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The creation of an *in vitro* germplasm collection of yam (*Dioscorea* spp.) for genetic resources preservation

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Summary

A genetic resources preservation program led to an *in vitro* germplasm collection of yam (*Dioscorea* spp.), obtained by nodal cutting and maintained under slow growth conditions with ((Knop, 1865) in George & Sherrington, 1984) modified medium. The collection comprises accessions of 14 species from Africa and Asia, including edible varieties from the humid intertropical areas, viz 10 wild species (*D. abyssinica, D. bulbifera, D. burkilliana, D. dumetorum, D. hirtiflora, D. mangenotiana, D. minutiflora, D. praehensilis, D. schimperana, D. togoensis*), 5 edible 'species' (*D. alata, D. bulbifera, D. cayenensis-D. rotundata* complex, *D. dumetorum* and *D. esculenta*) and 1 interspecific hybrid (*D. cayenensis-D. rotundata* complex, cv. Krengle× *D. praehensilis*). Three factors that may influence the success in transfer from the *in vivo* to the *in vitro* conditions have been studied. These are: the type of introducted material (nodal cutting fragments, seeds and exchanged microplants), the introduction date and the genotype. Some significant differences in success were due to the type of introduced material, whereas the introduction date had no effect. On the other hand, some species showed a greater success in the transfer from the *in vivo* to the *in vitro* conditions than others. The three tuberization types (basal tuberization, aerial tuberization and 'boulage' (tuberization without vegetative development) phenomena)), according to species, are discussed.

Introduction

The genus *Dioscorea* comprises nearly 600 species (Knuth, 1924; Coursey, 1967) distributed all over the humid intertropical zone (Bailey, 1960). Yam domestication would have occurred independently in Asia, in Africa and in America (Burkill, 1939; Chevalier, 1946; Alexander & Coursey, 1969). Today, 40 to 50 species are cultivated, or are collected from the wild (Martin & Degras, 1978). In West Africa, there are 5 cultivated species, *D. alata* L., *D. cayenensis* Lamk.-*D. rotundata* Poir. complex, *D. esculenta* (Lour.) Burkill, *D. dumetorum* (Knuth) Pax, *D. bulbifera* L. Nevertheless, wild yams (*D. praehensilis* Benth., *D. mangenotiana* J. Miège, *D. abyssinica* Hochst. ex Kunth, *D. hirtiflora* Benth., *D. minutiflora* Engl.) can still form an important component of the human diet (Miège, 1952; Hamon, 1987). Part of the bio-diversity has been collected for the breeding programs and is currently preserved in

field genebanks (Miège, 1952; Hamon, 1987). Significant risks of genetic erosion are linked to this kind of preservation. These risks are mainly due to diseases such as anthracnose and yam mosaic (Haque & Mantell, 1980; Toribio et al., 1980; Thouvenel & Fauquet, 1982; Notteghem, 1985), nematodes (Kermarrec et al., 1980; Bridge, 1982), insects (Sauphanor & Ratnadass, 1985) and rodents. To these risks, are added high maintenance costs, due to a high labour requirement, 370 up to 423 mandays per ha (Onwueme, 1982; Rankine & Ferguson, 1974 stated in Degras, 1986), necessary for moundmaking, weeding and stacking. The stacking, which is the most expensive process, is an essential operation due to the very high vegetative growth of the lianas, that may reach 30 m in length with more than 1000 leaves (Trouslot, 1985).

As recommended by IITA (IITA annual report, 1981; Ng & Hahn, 1985; Ng & Ng, 1990) and by IBPGR (Hanson, 1986), *in vitro* preservation of these collections avoids these problems. However, this process requires maximum survival rates, slow growth to limit sub-culturing, and should be applicable to all genotypes. In her bibliographic review on yam collection preservation, Hanson (1986) pointed out that currently too few species are maintained under aseptic conditions and that the long term maintenance of these cultures, without a loss of their genetic stability, is uncertain. Therefore, she suggested to sub-culture the clones every two years and to use growth retardants as additives to the culture medium. For example, 0.2 M of mannitol has been used for the *in vitro* shoot tips of *D. rotundata* (Henshaw, 1982).

