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In vitro propagation and cytology of wild yams, *Dioscorea abyssinica* Hoch. and *D. mangenotiana* Miège

D. Lauzer, G. Laublin, G. Vincent & M. Cappadocia*
Institut de recherche en biologie végétale, 4101 est rue Sherbrooke, Montréal, Qc, Canada H1X 2B2
(**requests for offprints*)

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Abstract

Two wild yams of West Africa, *Dioscorea abyssinica* Hoch, and *D. mangenotiana* Miège were micropropagated from nodal cultures. Both species produced 4–5 nodes per each node cultured. The size of nodal cuttings was critical, segments shorter than 0.5 cm being less suitable for micropropagation. The number of nodes produced was constant even after 5 cycles of subculture; however, *D. abyssinica* continuous subculture decreased propagation efficiency, resulting in a reduced number of reculturable nodes at each cycle. In *D. mangenotiana*, the decrease in multiplication efficiency affected both the number of total and reculturable nodes. Large-sized microtubers were induced on nodal segments maintained under 8-h daylength in both species. In *D. abyssinica*, however, microtubers were induced on media containing 20, 40, 60 and 80 g l^{-1} sucrose, whereas in *D. mangenotiana* only 40 and 60 g l^{-1} sucrose favoured tuberization. Cytological studies confirmed that the chromosome number of *D. abyssinica* was 2n = 40, although a high incidence of cytochimerism and cells with 2n = 38 were observed in root meristems. In *D. mangenotiana* clones, the chromosome number was 2n = 40, as against 2n = 72 and 2n = 80 reported in literature. This species also displayed karyological stability.

Abbreviations: BA – benzyladenine, NAA – naphthaleneacetic acid, PPF – photosynthetic photon flux

Introduction

The genus *Dioscorea* includes over 600 species (Ayensu 1972), and is of considerable economic importance (Forsyth & Van Staden 1984). A number of *Dioscorea* wild species are the source of compounds used in the synthesis of sex hormones and corticosteroids (Coursey 1967), and cultivated species are the source of food in some tropical countries (Coursey 1976). In both the wild and cultivated species, multiplication obtained by conventional methods is quite slow. Hence, increasing efforts have been made since the mid-1970s to improve clonal propagation of the valuable genotypes by tissue culture, using nodal segments, containing one or more axillary buds. In this regard, most of the studies have been made with medicinal yams such as *D. floribunda* (Chaturvedi et al. 1982). *D. deltoidea* (Grewal et al. 1977) and *D. composita* (Ammirato 1982), although a number of reports are also available on edible species (for a review see Ammirato 1985). More recently, an increasing attention has been paid to the clonal propagation via *in vitro* production of microtubers, e.g. in *D. alata* (Mantell & Hugo 1989) and *D. rotundata* (Ng 1988).

So far, little importance has been given to micropropagation of the wild edible relatives, potentially useful in yam breeding programs. Therefore, a joint effort is going on between Canada and the University of Abidjan (Ivory

Coast) in order to characterize genetically some of the wild relatives, and to establish *in vitro* cultures. In this paper, we report results of studies on shoot multiplication, *in vitro* tuberization and cytology in *D. abyssinica* and *D. mangenotiana*. *D. abyssinica* is a native of Ethiopia, and widespread in the savanna regions of Africa, where it is used as a famine food. *D. abyssinica* is one of the putative parents of *D. rotundata*, the most important cultivated African yam (Coursey 1967).

D. mangenotiana is among the most vigourous species of the genus *Dioscorea* with vines as long as 40 m, and produces tubers that can weigh up to 200 kg (Degras 1986). In addition, this species is reported to harbour nitrifying bacteria in the leaves on the lower part of the stem (Miège 1958).

