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REVIEW ARTICLE

Medium-term and long-term *in vitro* conservation and safe international exchange of yam (*Dioscorea* spp.) germplasm

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Yam edible tubers feed million of peoples in the intertropical area, where they represent 12% of human feeding. However, as a vegetatively propagated crop, yam is seriously affected by an accumulation of pathogens. Establishing *in vitro* germplasm collection is a process that cleans the plants from all diseases but viruses. It gives a good control on the preservation of the yam genetic resources and facilitates international exchanges of healthy plant material.

Two kinds of *in vitro* germplasm preservation were considered : slow growth condition culture for mid-term preservation, and cryopreservation using the encapsulation/dehydration technique for long-term preservation. Virus eradication was approached by meristem culture and chemo and thermotherapy. Production of virus-free plants was controlled by ELISA.

We succeeded in the introduction and maintenance of 20 yam species, under slow growth conditions. Cryopreservation was applied successfully on two edible yam species, *Dioscorea. alata* L and *D. bulbifera* L. Virus-free plants were obtained by meristem culture in *D. cayenensis-D. rotundata* complex and *D. praehensilis*. Indexation allowed the detection of different virus (poty-, potex-, badna- and cucumovirus), where the most important potyvirus was YMV.

Mid-term conservation of yam germplasm is used routinely, and from these conditions a direct acclimatization is possible. On the cryopreservation aspect, experiments are under way to apply the optimized protocol to genotypes which are more representative of the diversity, to insure a routinely use. More work can be conducted now on virus eradication,

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** ORSTOM is now called IRD, Institut de Recherche pour le Developpement.

based on knowledge accumulated on potyvirus diversity, on several tests available for yam indexing (ELISA, rt/PCR, monoclonal antibodies) and on new sanitation techniques.

Yam belongs to *Dioscorea* genus which has more than 600 species (Coursey, 1967) most of them distributed in the intertropical humid area. We will distinguish two types of yam: 1) medicinal yams, 2) edible yams and relatives. Medicinal yams concern about fifty species caracterized by their sapogenin content, which are steroidal components. For the edible yams and relatives, we will observe two groups: 1) domesticated species, 2) and wild species.
For domesticated species we consider that forty to fifty species are occasionally used (Martin and Degras, 1978). From these only eleven are cultivated (Table 1). From the 11 cultivated, 6 represent an important part of feeding (*D. alata, D. cayenensis-D. rotundata* complex, *D. bulbifera, D. dumetorum, D. esculenta, D. trifida*), 3 are scattered in all the intertropical humid area (*D. alata, D. bulbifera, D. esculenta*), and yam belonging to the *D. cayenensis-D. rotundata* complex take place most of them in West Africa and some in the Caribbean area. The other yams are cultivated in their origin area (Degras, 1986).

Table 1 : **Main edible species of yam**

Species [1]	**Zone of origin**	**Zone of culture**
Enantiophyllum Section		
D. alata L.	South East Asia	Inter-tropical humid
D. cayenensis Lamk. *D. rotundata* Poir. complex [2]	West Africa	West & Central Africa, and Caribbean
D. nummularia Lamk.	Indonesia, Oceania	Indonesia, Oceania and Micronesia
D. opposita Thunb. *D. japonica* Thunb. complex [3]	Temperate area from: China, Corea, Taiwan Japan	Temperate area from: China, Corea, Taiwan Japan
D. transversa Br.	South Pacific	South Pacific
Lasiophyton Section		
D. dumetorum (Kunth) Pax.	West Africa	West Africa
D. hispida Dennst.	India, South-China, New Guinea	India, South-China, New Guinea
D. pentaphylla L.	Himalaya and Oceania	Himalaya and Oceania
Combilium Section		
D. esculenta (Lour.) Burk.	South East Asia	Inter-tropical humid
Opsophyton Section		
D. bulbifera L.	South East Asia and Africa	Inter-tropical humid
Macrogynodium Section		
D. trifida L.	Guyana, Amazonian basin	Caribbean

Sources: Malaurie et al. (1998a)
[1] Species have been regrouped in Section by Knuth (1924), completed by Burkill (1960)
[2] Grouping together species of *D. cayenensis* and *D. rotundata* in a Complex has been proposed by Ayensu and Coursey (1972), Martin and Rhodes (1978), Miège (1982)
[3] Grouping together species of *D. opposita* and *D. japonica* in a Complex has been proposed by Tanaka (1977).