On the Côte d'Ivoire, the use of *in vitro* preservation was considered as early on as 1985. In this paper, the diversity of material introduced into the *in vitro* germplasm collection, and the problems encountered are discussed (the latter include the degree of inoculation success, callus induction difficulties, 'boulage' and tuberization).

In vitro preservation conditions

Basic conditions

The different organs and/or group of organs used to initiate the *in vitro* cultures belong to 3 types: uninodal cuttings from stem fragments (82,3%), seeds from collecting missions (13%) and *in vitro* plantlets from inter-institute exchanges of plant material[1] (4.7%).

The stem fragments and seeds are both sterilized by soaking in 1% mercuric chloride solution ($HgCl_2$) for 1 to 3 minutes. Sterilization is achieved in presence of an absorptive agent (Tween 20). After 4 to 5 washes with sterile water, the nodal cuttings are inoculated into test tubes, filled with 10 to 15 ml agar medium, in a laminar air flow cabinet. Test tubes (24 mm diameter; 15 cm high) are closed by a polycarbonate cap and sealed with an extensible transparent film.

In vitro cultures are maintained at a temperature of 28° C ± 2° C under a 16 h or 12 h per 24 hours photoperiod. Light flux of about 120 μ E $m^{-2}.sec^{-1}$ is provided by equal numbers of Grolux and Coolwhite fluorescent tubes. Each clone, represented by 14 replicates, is sub-cultured every 6 to 12 months depending the clone in question. Subculturing is carried out so as to maintain, for each clone, a number of physiological development stages.

The basal culture medium contains Knop's modified mineral nutrients, Murashige's and Skoog's (1962) modified vitamins, 3% sucrose and 0.8% agar, 0.2% activated charcoal and 200 $mg.l^{-1}$ glutamin (Table 1). This medium is used for *in vitro* maintenance under minimal growth conditions. This medium is adequate for the introduction of most of the clones (86%). For the difficult cases, a so-called 'introduction' medium was necessary. Here, the activated charcoal and glutamin are replaced by a balance of plant growth regulators NAA/BAP (1 $mg.l^{-1}$

[1] In addition to the plant material taken from the Côte d'Ivoire field collections since 1985, some plant material 'importations', for *in vitro* introductions, have occurred between 1986 and 1990 with the usual phytosanitary recommendations for foreign material received in the form of tubers: [Brazil (1987–89), Cameroun (1986–89–90), Guadeloupe (1986), New Caledonia (1986–89), Martinique and Polynesia (1988), Puerto Rico (1986)].

and 0.2mg.l^{-1}, respectively). After induction, the difficult clones are sub-cultured onto the basal culture medium. A certain number of studies refer to the *in vitro* micropropagation of yam nodal cuttings and/or to tuberization phenomenon observations, with or without growth regulators (Uduebo, 1971; Mantell et al., 1978; Arnolin, 1980; Cortes Monllor et al., 1982; Forsyth & Van Staden, 1982; Espiand, 1983; Ammirato, 1984; Forsyth & Van Staden, 1984; Fautret et al., 1985; Lacointe & Zinsou, 1987; Ng, 1988; Dalouman, 1989; Mantell & Hugo, 1989, Jean & Cappadocia, 1991).

Table 1. Low growth medium composition

	Low growth	
	2 GGC	medium
Macronutrients	mg·l^{-1}	mM
Ca (NO3)2, 4H2O	1000	4.2
(NH4) SO4	250	1.9
Mg SO4	250	1.0
7 H2O KH2 PO4	250	1.8
KCl	250	3.4
Micronutrients	mg·l^{-1}	µM
H3BO3	1	16
Mn SO4, H2O	0.755	4.5
Zn SO4, 7 H2O	1	3.5
Cu SO4, 5 H2O	0.03	0.1
Al Cl3	0.03	0.2
Ni Cl2, 6 H2O	0.03	0.1
Fe SO4, 7 H2O	27.8	100
Na2 EDTA, 2 H2O	37.25	100
Organic compounds	mg·l^{-1}	mM
Glycin	2	44.4
Myoinositol	100	550
Nicotinic acid	5	40.6
Pyridoxin HCL	0.5	2.5
Thiamin HCL	0.5	1.5
Biotin	0.05	0.2
Folic acid	0.5	1.1
Glutamin	200	1370
Sucrose (g·l^{-1})	30	
Activated charcoal (g·l^{-1})	2	

Factors influencing success

Three factors may influence the success of the passage from the *in vivo* to the aseptic culture condition: the type of explant material (uninodal cutting of stem fragment, seeds and exchanged microplants), introduction date and the genotype.

