In vitro Culture, Results and Prospects for Food Yams

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ABSTRACT

Although in vitro culture of yams for pharmaceutical purposes has made great advances, tissue culture techniques for food yams have been advanced less. Data from the literature and original work on Dioscorea alata, D. cayensis-rotundata, D. bulbifera, D. trifida and D. dumetorum in vitro culture are given and discussed in view of strengthening this approach.

Differential species requirements for complete plantlet organogenesis are described. The features of rejuvenation and aging leading to tuberization of in vitro plantlets are emphasized. Problems of obtaining a high multiplication rate in the field from in vitro materials, and methods for production of virus-free planting material using in vitro thermotherapy of plantlet and meristem tip cultures are also discussed.

Introduction

In vitro culture of yams dates back as far as 1958, at least, with the experiment by Sawada and al. of the effects of sucrose and peptones on the organogenesis and growth of bulbils from leafless nodes of Dioscorea opposita. Bulbil organogenesis again in 1968 (Asahira and Nitsch, 1968) and, then, with D. bulbifera in 1970 and 1971 (Uduebo) remains the first approach of the technique, until about 1975 a widest interest begins for in vitro yam culture, applied primarily to the diosgenine producing species.

Most researchers used directly part of the entire leafed or leafless node as material. Some research used other parts of the plant:

- leaf of D. deltoidea (Mascarenhas and al., 1976) or D. floribunda (Sinha and Chaturvedi, 1979).
- seedling hypocotyl of D. deltoidea (Grewal and Atal, 1976).
- tuber of D. deltoidea (Mascarenhas and al., 1976, Singh, 1978).
- apical meristem of D. deltoidea (Grewal and al., 1977).

Mapes and Urata (1970) could not obtain any proliferation from Dioscorea sp. internode.

As in many cases with other plants the medium of Murashige and Skoog (1962) has been the basal one in most research with diosgenine species. Mascarenhas and al. (1976) used also White (1963) or Smith (1967) media in combination with Murashige and Skoog (1962) basal medium. Some less common media have been used by

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some others (Chaturvedi and Chowdhury, 1980, or chinese scientists ,1981, for instance) when callus culture is considered.

The research for the best form and the best ratio of nitrogen compound in shoot and root organogenesis from tuber callus of D. deltoidea has been done by Singh (1978) and by Chaturvedi and Chowdhury (1980) for callus proliferation. But the more frequent research in medium adjustment is the levels and the balance of auxins and cytokinins for shoot and root organogenesis. (Lakshmi Sita and al., 1976, for instance).

A special feature of drug producing plants such as diosgenine Dioscorea species, is their ability to synthesize more drugs with indifferenciated callus or cell suspension cultures than is available from field cultivation. A number of reports bearing on the best techniques for reaching and maintaining this undifferenciated state has been published (Fonin and Voloshina, 1975; Stohs and al., 1975; Chaturvedi and Srivastava, 1976; Marshall and Staba, 1976). What could be useful for food yams in this direction is the investigation of inherent or induced genetic cell variability, in view of selecting new clones, an approach which appears valuable from the variations obtained in number of chromosomes and level of polyploidization with D. deltoidea cells by Karanova and Shamina (1978).

But, before in vitro culture of food yam rises to this level, efforts can be devoted to ensure high ratio of propagation and an easy cleaning of clones from virus.

The first goal of in vitro culture with food yams was an understanding of tuberization through bulbil organogenesis in D. opposita and D. bulbifera. However, the adding of growth substances in the mineral basal medium, which was sufficient for bulbil development, leads Uduebo (1971) to find the parts of the level and the balance of auxin (IAA) and cytokinin (kinetin) in callus, bulbil, shoot and root morphogenesis. Unfortunately, this approach remains a long time useless in its country (Nigeria) and it is only recently that, at IITA (Ibadan), in vitro culture has been again applied to food yams. Fifteen clones of D. alata at least are kept there in germplasm collections in vitro. Indeed, Frison (1981) considers the only important tool or the technique for exchange of free disease material, although he did not indicate if the yam clones were tested for virus cleaning (the explant used is node cuttings and not apical meristem).