Main edible species of yams are: 1) native of a continent, 2) and cultivated in the same continent, 3) or/and cultivated in an other. This observation implies very strong links to exchange problems. In Table 2, 36 countries have been observed by IBPGR in 1986 with *Dioscorea* germplasm. These countries are supposed to be concerned by an international exchange of yam germplasm. Some of them,

have, in our knowledge, already developed *in vitro* germplasm collection.

Table 2 : Countries [1] and geographic zones where yam collections have been observed

Europe	West Indies	America	Pacific	Asia	Africa
France [2]	Barbados *	Brazil [2]	Cook Islands	Bengladesh	Bénin
United Kingdom [2]	Cuba	Colombia	Fiji	India	Burkina Faso
	Guadeloupe [2]	Costa Rica	Niue Islands	Indonesia	Cameroun
	Jamaïca	Guatemala	New Calédoniaee [2]	Japan [2]	Ivory Coast [2]
	Saint-Dominique	Mexico	Papua NewGuinea	Malaisia	Ghana
	Trinidad-Tobago	Panama	Salomon Islands	Nepal	Nigeria [2]
		USA	Tonga	Philippines	South Africa
			Vanuatu	Sri Lanka	Togo
			Western Samoa [2]	Thailand	Uganda
				Viet Nam	

(Sources : IBPGR 1986, FAO 1996, Malaurie et al 1998a)
* *in vitro* maintenance for production purpose
[1] This country listing is not exhaustive, and take into account only sources in our possession
[2] Countries with *in vitro* collection (according to sources in our possession)

Different genebank preservation levels exist. A first group concerns non aseptic germplasm conservation with in field genebank and seed genebank, where important disadvantages and heavy constraint of quarantine measures explain the choice of *in vitro* germplasm conservation (Hanson, 1986; Malaurie et al., 1998a) (Table 3).

Table 3: Non aseptic germplasm conservation

	Non aseptic Genebanks	
	In field Genebanks	**Seed Genebanks**
Disadvantages		
Genetic erosion	+++	
Expensive	+++	
Hard to manage	+++	
Do not bread true		+++
Tuber shape		+++
Dormancy		+++

In vitro conservation

Three levels of *in vitro* genebank preservation levels could be considered: 1) short term conservation: this conservation

under normal growth conditions is suitable for temporary storage of germplasm collections, and for international distribution, 2) medium term conservation, which could be considered as an active *in vitro* genebank, 3) long term conservation, considered also as a base *in vitro* genebank.
These *in vitro* genebanks have been previously introduced *in vitro* from tuber or seed. These introductions have to be linked to an obligatory phytosanitary control from mother plants and from *in vitro* material after introduction. Medium term conservation, which correspond to *in vitro* culture under slow growth conditions, could be obtained by several ways: 1) physiological stage of the explant, 2) addition of osmotic agents and growth moderators, 3) low storage temperature, 4) low mineral or sucrose concentrations, 5) low oxygen pressure, 6) encapsulation in alginate (Charrier et al., 1991; Withers, 1991; Engelmann, 1991; Malaurie et al., 1998a).

Medium-term conservation

At ORSTOM, we choose to maintain the *in vitro* yam collection in a medium with low mineral nutrient and a low sucrose concentration. We succeeded in the introduction and maintenance of 14 species of yam (Malaurie et al., 1993). Since this time, this collection is continuously enriched by new genotypes and comprises 20 species (Table 4).
For yam, this simple solution of slow growth is used routinely and from these culture conditions a direct acclimatization is possible. This mode of conservation allows an international distribution of the material and corresponds to an active genebank (Malaurie et al. 1993,1998c, Malaurie and Trouslot 1995c).

This *in vitro* germplasm collection of yam is maintained in test tubes, at ORSTOM (Montpellier, France), with a total of 6 test tubes by accession, with two different places of storage for the replicates ; the minimal growth conditions allow to maintain most of the accessions up to 2 years. Technical constraints in the collection management lead to subculture the accessions every 6-8 months (Malaurie et al., 1998c).