In the first case, differences in success rates was found due to the type of introduced material; seeds show the highest rate of success (92%) which is significantly different from uninodal cutting stem fragments (78%). Introduction in the form of *in vitro* plantlets gave 61% success, but populations were too small to allow a statistical comparison (Table 2).

Next, the period over which plant material can be introduced *in vitro* through nodal cuttings is dependent upon the yam cultivation cycle for the tuber crop harvest, at the end of December to the beginning of January. The dormancy period, which is very variable from one clone to another, forced inoculations to be staggered in time, between February and July. Nevertheless, it was found that introduction date has no effect on *in vitro* inoculation success.

Lastly, the percentage of inoculation success varies from one species to an other. *D. alata* showed the highest success level (84%) which differed significantly from the *D. cayenensis-D. rotundata* complex (59%). *D. esculenta* displayed 80% success, but insufficient population sizes did not allow a statistical comparison. With *D. togoensis* Knuth and *D. du-*

Table 2. Influence of the type of material introduced on inoculation success

	Observed values		
	In vitro plantlets[1]	Nodal cuttings	Seeds
Introduction number	13	246	40
% success	61	78	92

[1]Insufficient theoretical sample size (<3).

Comparison Nodal cuttings/Seeds		
X^2=6.439	df= 1	S for the level 0.01 <p <0.02

metorum, with a smaller population size, the rate of success was respectively 7 of the 10 and 2 of the 3 introduced clones (Table 3). To sum up, only one attempt at inoculation was required for 244 clones of the 299 introduced. However, some of the clones required 2 to 3 attempts at inoculation over several years, before they could be maintained *in vitro* by sub-culture. In addition, after 5 years of *in vitro* micropropagation, the clones of *D. alata* displayed a significant difference in their *in vitro* survival (84%) compared to clones of the *D. cayenensis-D. rotundata* complex (71%) (Table 3). These latter were also more slow to adapt to *in vitro* culture conditions: nodal cutting bud(s) were often choked by the proliferation and bursting of tissues. Their initiation required callus induction, followed by sub-culturing onto the maintenance medium.

Tuberization

Types of tuberization

In vitro tuberization was observed for 9 species out of a total of 14 introduced into the *in vitro* germplasm collection and also for the interspecific hybrids (Malaurie & Tardieu, 1988). This occurs, depending on the clones, between 2 and 12 months, after a sub-culture. Three types of tuberization were observed:

- basal tuberization or formation, at the level of the original nodal bud, of a tuber or a microtuber in the agar medium. This phenomenon was observed in 9 species and the 12 interspecific hybrid clones (Fig. 1);
- aerial tuberization or aerial microtuber(s) formation on the vegetative part, at the level of the axillary buds (next to the leaf axil). This second type of tuberization was observed for all but three (*D. hirtiflora, D. mangenotiana* and *D. schimperana* Hochst. ex Kunth) of the species

Table 3. Number of clones, by species, capable or not to be introduced and maintained *in vitro*

Species	Total number of clones introduced	Introduction	Maintenance
		Clone introduction success[4]	Number of clones maintained *in vitro*[3]
D. alata	109	92^{a2}	74^{c2}
D. bulbifera	8	8^{1}	$8^{2,5}$
D. cayenensis-D. rotundata complex	117	69^{b2}	68^{d2}
D. esculenta	10	$8^{2,5}$	$3^{2,5}$
D. mangenotiana	14	14^{1}	$14^{2,5}$
D. togoensis	10	7^{1}	$7^{2,5}$
Interspecific hybrids	16	16^{1}	$12^{2,5}$
D. dumetorum	3	2^{1}	2^{1}
D. hirtiflora	1	1^{1}	1^{1}
D. praehensilis	3	3^{1}	3^{1}
D. schimperana	1	1^{1}	1^{1}
	X^2 global	17.77	4.114
	df	1	1