Virus cleaning on the contrary has been the main object of the work in Barbados by Haque, Mantell and Whitehall (1978). Mantell and Haque (1979), Mantell and al., (1980). They first established that just as for D. bulbifera (Uduebo, 1970), it was possible to grow D. alata in vitro from node cuttings in the basal mineral medium of Murashige and Skoog only. But this needs plants 4 to 6 month old, no complete organogenesis of shoot and root being obtained from a 2-month-old mother-plant or with auxine and/or cytokinine complement. Apical meristem culture for virus cleaning, however, needs growth substances for successful development. Naphtalene acetic acid and benzyladenine permit within 6 months a normal organogenesis and further micropropagation by node cuttings of the plantlets. As for node in vitro culture mother-plants and plantlets are conducted under 16h daylength. A scheme of virus free D. alata cv. White Lisbon seed is now operating from Barbados with an experimental multiplication net over several West Indian islands (Calixte, 1982). A yield surplus of about 95% can be expected.

Recently Ahoussou (personal communication) began to develop in vitro culture of D. alata and D. cayenensis-rotundata cultivars in the Ivory Coast. Intraclonal variation will be an interesting approach of this new program.

In vitro culture of D. alata has succeeded also in the Laboratories of Y. Demarly and R. Nozeran at the University of Paris-Orsay (Arsene, 1979; Espiand, 1980).

Since 1976 our laboratory began to experiment in vitro culture, first, with the assistance of Dr. Y. Demarly, in direction of androgenesis from D. trifida (Arnolin, 1976). This line of research has been temporarily put aside in favour of development of micropropagation from node cuttings, apidal meristem and callus techniques culture.

Methods, Technics and Material

These aspects having been extensively presented by one of us (Arnolin, 1980), broad traits only will be given here.

New technical developments:

- All work has been conducted from plantlets obtained in vitro (see Arnolin, 1980 for their advantage over primary explants from field or green house plants). They are lighted over 14 h with 2200 lux at 27 ± 2°C under 45% to 70% relative humidity.

Field transfer of plantlets benefits from:

- Pricking out in rich soil first under continuous, then interrupted, mist for 2 months before effective field growing;

- A 60% shading for the first 3 months in the field. All this for micropropagation.

Results

Micropropagation from Node Cuttings

The main result of the quoted work (Arnolin, 1980) was the interest of the N30K medium of Margara (1978) Murashige and Skoog (1962) medium needing a number of complements for a good growth. With N30K however, species differences are remaining:

- rooting ranks first for D. cayenensis-rotundata, less for D. alata and D. trifida, much less for D. dumetorum.

- 60-day old plantlets have between 1 and 2 stems for all species, D. cayenensis-rotundata being somewhat ahead again.

- at the same age, nodes are between 2 and 5, D. alata and D. cayenensis-rotundata being the more advanced species.

New progress in media appraisal obtained:

With a modified MS medium (169) proposed by C. Martin (see Arnolin, 1980), nodes production with D. cayenensis-rotundata cv. V17-2 have been raised to 6,8 at 2 months, that give micropropagation potential of more than 100 000 plantlets over one year.

Intra specific response to MS or N30K medium has been seen with *D. trifida*.

A position effect of in vitro node cutting level on the growth of subsequent plantlet has been noticed; the more basic level can be less prolific.

The response to different levels and balances of growth substances have been deeply studied.

Table 1 shows differential organogenesis from different balances and nature of growth substance in a 169-based medium. The association of kinetine + ANA raised rooting and depress shooting regarding BA or BA plus ANA plantlets.

Table 1. Action of associations of growth regulators on the plantlet organogenesis of *D. cayenensis-rotundata* cv. V17.2.

Characteristics	Associations		
	BA 10^{-6} -	BA 10^{-6} + ANA 5 10^{-5}	Kinetine 5 10^{-7} + ANA 5 10^{-5}
Average number of roots	3.96	1.58	17.00
Average number of stems	2.50	2.22	1.31
Average number of nodes/plants	6.82	3.81	3.61

Table 2 shows in a modified KNOP media other differential organogenesis responses linked to gibberellic acid interactions with ANA and BA. The triple-growth substances together seem to give a better balanced growth, the ANA + GA association giving a higher level of rooting. However, in all cases if rooting is early, roots and stems are thin.

Table 2. Effect of associations of growth regulators on *D. cayenensis-rotundata* cv. V17.2 and *D. trifida* cv. INRA 5-20.