Table 4 : Listing of different species of yam maintained in an *in vitro* collection, under slow growth culture condition *
(GeneTrop, GAP unit, ORSTOM, Montpellier, France)

Species	Number of accessions
D. abyssinica Hochst. Ex Kunth	6
D. alata L.	91
D. bulbifera L.	8
D. cayenensis Lamk. *D. rotundata* Poir. complex	63 (+ 17)
D. burkilliana J. Miège	11
D. dumetorum (Kunth) Pax.	2
D. esculenta (Lour.) Burk.	10
D. hirtiflora Benth.	1
D. mangenotiana J. Miège	15
D. minutiflora Engl.	2
D. opposita Thunb. *D. japonica* Thunb. complex	1
D. praehensilis Benth.	17
D. preussii Pax	1
D. sansibarensis Pax	1
D. schimperana Hochst. Ex Kunth	1
D. smilacifolia De Wild	2
D. togoensis Knuth	8
D. transversa Br.	1
D. trifida L.	2 (+ 1)
Interspecific Hybrids: *D. cayenensis-D. rotundata* complex cv. 'Krengle' X *D. praehensilis* so-called 'Igname de Pilimpikou'	14 (+ 9)

* (+): Accessions recently introduced

Different species maintained in the *in vitro* collection, such as *D. cayenensis-D. rotundata* complex are going to be enriched by cultivars from Burkina Faso for a sanitation program, and from Benin for a genetic program linkeds to virologic aspect. Others species supposed to be links to *D. cayenensis-D. rotundata* complex, such as *D. mangenotiana*, *D. praehensilis*, *D. minutiflora*, or *D. abyssinica*, *D. praehensilis*, are going to be enriched by further introduction.

Orstom virologists are interested by *D. trifida* because of its strong sensibility to virus, which provoked in Guadeloupe, French West-Indies, its quite disappearance. Serological and molecular works are developed to explain this virus sensibility.

Long-term conservation

Long term conservation correspond to cryopreservation in liquid nitrogen, at –196 °C. Plant cryobiology, which begun in 1971 by Latta works on carrot cell suspension, benefited from results on animals cell by Polge et al in 1949. Since these dates, different techniques have been set up: 1) on one hand, the so-called conventional techniques, using two steps of slow freezing, with the addition of cryo-protector (Sakai, 1984), 2) and on the other hand, new techniques, characterized by a very rapid freezing, about 1000°C/ min, by direct immersion in liquid nitrogen (Table 5) (Dereuddre et al., 1990, 1991; Tannoury et al., 1991; Uragami, 1993). The aim of these techniques is to try to control water flow and ice formation, and tend to a vitrificated state, avoiding crystal formation during thawing, and to protect the cell from thermic shocks.

Table 5 : Long term conservation : cryopreservation in liquid nitrogen, -196°C

Steps	**Conventional techniques**	**New techniques**		
		Air-drying	**Vitrification**	**Encapsulation / Dehydration**
Encapsulation				+
Sucrose pretreatment	+/-	+ (+ABA)		+
Cryoprotector	+		++++	
Desiccation		+		+
Slow-freezing	+ 0°C to -40°C (0.3 to 1°C/min)			
Rapid-freezing	+ -40°C to -196°C (200°C/min)	+ +25°C to -196°C (720°C/min)	+ +25°C to -196°C (400 to 1100°C/min)	+ +25°C to -196°C (720°C/min)
Thawing	500°C/min	120°C/min	120°C/min	120°C/min

Sources : Uragami (1993), Malaurie et al. (1998a).

Most of the results about cryopreservation have been obtained from conventional techniques on suspension cells of medicinal yam, *D. deltoidea* being the most used (Butenko et al., 1984; Popov and Fedorovskii, 1992; Popov and Volkova, 1994). More recent works have been done on rapid cryopreservation of callus (Chulafich et al., 1994), by direct immersion in liquid nitrogen, of two other medicinal yams (*D. balcanica*, *D. caucasica*).

Since 1996, new results have been obtained by two different research teams, using encapsulation/dehydration of shoot apices. On the one hand, Mandal *et al.* (1996) compared the survival capacities of apices after the osmotic

and thermic stress of the technique of four species of yam - three edible (*D. alata, D. bulbifera, D. wallichii*), and one medicinal (*D. floribunda*). Four of them have survived after immersion in liquid nitrogen, with 26 to 71%, depending on the species. Meanwhile, only two of them (*D. alata*, *D. wallichii*) allowed the recovery into shoots after immersion in liquid nitrogen, with 21 and 37%, respectively.