[1]Species not analyzed by a statistical comparison for *in vitro* introduction and maintenance.
[2]Species examined by statistical comparison.
[3]Values followed by different letters are significantly different for $0.02 < p < 0.05$, after comparison with the X^2 Pearson test.
[4]Values followed by different letters are significantly different for $p < 0.001$, after comparison with the X^2 Pearson test.
[5]Insufficient theoretical sample size (<3).

which developed *in vitro* basal microtuber (Fig. 1);

- tuberization resembling 'boulage' (Madec in Courduroux, 1967) which results in the formation of a microtuber without development of a leafy stem. This third type of tuberization was observed on a number of clones belonging to 3 species: *D. alata* (10 clones over 71[2]; *D. bulbifera* (1 clone over 8); *D. cayenensis-D. rotundata* complex (19 clones out of 105[2]).

Tuberization ability

The five species most represented in the collection (*D. alata, D. bulbifera, D. cayenensis-D. rotundata* complex, *D. mangenotiana, D. togoensis* and the interspecific hybrids) were compared for their ability to produce tubers under the described tissue culture conditions (Fig. 1).

[2] The clones introduced into the *in vitro* germplasm collection, in 1990, were not taken into account.

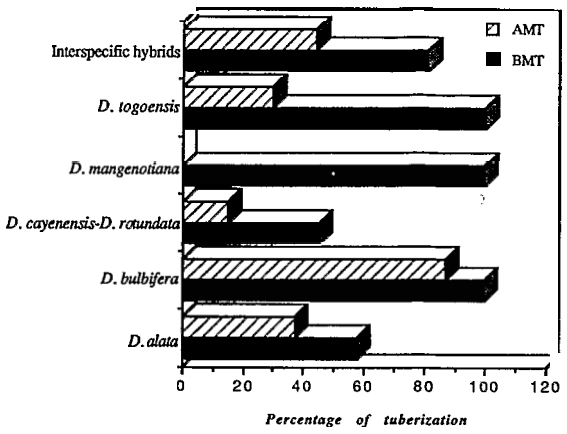

Fig. 1. Percentage of tuberization (basal microtuber-BMT and aerial microtuber-AMT) of 'species' with at least 8 introductions. The other species (*D. dumetorum; D. hirtiflora; D. praehensilis* and *D. schimperana*) with less than 8 introductions have not been considered on this histogram.

For aerial microtuber formation, a significant difference at the 1% level was observed between *D. alata* (56% of clones with aerial microtuber formation) and the *D. cayenensis-D. rotundata* complex,

Table 4. Number of clones per species with aerial and basal microtubers

Species	Tuberization		
	Total observed clones	Clones with aerial microtubers[3]	Clones with basal microtubers[4]
D. alata	71	40^{e2}	66^{i2}
D. bulbifera	8	8^{2}	$8^{2,5}$
D. cayenensis-D. rotundata complex	105	19^{fgh2}	55^{j2}
D. esculenta	3	0	0
D. mangenotiana	14	0	$14^{2,5}$
D. togoensis	7	$3^{2,5}$	$7^{2,5}$
Interspecific hybrids	14	7^{fgh2}	$14^{2,5}$
D. dumetorum	2	2^{1}	2^{1}
D. hirtiflora	1	0	1^{1}
D. praehensilis	1	1^{1}	1^{1}
D. schimperana	1	0	1^{1}
	X^2 global	28.78	32.401
	df	2	1

[1] Species not considered by a statistical comparison for *in vitro* tuberization.
[2] Species analyzed by statistical comparison.
[3] Values followed by different letters are significantly different for p= 0.01, after comparison 2 by 2 with the Ryan test.
[4] Values followed by different letters are significantly different for p <0.001, after comparison with the X^2 Pearson test.
[5] Insufficient theoretical sample size (<3).