Materials and methods

All plant material was kindly provided by the Laboratory of Plant Genetics, University of Abidjan, Ivory Coast. Tubers of *D. abyssinica* and seeds of *D. mangenotiana* were grown in a glasshouse (23 ± 3°C) at the Montreal Botanical Garden, until the vines were about 50 cm long. Single-node cuttings about 2.0 cm long after removal of the leaf and trimming of the petiole were surface disinfested with a rapid dip in 70% ethanol, and then with calcium hypochlorite unfiltered solution (7%) for 15 mn, rinsed four times in sterile distilled water, and left for 10 mn in 1% filter-sterilized ascorbic acid solution. The nodal segments were cultured on a basal medium containing Murashige and Skoog (1962) mineral salts, thiamine HCl 0.1 mg l^{-1}, myo-inositol 100 mg l^{-1}, nicotinic acid 0.5 mg l^{-1}, pyridoxine 0.5 mg l^{-1}, sucrose 30 g l^{-1}, and agar (Difco Bacto) (8 g l^{-1}); pH was adjusted to 5.8. The medium was autoclaved at 121°C for 20 min. The cultures were incubated at 25 ± 2°C under a 16-h photoperiod (fluorescent lights, Westinghouse cool-white, 80 μmol m^{-2} s^{-1}). Thereafter, only *in vitro*-produced plant material was used.

Initially, single-node cuttings, up to 0.5 cm (short) and longer than 0.5 cm (long) were compared for their suitability for micropropagation. Later, only long segments were cultured in Kimble borosilicate glass tubes (25 × 150 mm) covered with Bellco caps with 10 ml of the basal medium supplemented with NAA (0.5 μM) and BA (4.4 or 8.9 μM depending upon the species). The growth regulators were added to the medium before autoclaving. The effect of PPF, 35 and 80 μmol m^{-2} s^{-1}, on micropropagation efficiency was also tested. Observations and subcultures were made every 6 weeks.

In order to assess the capacity of the cultures to form *in vitro* microtubers, the long nodal cuttings were cultured on basal medium supplemented with four concentrations of sucrose (20, 40, 60 or 80 g l^{-1}), and 2.7 μM NAA. Cultures were incubated under 8-h or 16-h photoperiod (fluorescent lights, 80 μmol m^{-2} s^{-1}), and compared with those maintained in dark. In *D. abyssinica*, where more abundant plant material was available, 4 replicates of 8 explants each were usually cultured for each photoperiod and sucrose concentration, while in *D. mangenotiana* only one set of 8 explants for each condition could be made. The number and weight of microtubers were recorded after 35 weeks.

Chromosome number of *in vitro* growing material was determined, in each species, from somatic cells at metaphase of the root meristems of 3–5 randomly selected plants 7–8 weeks old. To determine chromosomes, the root tips were treated with a saturated solution of 1-bromonaphthalene for 90 min, stained with Schiff's reagent, and squashed in 1% acetocarmine. For anaphase chromosomes, the 1-bromonaphthalene treatment was omitted. Drawings were made with a Leitz Camera Lucida.

Statistical analyses were done using subprograms ANOVA, ONEWAY and SCATTERGRAM of the SPSS package (Nie et al. 1975).

Results

Shoot multiplication

The culture media used in the present study gave a satisfactory rate of multiplication. The *in vitro* growth habit of the two species, however, differed. After 6 weeks of culture, cuttings of *D. abyssinica* usually produced two or more shoots originating from the nodal region (Fig. 1),