On the other hand, ORSTOM ability in different aspects of the long term conservation on tropical plants (Engelmann, 1991), and on encapsulation/dehydration technique applied on coffee, cassava, oil palm...etc, permitted to apply the process on apical shoot-tips of *in vitro* plantlets of yam (Malaurie and Trouslot 1996). Malaurie et al. (1998b) obtained survival rates over 50% for the two species (*D. alata*, *D. bulbifera*), and recovery to rooted leafy shoots after immersion in liquid nitrogen of at least 60% for *D. bulbifera* and 20% for *D. alata*, three months culture after thawing.

Comparatively to previous works on cryopreservation using encapsulation/dehydration technique, Malaurie et al. (1998b) have used higher sucrose concentration (0.9, 1.0 and 1.1M), a wider range of dehydration duration, up to 23h and a new and more accurate method for measuring of dry weight.

The new and more accurate method for measuring of dry weight used in our experiments consisted of desiccating alginate beads for 30d in airtight boxes containing dry silica gel, to avoid mass loss due to caramelization of sugar when drying at a temperature higher than 100°C. We obtained a strong linear correlation between dry mass (DW_{30}) and sucrose molarity for sucrose-pretreated alginate beads. During the whole experiment, we used DW_{30} values estimated by linear regression (Table 6).

Table 6: Dry mass and water content of sucrose-pretreated alginate beads, determined after 30d of drying with silica gel in airtight boxes at room temperature (1).

Sucrose concentration	DW_{30} (% FW) estimated by linear regression (2)	Water content before dehydration ($g.g^{-1}$ DW)
0.75M	28.8	2.47
0.9M	33.3	2.00
1.0M	36.3	1.76
1.1M	39.3	1.54

(1) Source: Malaurie et al. (1998b)

(2) >From mean values over 13 to 15 replicates for each of the four sucrose concentrations (y= 6.4319 + 29.872x; N= 4; r= 0.999). Similar results were obtained from replicate data (y= 6.4177 + 29.883x; N= 55; r= 0.960). Data not shown.

For the best sucrose pretreatments depending species, figure 1 (Malaurie et al., 1998b) shows that *D. bulbifera* still has high survival with high sucrose concentration and after long duration dehydration (up to 23h). For the two species, the water content of encapsulated apices had to be decreased down to 0.15g H2O g^{-1} DW in order to obtain high survival after freezing. The percentage of water loss was of 67, 62, 58 and 55% FW (± 1%) for 0.75, 0.9, 1 and 1.1M sucrose pretreatments, respectively. Our results demonstrated that, in most cases, survival increased when dehydration was extended to a defined threshold, around 0.13-0.15g. H2O g^{-1} DW, which was obtained after desiccation periods from 10 to 18h. It seemed that, with this soft dessication process, we could rub out differences in residual water-free, which certainly exist between apices from a same plot.