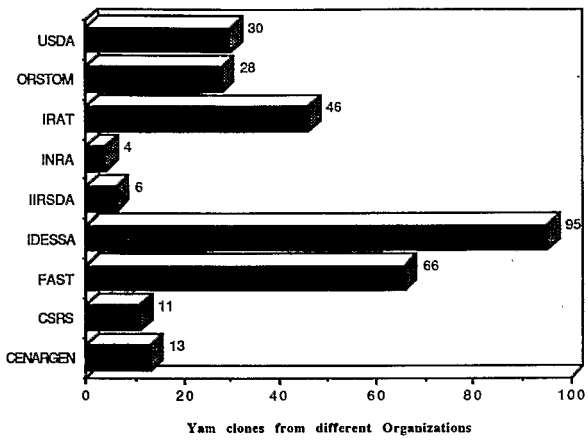

Fig. 2. Organization and importance of their contributions to the creation of a yam (*Dioscorea* spp.) *in vitro* germplasm collection by nodal cutting. Cenargen/Embrapa, Brazil; CSRS, Orstom and Iirsda (Institut International de Recherche Scientifique pour le Développement en Afrique), Research Centre of Adiopodoumé, Côte d'Ivoire; Fast, Abidjan, Côte d'Ivoire; Inra-Guadeloupe; USDA, Mayaguez Institute of Tropical Agriculture, Puerto Rico.

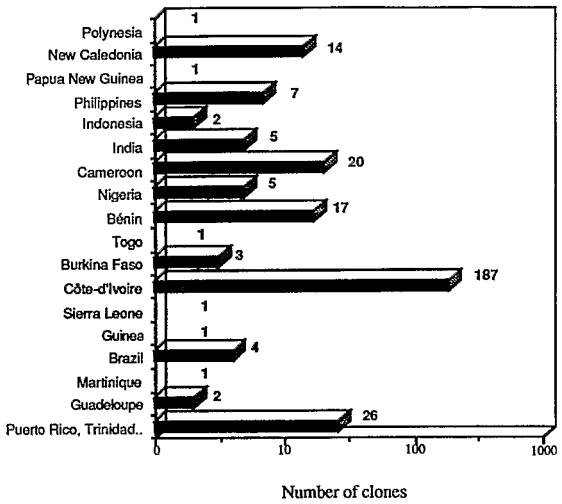

Fig. 3. Countries of origin of the plant material and importance of their contribution to the constitution of a yam (*Dioscorea* spp.) *in vitro,* genetically diversified, germplasm collection maintained in the form of nodal cuttings.

D. togoensis and the interspecific hybrids (18 to 50% of clones with aerial microtuber formation) (Table 4).

For basal tuber formation, only *D. alata* and the *D. cayenensis-D.rotundata* complex, with sufficient population sizes, were compared. A very high significant difference was found with a high basal tuberization percentage for clones *D. alata* (93%) compared to the *D. cayenensis-D. rotundata* complex (52%) (Table 4). These two types of tuberization, basal and aerial, can develop on the same microplant.

Basal and aerial microtubers allow potential by higher success rates upon transfer to the field (Ng, 1988).

Genetic diversity of the *in vitro* collection

Origins of the introduced material

The plant material came from different live collections maintained in the field located in Côte-d'Ivoire[3], Guadeloupe[4], Martinique[5], New Caledonia[5], French Polynesia[5], or from collecting missions or from an *in vitro* collection based in Brazil[6] (Fig. 2). Some of the in-field collections, had been enriched in the past, by the transfer of traditional clones from diverse geographical areas (Degras, 1986)[7]. Therefore, the clones introduced *in vitro* into our collection, came from a range of 18 different geographical regions (Fig. 3). However, the countries from which the traditional clones came were not necessarily those from which the particular species originated or in which it was cultivated (Table 5, (1)). For example, for the Asian species *D. alata,* only 17% of clones came from the diversity centres of South-east Asia, as opposed to 78% originating from South America and the Caribbean.

[3] Fast/Ensa, Fast/Bouaké, Idessa/Cirad-Irat/Bouaké, Orstom/Iirsda/Adiopodoumé.

[4] Inra/Petit-Bourg.

[5] Cirad/Irat.

[6] Cenargen/Embrapa.

[7] The transfer of certain clones of 3 'species' (*D. alata, D. esculenta* and the *D. cayenensis-D. rotundata* complex) present in the 'M.I.T.A.' collection [Mayaguez Institute of Tropical Agriculture (Puerto Rico)] in the Orstom collection had been performed by Martin in 1974.