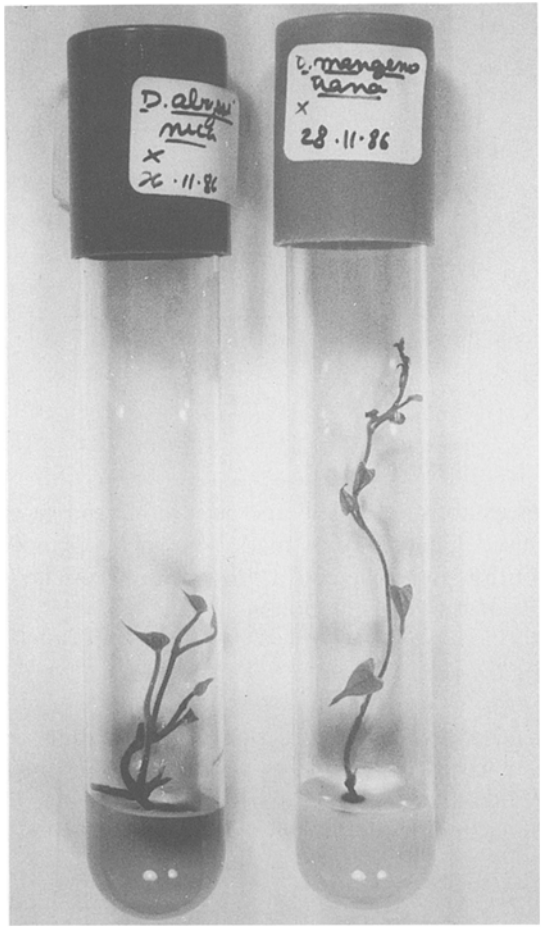

Fig. 1. Shoot development from nodal culture in *D. abyssinica* (L) and *D. mangenotiana* (R) after six weeks.

whereas in *D. mangenotiana* a single elongated shoot was generally observed (Fig. 1). In *D. abyssinica*, each shoot had 2 to 3 nodes, whereas in *D. mangenotiana* up to 12–14 nodes could be observed on the longest shoots (Fig. 1). In this species, the apical region of the shoot usually carried 2, 3 or more nodes separated by short internodes (Fig. 1). The presence of short internodes was also observed in *D. abyssinica*, although they were not necessarily located at the apical region. In both species, short explants produced shoots with a significantly lower number of nodes than long explants (Table 1). The difference was also qualitative, since only long cuttings were able to produce shoots with nodes suitable for further cultures. By contrast, the culture of short cuttings resulted in swelling of the explants, and subsequent production of shoots with reduced vigour and short internodes. Therefore, only long nodal pieces were used in the subsequent experiments. Under the environmental conditions described, both species showed a similar rate of multiplication, i.e. a mean of 4–5 nodes per cultured node, and started to produce roots after about 5 weeks.

D. abyssinica produced a greater number of nodes at a low PPF than under a high one. In addition, the number of nodes preceded by long internodes was also significantly higher at low PPF (Table 2). By contrast, PPF did not affect node production in *D. mangenotiana*. In both cases, low PPF favoured the development of plants with green leaves, whereas high PPF favoured the production of anthocyanin-rich

Table 1. Effect of initial size of nodal cuttings on the efficiency of *in vitro* propagation of *Dioscorea abyssinica* and *D. mangenotiana*.

Species	Initial size[1] of the cutting	No. of cultured nodes	Mean number of nodes obtained	
			Total	Long
D. abyssinica[2]	Short	25	1.85	0
	Long	60	3.65	1.65
			***	***
D. mangenotiana[3]	Short	56	2.82	0.03
	Long	121	4.15	0.95
			**	***

According to Student's test: ** Significant at $p < 0.01$ *** Significant at $p < 0.001$

[1] Short: up to 0.5 cm; long: more than 0.5 cm.

[2] Cultured on basal medium with NAA 0.5 μM and BA 8.9 μM.

[3] Cultured on basal medium with NAA 0.5 μM and BA 4.4 μM.

Table 2. Effect of photosynthetic photon flux (PPF) on the production of nodes in *D. abyssinica* and *D. mangenotiana* cultured *in vitro*.

Species	PPF μmol m^{-2} s^{-1}	No. of cultured nodes	Mean number of nodes obtained	
			Total	Long
D. abyssinica[1]	35	35	5.86	2.37
	80	36	4.47	0.97
			**	***
D. mangenotiana[2]	35	33	2.94	0.48
	80	35	3.49	0.53
			n.s.	n.s.