Character-istics	Cultivars	Associations				
		Modified GRENAN Medium[1]	ANA 10^{-7} BA 10^{-6} GA 10^{-6}	0 BA 10 GA 10^{-6}	ANA 10^{-7} 0 GA 10^{-6}	ANA 10^{-7} BA 10^{-6} 0
Root number	V17/2	-	3.39	6.30	8.25	1.93
	5-20	2.26	1.82	1.36	4.04	1.17
Stem number	V17/2	-	2.22	1.70	2.60	1.87
	5-20	1.33	2.95	1.66	2.32	1.18
Node number	V17/2	-	3.64	2.90	3.45	2.90
	5-20	2.78	4.08	3.62	1.86	2.13

[1]Modified Knop + Skoog microelements + Fe + sucrose + agar 4,5 g/l.

Preliminary results suggest positive growth response to agar rising from 4,5 to 8 g/l, sucrose rising from 20 to 50 g/l and a timing for different successive media.

Among other media needing further testing, vitamins and amino-acid levels and balances will be introduced in relation with the mineral elements and growth substances which appears determinant for the best adjustment to each species or cultivar.

Thermotherapy of Plantlets against Virus

Plantlets obtained from field node cuttings of *D. trifida* have been tested (Miss M. Balagne, personnal communication) over a range of temperature to combine best survival with lethal temperature for virus. Data are soon to be published.

Gamma Irradiation of Plantlets for Mutation

D. alata and *D. trifida* plantlets obtained in our laboratory have been submitted to gamma irradiation (varied duration of a weak source) at Montpellier (R. Marie), before pot transfer. They are now in observation in Guadaloupe.

Apical Meristem Culture

Apical meristem culture of *D. alata*, *D. cayenensis-rotundata* and *D. trifica* are on observation. Full organogenesis has been obtained within 5 months with *D. cayenensis-rotundata*, and within 6 month with *D. alata*. However, successes remains low but from the plantlets, as practised in Barbados, node cuttings will be done to grow more plantlets, and to assess their lack of virus.

Field Transfer of In Vitro Plantlets

Table 3 shows the high differences of behaviour from three cultivars transferred to the field in June 1982 after 2 months in vitro and 3 months under mist. They reached maturity together and were harvested in January 1983. No flowering occurred. The most striking features of these results are:

- the high numbers of stems per plant,
- the narrow association between this number and the tubers one,
- the high level of the *D. alata* cv. Ti-joseph yield; mean yield of *D. alata* cv. Belep average the weight of a good experimental seed-set for this cultivar.

Discussions - Conclusions

This review of results with in vitro culture of food yams confirm the high prospects this technique offers.

Some problems of general interest will be stressed here.

Though rather quick success is obtained in micropropagation, the best ratio of multiplication through the more adequate medium to each cultivar deserves additional attention.

Table 3. Characteristics of plants from vitro culture.

Characteristics	V17/2 D. cayenensis/rotundata	Belep D. alata	Ti-Joseph D. alata
	Cultivars		
Average number of stems	1.98	6.29	9.66
Number of plants with flowers	0	0	0
Number of harvested plants	55	50	12
Total number of tubers	90	299	110
Average number of tubers/plant	1.63	5.98	9.16
Total weight of tubers (in g)	8.92	25.740	24.015
Average weight per plant (in g)	16.22	514.80	2,001.25
Average weight of tuber (in g)	9.91	86.08	218.31

Attention might be given to the clonal conformity after in vitro culture. A number of data from other plants suggest that in vitro culture could affect the clonal stability if not its definitive functioning. At least, in *D. trifida*, juvenile stage is clearly carried on with in vitro culture. The plantlet has indefinitely the morphology of a seedling, after a short three-lobed leaf stage when it comes from field node cuttings (see, on Dahlia, Watelet Gonod (1977), for instance). With a modified physiological age of the clone at the plantlet stage, field behaviour could be at variation over some time with normal culture.

The limited field production of *D. cayenensis-rotundata* as well as the high number of stems and tubers observed are perhaps in relation with physiological plantlets condition or environmental conditions. Relation between stems and tuber number has divergent aspects (see Arnolin and Mathurin this Symposium).

The high tuber yield obtained with *D. alata* cv TI-Joseph is encouraging although the tuber shape is not attractive (plantlet x field interactions perhaps). But as proved by the Commonwealth West Indian scheme based in Barbados, the use of micropropagation in yams for *D. alata* cultivars is now at hand for commercial growing.

If *D. trifida* seems less easy to manage, progress is clear in media and techniques.

Now that in vitro culture of food yam seems to enhance their vegetative multiplication, new efforts are needed to develop its contribution to variation. Gamma irradiation could be an interesting path.

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