Interspecific diversity of the introduced material

The interspecific diversity of the *in vitro* germplasm collection is important. Among the 20 species of *Dioscorea* in Tropical West Africa (Miège, 1968), 13 were introduced *in vitro* germplasm collection, 3 of which were edible and widely cultivated (*D. alata, D. cayenensis-D. rotundata* complex, *D. esculenta*), 2 included edible forms as well as toxic wild varieties (*D. bulbifera, D. dumetorum*), 8 were wild (*D. abyssinica, D. burkilliana* J. Miège, *D. hirtiflora, D. mangenotiana, D. minutiflora, D. praehensilis, D. schimperana, D. togoensis*). Moreover, 1 interspecific hybrid (*D. cayenensis-D. rotundata* complex, cv. Krengle × *D. praehensilis*) was also introduced (Fig. 4). These 14 'species' are all annuals except for *D. burkilliana* and *D. minutiflora* which are perennials and *D. mangenotiana* which is half-perennial. Only 7 wild West-African species[8] are not yet included in the *in vitro* germplasm collection.

[8] *D. lecardii* De Wild, *D. liebrechtsiana* De Wild, *D. preussii* Pax, *D. quartiniana* A. Rich, *D. sagitifolia* Pax, *D. sansibarensis* Pax and *D. smilacifolia* De Wild.

Intraspecific diversity of the introduced material

To this interspecific diversity may be added a great intraspecific variation. Two 'species' (*D. alata* and *D. cayenensis-D. rotundata* complex) are represented by over 100 different accessions. Four other species (*D. esculenta, D. bulbifera, D. mangenotiana, D. togoensis*) and the interspecific hybrid have between 8 and 16 accessions each. The other species which are represented by between 1 and 4 should be enlarged by new introductions so as to enhance intraspecific variability.

An important percentage of the field collections of Côte d'Ivoire have been introduced into the *in vitro* collection. In addition, 53% (95 introduced clones), 52% (66) and 81% (57), respectively, of the in-field collections of Idessa and Fast, based in Bouaké, together with those from Orstom/Iirsda, based in Adiopodoumé, are represented.

Moreover the groups established within *D. alata* (N'za group, purple flesh (25 accessions); Bété Bété group, white flesh (28 accessions); not presently identified (56 accessions)) have *in vitro* representatives. Those introduced from the *D. cayenensis-D.*

Table 5. Distribution of the introduced clones according to region of origin

		DA[6]		DE[6]		DB[6]	DCR[6]
		(1)	(2)	(1)	(2)	(2)	(2)
Zone I[3]	New Caledonia	10	10			2	2
	Pacific			1	1		
Zone II[4]	Cameroon	1	1			2	18
	Nigeria	2	2				
Zone III[5]	Brazil	13	13				4
	Caribbean	34	12	9			4
Total		60	38	10	1	4	28

(1) Total clones present in the *in vitro* germplam collection of which the importation origin depends on foreign collecting missions and importation field collection transfer.

(2) Recently introduced clones in the *in vitro* germplasm collection which didn't appear previously in field collection of the Côte d'Ivoire and whose initial origin didn't belong to Côte d'Ivoire.

[3] South-East Asia.

[4] West and Central Africa.

[5] South America and the Caribbean islands.

[6] DA, *D. alata;* DB, *D. bulbifera;* DE, *D. esculenta;* DCR, *D. cayenensis-D. rotundata* complex.

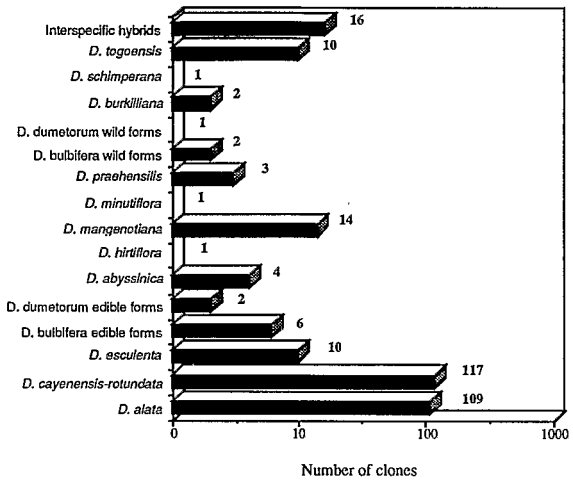

Fig. 4. Different edible and wild cultivated species, and wild toxic species introduced into the yam (*Dioscorea* spp.) *in vitro* germplasm collection maintained by nodal cutting.

rotundata complex (Hamon et al., 1986) represent the majority (17 groups or 85%) (Table 6).