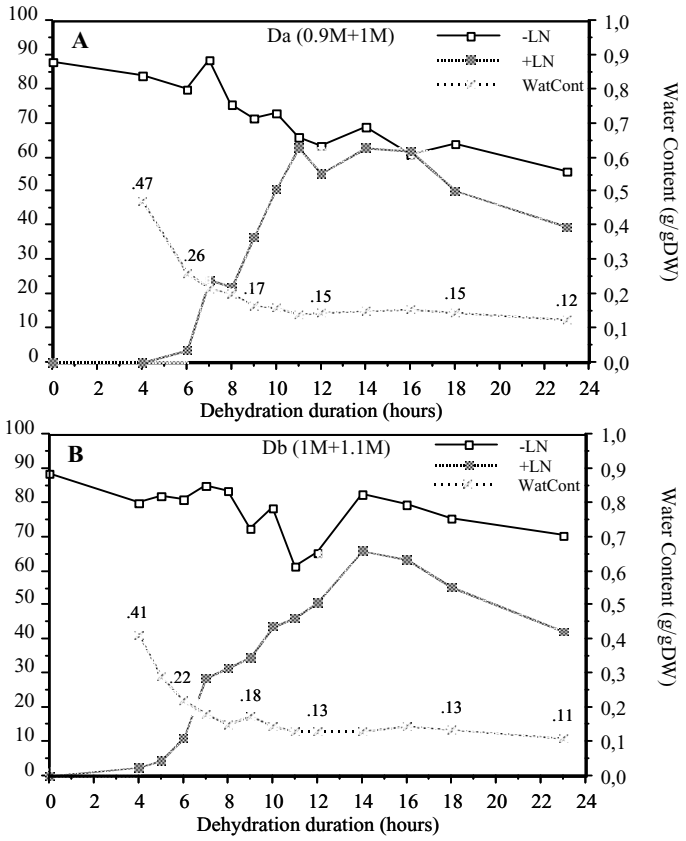

Figure 1. Effect of dehydration duration on the water content of beads and survival rate of control (-LN ❒) and cryopreserved (+LN ■) encapsulated apices of *D. alata* 'Brazo Fuerte' after pretreatment with sucrose (0.9M+1M)- (A), and *D. bulbifera* 'Nouméa Imboro' after pretreatment with sucrose (1M+1.1M)- pretreated ones (B). Each point corresponds to a mean over 2 sucrose concentrations and all the 6 or 8 pretreatment durations used depending on the clone (Malaurie et al., 1998b).

Indexation and Disease-free germplasm production

Indexation

In vitro germplasm conservation presents different advantages such as: 1) to be free from genetic erosion, 2) to have the possibility for the establishment of core collection with long term genebanks, 3) to be free from fungis and bacteria, 4) to be not expensive, when *in vitro* facilities are already present, 5) easy and convenient for international distribution. But International exchanges need more for safe international exchange. We need to know the plant material on genetic level, and over all on the phytosanitary level. On the phytosanitary level, various viruses have been described on edible and medicinal yams on their production area. Different works, depending virus and virus group, are reported (Table 7). Indexing techniques allow to highlight a certain number of viruses on yam: Poty, potex, badna and cucumo-viruses, where yam mosaic virus (YMV) provokes the most important loss.

Table 7: Viruses of yam: group and type viruses, yam species affected and reference works

Virus Group	Virus	Yam species affected	Geographic spreading	Disease importance	Authors
Cucumovirus	CMV	*D. alata*, *D. cayenensis-D. Rotundata* complex, *D. Trifida*	Caribbean and West-Africa	-	Migliori, 1977; Fauquet and Thouvenel, 1987
'Carlavirus'	ChYNMV	*D. batatas*	Japan	-	Fukomoto and Tochihara, 1978;

cf.	(Chinese yam necrotic mosaic virus)				Shirako and Ehara, 1986
Badnavirus	DBV (*Dioscorea* bacilliform badnavirus)	*D. alata*	Barbade	+/-	Mantell and Haque, 1978
	DaBV (*D. alata* bacilliform virus)			-	Degras, 1986
	DbBV (*D. bulbifera* bacilliform virus)			-	Degras, 1986
Potexvirus	DLV (*Dioscorea* latent virus)	*D. floribunda* *D. composita*	Puerto Rico	-	Hearon et al., 1978; Phillips and Brunt, 1988; Waterworth et al., 1974
	PVX (Potato virus X)		*In vitro* collection	-	Urbino et al., 1998
Potyvirus	YMV (yam mosaic virus)	*D. alata, D. Cayenensis-D. Rotundata* complex,	All the intertropical area	+++	Thouvenel and Fauquet, 1979;Goudou-Urbino,1995; Goudou-Urbino et al., 1996a,b
	YMMV [1] (yam mild mosaic virus)	*D. alata, D. Cayenensis-D. Rotundata* complex,	West-Africa	+++	Mumford and Seal, 1997
	D. trifida virus [2]	*D. trifida*	Guadeloupe	+++	Migliori, 1977
	DGBMV [2] (*Dioscorea* green banding mosaic virus)		Togo	++	Porth and Nienhaus, 1983
	DaRMV [3] (*D. alata* ring mottle virus)	*D. alata*	Togo	++	Porth and Nienhaus, 1983
	DaV [4] (*D. alata* virus)	*D. alata* *D. rotundata*	Togo	+	Reckhaus and Nienhaus, 1981
	D. dumetorum potyvirus		South-Pacific	-	Mumford and Seal, 1997
	D. esculenta potyvirus		South-Pacific	-	Mumford and Seal, 1997
	DGBV (*Dioscorea* greenbanding potyvirus)	*D. composita* *D. floribunda*	Puerto Rico	-	Hearon et al., 1978; Phillips et al. 1986
	PVY (Potato virus Y)		*In vitro* collection		Urbino et al., 1998

