Effects of sucrose and growth regulators on the multiplication and the microtuberization of yams (*Dioscorea* spp.)

A.Carlier [1]*, K. Odah [2], D. Filloux [3a], P. Vernier [3b] et P. du Jardin [1]*

gembloux faculté universitaire des sciences agronomiques

1 Faculté universitaire des Sciences agronomiques de Gembloux, Unité de Biologie végétale - Passage des Déportés, 2 - 5030 Gembloux, Belgique * *Corresponding authors :* carlier.a@fsagx.ac.be et dujardin.p@fsagx.ac.be
2 Université de Lomé, Laboratoire de Physiologie et de Biotechnologie végétale – BP 1515 Lomé - Togo
3 CIRAD, [a]UMR BGPI et [b]UR Horticulture – F- 34398 Montpellier, France

CIRAD

Introduction

Carlier, 2004

Baudoin, 2002

Yam tubers feed million peoples in the intertropical area and are an important traditional staple food in West Africa. The complex *Dioscorea cayenensis-rotundata* and *D. alata* are the most widely cultivated yam species in the world. As a vegetatively propagated crop, yam is seriously affected by a range of pathogens. Productivity is hampered by pests, diseases and high costs of planting materials.

An *in vitro* germplasm collection and a rapid multiplication process should allow a good preservation of the yam genetic resources. *In vitro* culture also permits the distribution of pathogen-free clonal material in international yam germplasm exchanges programmes. Two *in vitro* conservation methods have been developed in yams : tissues culture microcuttings and microtuberization. *In vitro* plantlets are easily damaged and poor field establishment of *in vitro*-grown plantlets has restricted the implementation of *Dioscorea* tissue culture techniques (Mantell and Hugo, 1989). Microtubers, small basal tubers originating from plant tissues in *in vitro* culture, may provide an alternative to *in vitro*-grown plantlets as means of germplasm exchange, because they are more tolerant to light and temperature variations and less prone to damage than cultured shoots (John et al., 1993). Moreover, yam shoot cultures maintenance often requires frequent subculturing. Microtubers can be stored for long periods without losing their viability, and the field establishment is relatively easy and inexpensive (Ng, 1988).

In many *Dioscore*a species, *in vitro* cultured shoots are able to produce microtubers under certain induction and growth conditions. Factors known to influence the tuberization process include growth regulators, sucrose concentration, nitrogen supply, day length, culture medium and temperature. This paper described the effects of sucrose and growth regulators on the multiplication and the microtuberization of yams (*Dioscorea* spp.)

Materials and methods

Plant material and culture conditions

D. cayenensis-rotundata (cv. Singou) and *D. alata* (cv. Malingova, cv. Tero Osi, cv. Letslets Bokis ans cv. Peter) *in vitro* plantlets are from the germplasm collection of *CIRAD, Montpellier, France*. All cultures were grown in glass tubes containing 10ml of basal medium and were incubated under 16h photoperiod at 25 ± 5 °C. The basal medium for multiplication of nodes consisted of MS salt macro- and microelements (Duchefa) supplemented with 2 ml Morel vitamins solutions, 30 gl-1 sucrose (VWR International), 2 mgl-1 kinetin (Sigma), 2 gl-1 active coal (Sigma) and 4 gl-1 agar-agar (Duchefa). Singles nodes with leaves from material maintained *in vitro* for 2 months in these conditions were used for microtuberization studies.

Odah, 2002

Experiment design and analysis

For all varieties, nodal cuttings were induced to produce nodes and microtubers on modified MS medium supplemented with various concentrations of sucrose (3, 5 and 8 % w/v). In a second time, nodal cuttings of three varieties of D. alata (cv. Tero Osi, cv. Letslets Bokis and cv. Peter) were induced to produce nodes and microtubers on modified MS medium supplemented with various concentrations (0 µM, 0.5 µM, 2.5 µM and 5 µM) of growth regulators. The effects of NAA on the multiplication and the induction of microtuberization were studied on the three varieties of D. alata. The effects of kinetin were studied on D. alata cv Tero Osi and cv. Peter. The effects of zeatin and ABA were studied on D. alata cv. Tero Osi.

All experiments were completely randomized and 15 plantlets were used per treatment, excepted for control plant where 20 nodal cuttings were used. The number of nodes of each plantlet and the plantlets with microtubers (diameter ≥ 2mm) were determined. The statistical analysis was realized with Minitab and Excel software. Mean number of nodes per plantlets and the percentage of microtubers (number of plantlets with microtuber / total number of plantlets) were calculated.

Results and discussion

Effect of sucrose concentration

For the five cultivars, explants growth is favoured by a low concentration (3 or 5 %) of sucrose. But, the highest number of microtubers was obtained with a concentration of 8 % (see table 1). Similar observations have been made for *D. composita* by Alizadeh et al. (1998) and for *D. bulbifera* by Forsyth & Van Staden (1984) and Mantell & Hugo (1989). For *D. alata* and *D. cayenensis-rotundata*, reported results are extremely variable probably due to interactions with others culture conditions. Actually, Ng (1988) found that an important sucrose concentration (8 %) in culture medium, associated with 2.5 µM of kinetin reduces the tuberization percentage for *D. rotundata*. Nevertheless a positive effect of a high sucrose concentration (12 %) associated with a 0.2 mgl-1 kinetin was reported by Odah (2002) for *D. cayenensis-rotundata*. Odah (2002) also found that an increase of sucrose concentration (3 to 6 %) favored tuberization for *D. alata* although Mantell & Hugo (1988) reported an inhibitory effect of a sucrose concentration superior to 2 % for other varieties of this species.