On the other hand, a significant proportion of the *in vitro* germplasm collection comes from the introduction by *in vitro* methods of new varieties to Côte d'Ivoire (Table 5, (2)); their membership of the established groups within the two most represented 'species' will be possible by enzymatic characterization using starch gel electrophoresis with the *in vitro* germplasm (unpublished results) and morphological and enzymatic characterization after the *in vitro-in vivo* transfer.

In vitro preservation security

In order to provide better protection for the plant material, Hanson (1986) recommended that a number of replicates, at least 5, be kept for each clone. In our case, we have limited the *in vitro* collection to a minimum of 12 test tubes per clone. A replicate of the *in vitro* collection, with 2 replicates per clone is stored in another tissue culture room, which increase the safety of the collection.

This author also suggested at least 2 replicates for an *in vitro* collection be kept in different geographical areas (IBPGR, 1985). Currently, the yam *in vitro* nodal cutting germplasm collection exists in triplicate, of which two are in the same geographical site (Iirsda and Fast, in Côte d'Ivoire). The third replicate has been transferred to France to the Genetic Resources and Tropical Plant Breeding Laboratory (LRGAPT) of the Orstom centre in Montpellier. These transfers of *in vitro* material are usually not particularly sensitive, with a loss below 5%. The latter resulted in a loss of 14% of the duplicate clones.

Conclusions and perspectives

We have shown that an 'active *in vitro* genebank' (IBPGR, 1985) was feasible in a genetic resources preservation program with a yam nodal microcuttings under minimal growth conditions. We succeeded in the introduction and maintenance by micropropagation of other species by nodal cutting (*D. burkilliana, D. mangenotiana, D. minutiflora, D. praehensilis, D. schimperana* and *D. togoensis*), which, to our knowledge, are not mentioned in the literature. The range of *in vitro* recovery and maintenance problems observed could be due to the monocotyledonous material, as described by Hunault (1979). The establishment of the collection in a humid tropical area did not create any major technical problems and its transfer to distant geographical zones lead to the loss of 14% of the total accessions. This loss is explained by a transfer of some nodal cuttings recently inoculated *in vitro* without any shoot development of the axillary buds, most of them belong to the *D. cayenensis-D. rotundata* complex and did not develop any leafy shoot. However, some of the species showed a higher aptitude to *in vivo-in vitro* transfer.

In vitro germplasm collections have the advantage of providing a collection of preserved material taking up little space, under phytosanitary conditions, after virus indexing, and free from fungi and bacteria. This enables the transfer of plant material without quarantine problems (Hanson, 1986). The preservation of genetic resources requires the establishment of a minimal collection or 'core collection' (Frankel & Brown, 1984) to be created in order to maintain a high level of genetic diversity within a restricted number of individuals. In Côte

d'Ivoire, the last yams added to the field collections were selected according to these recommendations (Hamon et al., 1986).

The reduced number of sub-culturing operations due by the use of dilute medium, and the lack of plant growth regulators in the medium allows somaclonal variation to be reduced and so preserve material conformity (Ammirato, 1984; Ducreux et al., 1986). *In vitro* methods, such as *in vitro* micropropagation by nodal cutting, basal microtuber, aerial microtuber, somatic embryos permit an easy transfer of material from one point of the globe to another (Ng, 1988). Moreover, after virus indexation, the use of meristem tip cultures should assist in the production of virus-free and other pathogen-free microplants (Saleil et al., 1990).

In the future the use of cryopreservation, from meristems, zygotic or somatic embryos, should enable the consitution and maintenance of 'base *in vitro* genebanks' (IBPGR, 1985). This type of preservation should protect specific clonal stocks and allow the long term maintenance of a large genetic diversity.

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