[1] YMMV: is it a new potyvirus or a strain of the YMV?
[2] *D. trifida* virus and DGBMV have been shown as YMV strains (Porth et al.,1987)
[3] DaRMV should be a 'yam strain' of the beet mosaic potyvirus transmissible on N. benthamiana (Porth et al.,1987)
[4] DaV is serologically links toYMV but differ by is non-transmissibility (Porth et al.,1987)

During the establishment of the yam *in vitro* germplasm collection, in the biotechnology laboratory of ORSTOM (Malaurie et al., 1993), afterwards IIRSDA - Adiopodoumé research station, near Abidjan, Ivory Coast - clones were systematically indexed by ELISA directed to YMV, when introduced *in vitro* (Malaurie and Thouvenel, 1988; Malaurie et al., 1988a,b; Charrier and Hamon, 1991).

Later on, one indexation was carried out by a virologist team on the duplicate of the yam *in vitro* germplasm collection enriched by introduction of new genotypes. 92 samples, belonging to several yam species, were used for the indexation : *D. alata, D. bulbifera, D. cayenensis-rotundata* complex*, D. dumetorum, D. esculenta, D. mangenotiana, D. praehensilis, D. shimperiana, D. togoensis, D. trifida*. These samples were originating from various geographic areas: Africa, Caribbean, South America and Asia. Four viruses were fetched by ELISA technique: PVX (potato virus X, potexvirus) PVY (potato virus Y, potyvirus), CMV (cucumber mosaic cucumovirus), and YMV (Urbino et al. 1998). Results are presented in Table 8.

Table 8: Indexation of the ORSTOM *in vitro* yam collection

ELISA results	PVX	PVY	CMV	YMV
% Positives	5,5	6,3	2,1	7,7
% Negatives	94,5	93,7	97,9	92,3

Source: Urbino et al. (1998)

This study allowed to show that detection of viruses serologically linked to PVX and to PVY, in different yam species, was possible, even with the same frequencies than with YMV. Further works have to be done for a precise caracterization of these virus isolates, and check their respective importance in natural environement. Experiments using more sophisticated techniques for virus diagnostic (IC/rt/PCR) are developed (Bousalem, 1995). Yam on molecular caracterization and molecular diversity on potyvirus of the yam mosaic virus (YMV) have been developed on the ILTAB/ORSTOM-TSRI laboratories (Aleman, 1996; Aleman et al 1996a,b) and from the LPRC laboratory (Bousalem, 1995; Urbino et al., 1998).

Virus eradication techniques

The use of *in vitro* techniques allows to be free from fungis, bacteria, and other pests. Only viruses could be present on the plant and have to be eradicated. Different techniques exist and are already applied on yam. There are meristem culture, thermotherapy and/ or chemotherapy (36°C during 1 to 2 weeks on *in vivo* or *in vitro* plants, use of chemicals such as vidarabine, ribavirin and 2-thiouracil). They could be used alone or associated (Table 9).

Table 9 : Yam disease eradication techniques

	Eradication techniques	**Species**	**Authors**	**Type of use***	**Virus eradication**
(A)	meristem culture	*D. cayenensis-rotundata*, *D. japonica, D. opposita, D. praehensilis, D. rotundata, D. trifida, Dioscorea* spp.	Cortes Monllor et al., 1982; Kobayashi, 1991; Malaurie et al., 1988a,b, 1992, 1995a,b; Malaurie and Thouvenel, 1988; Matsubaru and Ishira, 1988; Mikami, 1984; Saleil et al., 1990;	E	+ / -
(B)	Thermotherapy *in vivo* + meristems culture	*D. alata*	Mantell et al., 1980	E	+
(C)	Nodal microcutting or apices + Thermotherapy	*D. alata, D. Trifida*	Balagne, 1985; Salazar and Fernandez 1988	E, R (+ / -)	+
(D)	Nodal microcutting +	*D. alata*	Mantell, 1993	E, R (+ / -)	+