According to Student's test: ** Significant at $p < 0.01$ *** Significant at $p < 0.001$ n.s. not significant
[1] Cultured on basal medium with NAA 0.5 μM and BA 8.9 μM.
[2] Cultured on basal medium with NAA 0.5 μM and BA 4.4 μM.

leaves. In *D. abyssinica*, the total number of nodes obtained after each subculture remained constant even after 5 cycles; however, in *D. mangenotiana* a gradual decrease in propagation efficiency was observed (Table 3). In both species, however, the production of nodes suitable for further culture decreased with time. Although glasshouse evaluations of *in vitro*-produced plant material were beyond the objectives of the present study, a sample for each species of 10 rooted plantlets, about 9 weeks old, was established in soil and cultured in the greenhouse. Observations made on these plants 2 months later revealed a growth habit similar to that of the original clones.

In vitro tuberization

In *D. abyssinica*, production of microtubers and their weight was strongly affected by the photoperiodic regime. The largest tubers were obtained under 8-h daylength, with all the sucrose

Table 3. Effect of subculture on the efficiency of *in vitro* propagation of *Dioscorea abyssinica* and *D. mangenotiana*.

Species	Subculture	No. of cultured nodes	Mean number of nodes obtained	
			Total	Long
D. abyssinica[1]	1	52	4.42	2.98
	2	48	3.40	1.83
	3	30	4.06	1.37
	4	20	4.55	0.95
	5	16	4.38	1.00
		Person's correlation coefficient	$0.04^{n.s.}$	−0.44***
D. mangenotiana[2]	1	74	4.52	1.08
	2	29	3.86	1.40
	3	20	3.80	0.40
	4	15	3.07	0.87
	5	21	3.24	0.28
		Person's correlation coefficient	−0.20**	−0.15*

* Significant at $p < 0.05$ ** Significant at $p \leq 0.01$ *** Significant at $p \leq 0.001$
$^{n.s.}$ not significant
[1] Cultured on basal medium with NAA 0.5 μM and BA 8.9 μM.
[2] Cultured on basal medium with NAA 0.5 μM and BA 4.4 μM.

Table 4. Influence of sucrose concentration and photoperiodic regime on *in vitro* tuber production in *D. abyssinica*.

Sucrose ($g\,l^{-1}$)	Photoperiodic regime					
	Dark		8/16		16/8	
	Tuber no.	Tuber wt (g)	Tuber no.	Tuber wt(g)	Tuber no.	Tuber wt (g)
20	43[1]	0.08[2a]	32	0.46[b]	49	0.06[a]
40	50	0.15[a]	33	0.71[b]	61	0.09[a]
60	32	0.18[a]	52	0.41[b]	25	0.11[a]
80	37	0.13[a]	47	0.30[b]	17	0.07[a]

[1] Number of tubers produced.
[2] Mean weight of tuber (g).
Means within a line followed by the same letter are not significantly different according to Duncan's multiple range test at the 5% level.

concentrations tested (Table 4). In this species, the number of microtubers produced was not influenced by either photoperiod or sucrose concentration. However, at 16 h light and $80\,g\,l^{-1}$ sucrose, the growth of the tuber-bearing plantlets was negatively affected and their root system was very reduced. Callus formation was observed on the root system under all conditions tested. In *D. mangenotiana*, no tubers were obtained on media containing either 20 or $80\,g\,l^{-1}$ sucrose, under all the light conditions tested. On media containing $40\,g\,l^{-1}$ sucrose, the average tuber weight was 0.02 (dark), 0.15 (16-h photoperiod) and 0.42 g (8-h photoperiod), whereas with $60\,g\,l^{-1}$ sucrose it was 0.25 (dark), 0.39 (16-h photoperiod) and 0.77 g (8-h photoperiod).

