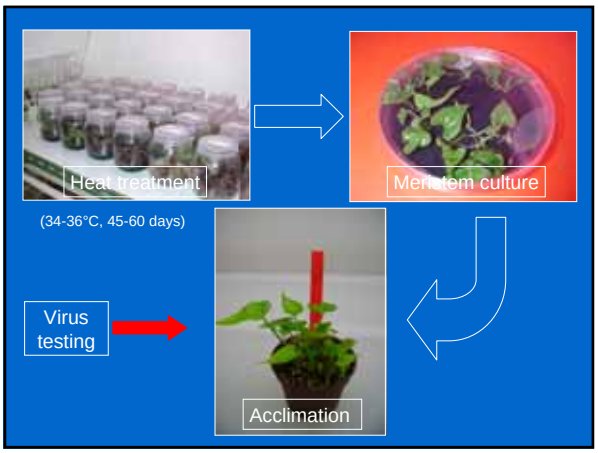
Duplex RT-PCR test for YMMV and YMV detection

Coating-duplex-RT-PCR (modified from Mumford and Seal, 1997)

Viruses detected

Targeted virus	No. of infected accessions (% analysed samples)					
	Vanuatu [a]		Benin [b]		CIRAD [c]	
Potyvirus:						
YMMV	44	(69.8)	0	(0)	1	(20.0)
YMV	0	(0)	1	(3.7)	2	(40.0)
Badnavirus:						
all strains	5	(7.9)	26	(96.3)	3	(60.0)
Potexvirus:						
all strains	1	(1.6)	0	(0)	1	(20.0)
Cucumovirus:						
CMV	0	(0)	0	(0)	0	(0)

[a] 55 *D. alata* acc., 7 *D. nummularia* acc., 1 *D. trifida* acc.
[b] 1 *D. alata* acc., 1 *D. praehensilis* acc., 25 *D. rotundata* acc.
[c] 1 *D. alata* acc., 1 *D. rotundata* acc., 2 *D. trifida* acc.



Regeneration efficiency of meristem culture

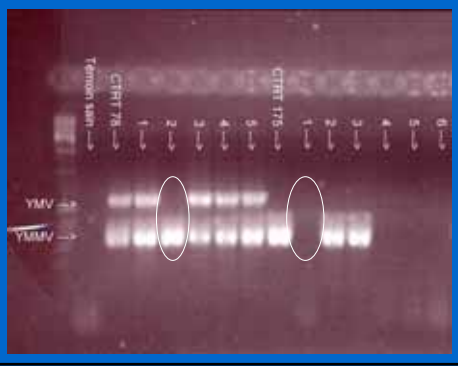
Species	No. accessions	No. of cultivated meristems	No. of regenerated plantlets	Regeneration ratio (%)
D. alata	41	820	325	39.6
D. nummularia	4	140	28	20.1
D. praehensilis	1	20	17	84.6
D. rotundata	26	520	174	33.5
D. trifida	3	60	9	14.6
Total	75	1560	553	35.4

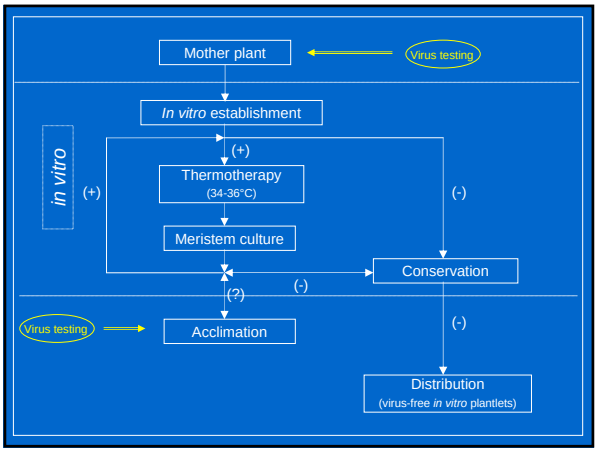
Efficiency of meristem culture for virus elimination

Virus	Plants			Accessions [a]		
	No. regenerated	No. cleaned	Virus elimination (%)	No. regenerated	No. cleaned	Virus elimination (%)
Badnaviruses (all strains)	148	3	2.0	34[b]	1	2.9
Potexviruses (all strains)	5	3	60.0	2[c]	1	50.0
YMMV	238	110	46.3	45[d]	40	88.9
YMV	14	3	21.4	3[e]	1	33.3

[a] Each accession is represented by 1 to 6 plants
[b] 3 *D. alata* acc., 1 *D. nummularia* acc., 1 *D. praehensilis* acc., 26 *D. rotundata* acc., 3 *D. trifida* acc.
[c] 1 *D. nummularia* acc., 1 *D. rotundata* acc.
[d] 40 *D. alata* acc., 3 *D. nummularia* acc., 2 *D. trifida* acc.
[e] 2 *D. rotundata* acc., 1 *D. trifida* acc

YMMV and YMMV elimination





First transfers

47 *D. alata* (Vanuatu et Benin)
6 *D. nummularia* (Vanuatu)
→ Guadeloupe (CIRAD)
→ breeding

28 *D. alata* (Vanuatu)
→ Benin (CIRAD-IITA)
→ agricultural exploitation

38 *D. alata* (Vanuatu)
→ Fiji (SPC-RGC)
→ *in vitro* conservation

~~8 *D. alata* (Vanuatu)~~
~~1 *D. praehensilis* (Benin)~~
~~25 *D. rotundata* (Benin)~~
~~1 *D. trifida* (Vanuatu)~~
→ not released from quarantine

Conclusions

- Elimination of YMMV in most of infected accessions
- Elimination of badnaviruses, potexviruses and YMV in some accessions

→ **Distribution of healthy germplasm (*D. alata*, *D. nummularia*)**

- All *D. rotundata* accessions were found badnaviruses positive with PCR test and badnavirus elimination failed

→ **No *D. rotundata* distribution**

Future work

- More investigations on badnaviruses infecting yams, particularly *D. rotundata*: characterization of badnaviruses, badnavirus elimination,…
- Characterization of potexviruses detected in yams
- More investigations on other virus cleaning techniques: chemotherapy, cryotherapy,…
- Other transfers of yam germplams…

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