(E)	Chemotherapy Nodal microcutting + Thermotherapy &/or Chemotherapy	*D. praehensilis*	Malaurie, unpublished results	E	+ / -
(F)	Meristem culture + Thermotherapy &/or Chemotherapy	*D. cayenensis-rotundata*, *D. praehensilis*	Malaurie, unpublished results	E	+ / -

* E: experimental use; R: routine use

Success in meristem culture depends on the size and location of the explant excised, and on the growth regulator ratio. 'Meristem culture', on Table 9, concerns works using meristem-tips (0.2-0.5 mm long) as well as shoot-tips (0.6-2.5 mm long). Experiments on viability and *in vitro* morphological development of meristem-tips of two sizes, 'small' (0.3-0.5 mm) and 'large' (0.6-0.8 mm), have shown that it was better to use large meristem size to increase the shoot elongation percentage. The use of axillary or apical meristems did not induce difference and should allow an important yield in micropropagating such material from excised meristem-tips. Eleven months after meristem excision, production of plantlets was observed with a rate of 82% and 39% from the survivors, for a clone of *D. cayenensis-D. rotundata* complex and *D. praehensilis* genotype, respectively (Malaurie et al., 1995a,b).

Meristem cultures have been done on 8 clones of 5 *Dioscorea* species belonging to the *in vitro* germplasm collection. Morphological development has been observed and data were recorded 60 days after meristem inoculation. In our case, the production of rooted leafy shoots, 60 days after meristem inoculation, occurred in five clones out of eight, with percentage shoot leaf production of 5 to 26 %, depending on the clone. Six months later, the excised meristems of all clones developed into rooted leafy shoots, where *D. bulbifera*, and *D. dumetorum* was not, to our knowledge, mentioned in the literature (Table 10).

Table 10: Genotypic effect on morphogenetic orientation 2 months after meristem excision of *Dioscorea* spp *

	Total meristems observed	Necrosis [1] %	Organogenesis [2] %	Regeneration [3] %
D. alata	67	27	55	18
D. bulbifera	252	46	37	18
D. bulbifera	146	52	48	0
D. cayenensis-D. rotundata complex	23	65	35	0
D. cayenensis-D. rotundata complex	81	24	51	26
D. cayenensis-D. rotundata complex	81	26	54	20
D. dumetorum	24	83	17	0
D. praehensilis	117	41	54	5

[1] Necrosis. [2] Organogenesis: callusing, rooting, swelling were added together, [3] Regeneration: meristem development into rooted leafy shoots and axillary bud development or bud neoformation.

* (Malaurie, unpublished results)

Works about production of virus-free *in vitro* plants of yam through yam meristem culture alone are very rare. Saleil et al. (1990) on *D. trifida* obtained YMV-free plants, after ELISA indexation with a 27% rate through the total indexed plants. Nevertheless, other unpublished results on 2 genotypes YMV-infected of 2 species (*D. cayenensis- D. rotundata* complex, *D. praehensilis*) showed that meristem culture allowed the production of virus-free plants with 76% and 17% plants indexed, respectively (Malaurie, unpublished results).

Production of virus-free *in vitro* plants of yam has been attempted through thermotherapy, chemotherapy associated or not, from *in vivo* mother plants, nodal cuttings or apices (Balagne, 1985; Mantell, 1993; Mantell et al., 1980; Salazar and Fernandez, 1988). None of them described clearly the percentage of virus-free plants obtained through these techniques. Meanwhile, the production of plantlets free from virus is described by Mantell (1993) on *D. alata* cv. Kinabayo, after the action of antiviral agents (vidarabin, ribavirin) on nodal microcuttings infected by a potyvirus. The production of virus-free plants have been obtained 210

days after *in vitro* inoculation, after 3 subcultures of 60, 120 and 30 days on a liquid/solid biphasic cuture system with 10^{-5} M of antiviral agent.

Other available techniques could be electrotherapy used on potato, with 60 to 100% success as compared to 25-40% with thermotherapy (Lozoya-Saldaña et al., 1996; Bernal et al., 1998), or apex micrografting, used on Lemon tree or vine, routinely.

If different works have already been done on yam sanitation, only a few of them conducted to an eradication of virus with more or less importance.