Cytological analysis

Cytological analysis of the mother clone of *D. abyssinica*, used in the present study, revealed that mixoploidy was present in the root tip cells with $2n = 38$ and 40 chromosomes. Overall, 37 (43%) metaphases with 40 chromosomes and and 29 (33%) with 38 chromosomes were observed, along with 13 (15%) metaphases with 80 chromosomes and 7 (8%) where 73–76 chromosomes could be seen. Polyploid cells were present in roots both treated and untreated with 1-bromonaphthalene. In the latter material anaphase analyses did not show any structural abnormality, such as bridges or fragments, that would generally appear if one or more chromosomes were dicentic, which would account for the observed aneuploid constitution of some cells. The chromosomes were small (0.3 up to 1.6 μm) and both rod and dot-like chromosomes were present; the largest pair of rod-type carried a satellite (Fig. 2). The chromosomes of *D. mangenotiana* were relatively large (up to 2.2 μm, Fig. 3), and their number was constant ($2n = 40$) in all the cells analyzed.

Discussion

One of the advantages of *in vitro* micropropagation lies in its ability to yield, in short time and limited space, a much larger number of true-to-type individuals than is possible by conventional methods. We found that multiplication rate of the two wild species *D. abyssinica* and *D. mangenotiana* was lower than that reported for the edible species (Ammirato 1985; Chaturvedi et al. 1982; Mantell et al. 1978). Differences in *in vitro* response among various species have been commonly reported and could also easily account for the differences observed between *D. abyssinica* and *D. mangenotiana* in the present study. The explant size was found to affect the rate of multiplication, and was critical for the production of plant material suitable for further subculture. It is interesting to note that for both *D. abyssinica* and *D. mangenotiana*, after each subculture, the number of long internodes, (i.e. most productive and therefore most suitable for further multiplication) decreased gradually, although the total number of nodes produced at least for *D. abyssinica*, was not found to vary