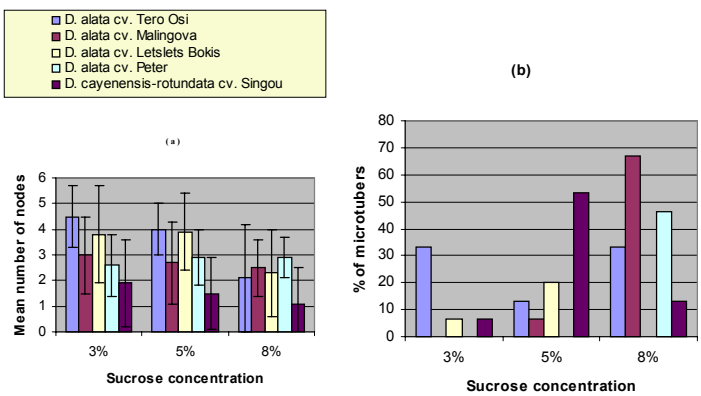

Table 1 : Effect of sucrose concentration on multiplication (a) and microtuberization (b)

Effect of growth regulators

In these experiments, we noted that differences observed among growth regulators treatments were not significant for any varieties. And the results are in general agreement with those presented in previously published reports on microtuber induction in Dioscorea yams. However they are in contradiction with results obtained by Ammirato (1982) and Mantell &Hugo (1989), where growth regulators like NAA, kinetin and ABA have all been shown to promote microtuber induction in *D. bulbifera* and *D. alata*.

However, our study indicated a significant cultivar effect on the efficacy of microtuberization. Previous works indicated different responses between *Dioscorea* species, but our work thus suggests the existence of an intraspecific variability in the microtuberization potential of yams.

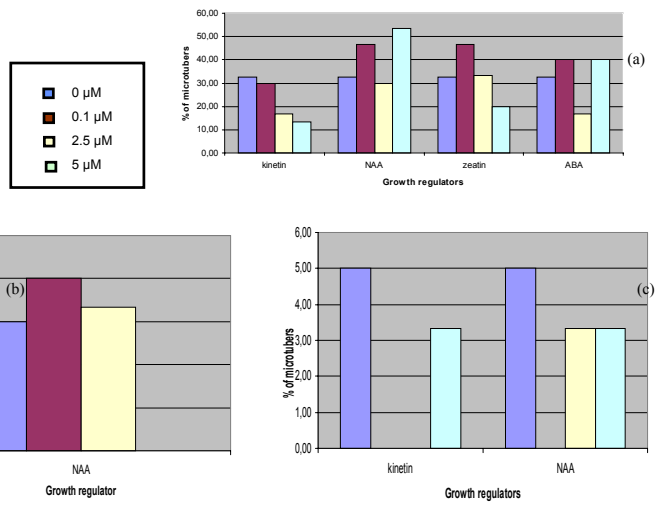

Table 2: Effect of growth regulators on microtuberization on D. alata cv. Tero Osi (a), D. alata cv. Letslets Bokis (b) and D. alata cv. Peter (c)

References:

- Alizadeh AY and Mantell SH (1994) Manipulation of cultural factors to increase microtuber size and frequency in shoot cultures of food yam, *Diosorea alata* cv Oriental Lisbon. ISHS Acta Horticulturae 380.
- Ammirato PV (1982) Growth and morphogenesis in cultures of the monocot yam, *Dioscorea*. In Fujiwara A, Plant tissue culture. 169.170.
- Forsyth C and Van Staden J (1984) Tuberization of Diosocrea bulbifera stem nodes in culture. J. Plant Physiol. 115: 79-83
- John JL, Courtney WH ans Decoteau DR (1993) The influence of plant growth regulators and light on microtuber induction and formation in *Dioscorea a alata* L. cultures. Plant Cell Tiss. Org. Cult. 34: 245-252
- Mantell SH and Hugo SA (1989) Effects of photoperiod, mineral strenth, inorganic ammonium, sucrose and cytokinin on root, shoot and microtuber development in shoot cultures of Diosocrea *alata* L. and *D. bulbifera* L. yams. Plant Cell Tiss. Org. Cult. 16: 23-37
- NG SYC (1988) In vitro tuberization in white yam (*Dioscorea rotundata* Poir). Plant Cell Tiss. Org. Cult. 14: 121-128
- Odah K. [2002]. Etude de l'influence de quelques facteurs exogènes sur le développement in vitro de ***Dioscorea cayenensis-rotundata*** et *D. alata* (*Dioscoreaceae*) cultivées au Togo. Thèse de doctorat. Université de Lomé. Togo. 122 p.

Acknowledgements:

This research was partially funded by the Ministry of Agriculture of the Walloon Region (Belgium). We are grateful to Adeline Blondiaux for her excellent technical assistance.