Safe international exchange

Exchange and distribution of plant material could be done by two ways: 1) with non aseptic plant material (tubers, aerial tubers, seeds, nodal cuttings from the vine), 2) with plant material in aseptic conditions (micro-nodal cuttings, microtubers, aerial microtubers, apices, zygotic or somatic embryos, callus and cells suspension).

Exchange in non-aseptic conditions was used in the past, but required severe quarantine measures. Since 1989, with the FAO/IBPGR technical guidelines for the safe movement of yam germplasm, recommendation has been given to use *in vitro* conditions for exchange and distribution. For that, safe movement of yam germplasm could be done easily by three ways: 1) micro-nodal cuttings, 2) micro-tubers, 3) or encapsulated apices.

Safe movement of yam germplasm by micro-nodal cuttings is the most common way and has been frequently used (Malaurie et al., 1998a). In Table 11, the use of laboratories with *in vitro* and quarantine facilities allowed the indexation, *in vitro* introduction and micropropagation for a safe diffusion of various genotypes from different geographical origin.

Table 11: Enrichment of the genetic diversity of a country by transfer and introduction of *in vitro* yam genotypes from different geographic origins*

Species	Number of accession	Sending countries	Receiving countries
D. alata	6	Ivory Coast	New Caledonia
D. alata	5	West Indies	New Caledonia
D. alata	1	Brazil	New Caledonia
D. alata	5	West Indies	Ivory Coast
D. alata	3	New Caledonia	Ivory Coast
D. alata	3	Brazil	Ivory Coast
D. bulbifera	1	New Caledonia	Ivory Coast
D. cayenensis-D. rotundata complex	4	Ivory Coast	New Caledonia
D. cayenensis-D. rotundata complex	1	Brazil	Ivory Coast

* All plant material from the sending countries were, at first, tubers sent to laboratories with quarantine and *in vitro* culture facilities (1988-89: Orstom & Iirsda, Adiopodoumé, Côte d'Ivoire; 1992-95: Orstom, LRGAPT, Montpellier) for their *in vitro* introduction and micropropagation, preliminary to all safe international exchange.

Tuber potentiality shown by a great number of *in vitro* yams (aerial and basal micro-tubers) could be also used for a safe transfer of yam germplasm. They could increase the percentage of success during their acclimatation in field (John et al.,1993; Malaurie et al., 1993; Mantell, 1993; Ng, 1988; Ng and Mantell, 1997). These tubers developed *in vitro* are dormant at maturity and they still keep their dormancy from 2 to 5 months, as tubers developed *in vivo*.
Recently a new method, experimented over three yam species (*D. alata*, *D. opposita*, *D. rotundata*), has been proposed by Hasan and Takagi (1995). They use encapsulation technique, with the embeddment of nodal cuttings in alginate beads, for a concept of a material transfer. This process allow to maintain in the dark for at least 2 weeks. These 2 weeks in the dark allow to envisage a safe and easy international exchange of genetic resources.

Concluding Remarks

This paper tries to describe different studies done and to be done on yam *in vitro* germplasm conservation and its safe international exchange. Yam *in vitro* culture contributes to the safeguard of the biodiversity of the genus *Dioscorea*. An application of the results obtained on cryopreservation to more species should allow a transfer of technology. The use of new techniques, in a one hand, for pathogen eradication (electrotherapy, micrografting), in addition to the existent ones, and in the other hand, for the obtention of plants resistant to somes viruses (transformation), should guarantee to yam a state of virus-free plant and allow

international exchanges, and in long term, distribution to the farmer of cultivar free from virus.

To conclude we can say that we are already able to manage routinely yam *in vitro* genebanks in slow growth culture, to index for more viruses, and to produce some virus-free *in vitro* plantlets.

For an efficient distribution - transfer - utilisation of yam germplasm, we should develop: 1) virus-free germplasm, 2) restricted size collection, with large diversity, so-called core-collections. For that, *in vitro* conservation under slow growth condition and cryopreservation, have to be applied routinely to more genotypes; virus-indexing has to be done with more precise techniques (rt/PCR); therapy has to be done with several combined techniques to become genotype independant.

But, we should not forget, as previously said by Hanson (1986), that, for a better security of germplasm conservation, different methods of conservation have to be combined (*in situ* - Field Genebanks - , *ex situ* - Seed Genebanks, *in vitro* Genebanks).

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