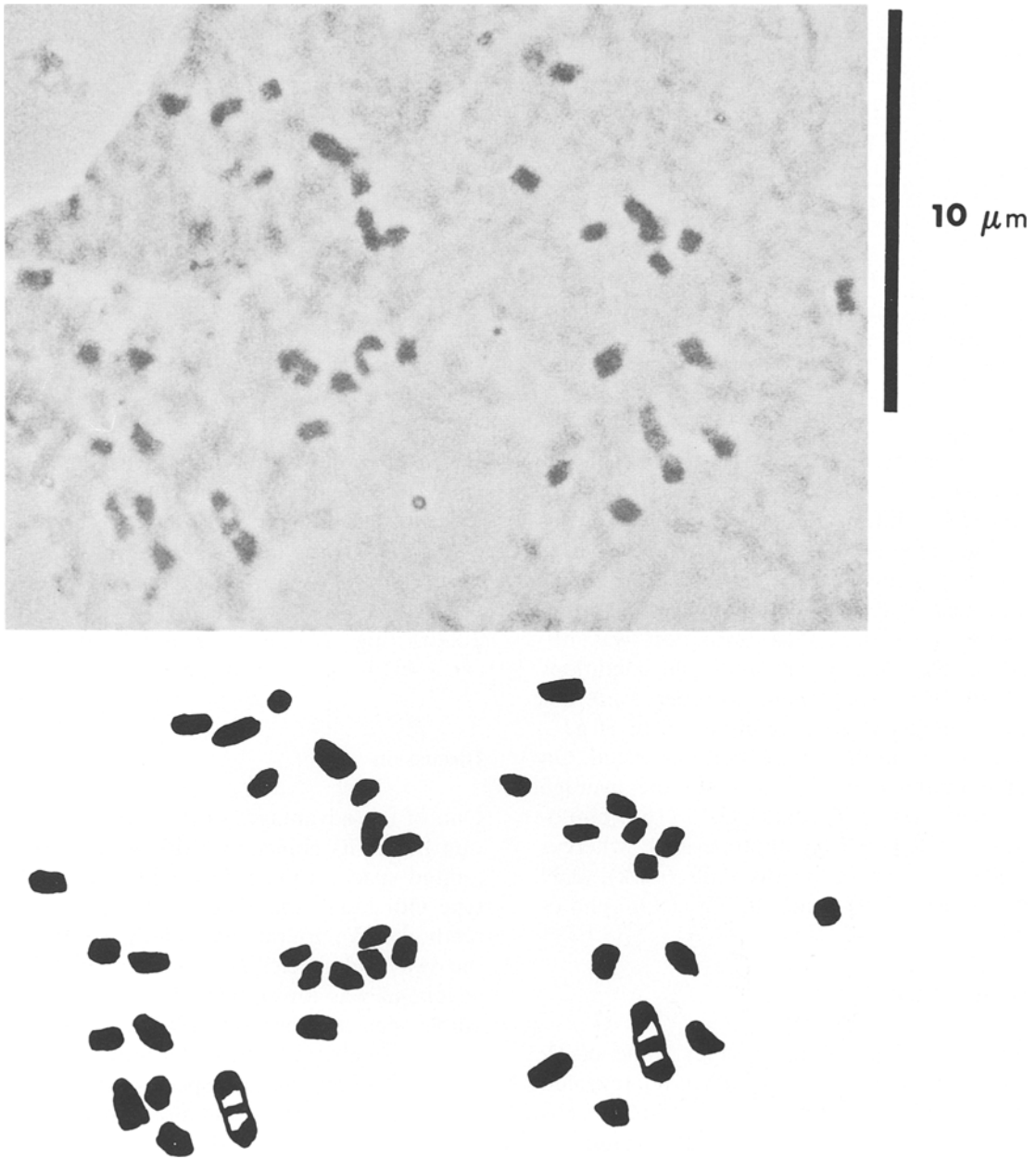

Fig. 2. Somatic cell of *D. abyssinica*, 2n = 38. Note the presence of the large pair of chromosomes carrying a satellite.

over the duration of experiments (Table 2). In this respect, our findings on the gradual reduction of the propagation efficiency seem to be in contrast with observations on other *Dioscorea* species (Charturvedi et al. 1982; Lakshmi Sita et al. 1976; Mantell et al. 1978). In some of these studies, the rate of multiplication was reported not to change with the time (Chaturvedi et al. 1982; Mantell et al. 1978). The difference, however, could be more apparent than real and caused by the pronounced differences of efficiency between long and short nodal cuttings, the latter ones being less productive in micropropagation

The production of microtubers was also observed in both species, although the experiments

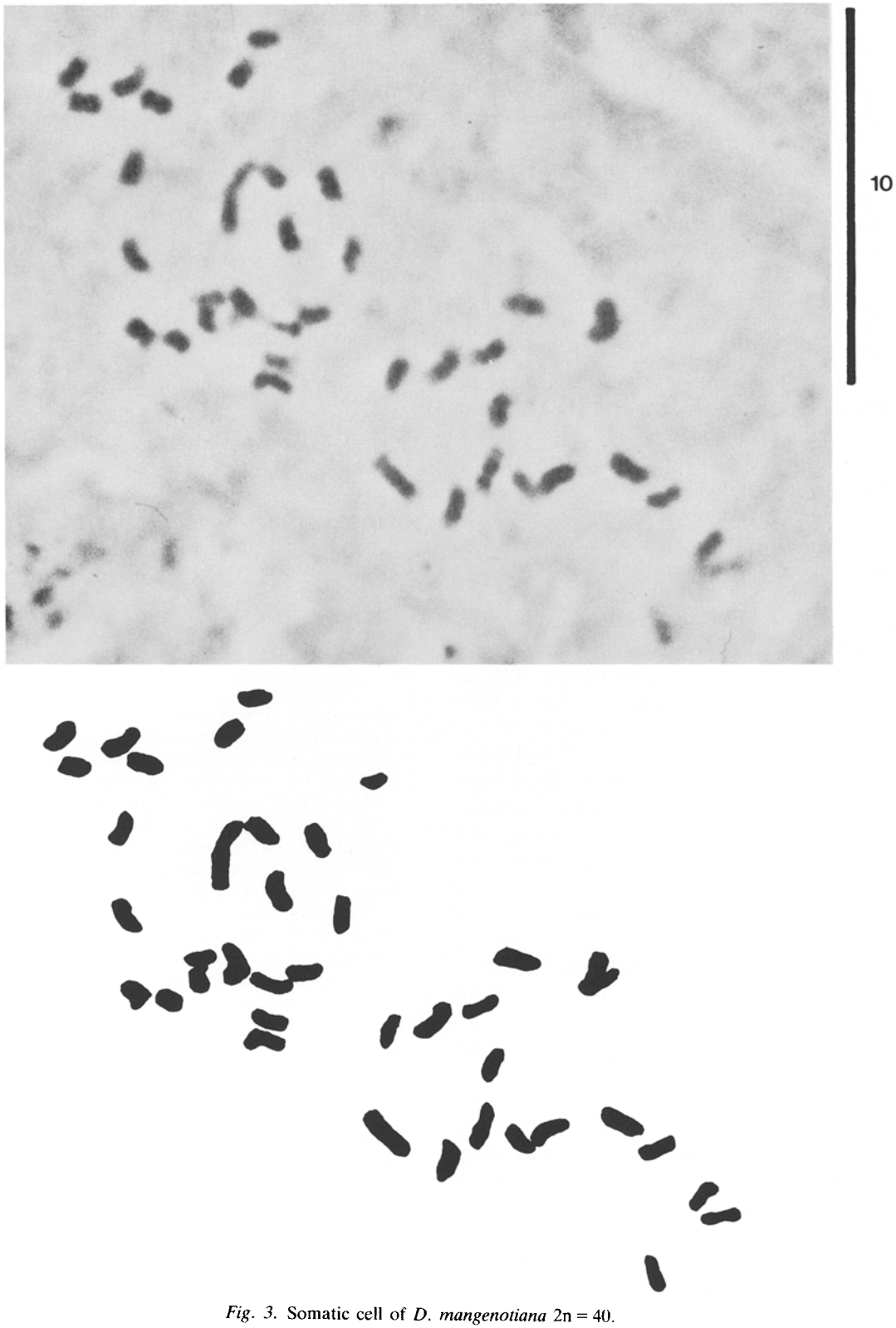

Fig. 3. Somatic cell of *D. mangenotiana* 2n = 40.

with *D. mangenotiana* should be considered only preliminary because of the low number of explants tested. Nevertheless, the results obtained with both *D. abyssinica* and *D. mangenotiana* indicate that the size of microtubers was similar in each treatment for both the species and had tendency to increase under short photoperiod (Tables 4 and 5). In contrast, microtuber number did not vary significantly within each species, but was significantly lower in *D. mangenotiana*, which failed to form tubers at two sucrose concentrations. Sucrose concentration and daylength are known to affect tuberization in yams (Forsyth & Van Staden 1984; Mantell et al. 1978; Ng 1988), the optimal amount depending upon the species, culture medium and growth regulators used. Thus, in *D. alata* increased sucrose concentration of 40 and 80 $g\,l^{-1}$ inhibited microtuber induction (Mantell & Hugo 1989), although not so drastically as we observed in *D. mangenotiana*. Yet, in the same species, microtubers were formed when explants were kept under 8-h daylength (Mantell & Hugo 1989).

Dioscorea species have been subjected to relatively few cytological investigations, probably because the chromosomes are numerous, and too small to allow karyological analysis. So far, chromosome numbers are known for about 12% of the species belonging to this genus (Essad 1984). A previous investigation reported chromosome number of $2n = 40$ for *D. abyssinica* (Martin & Ortiz 1963). We found the same chromosome number, but detected a high incidence of cytochimerism. Polyploid cells along cells with 38 or 40 chromosomes were found in the same root tip. This instability apparently was not linked to structural changes such as paracentric inversions since no bridges were found at anaphase, which would indicate dicentric chromosomes. Since *D. abyssinica* is propagated from tubers, some degree of cytochimerism is not completely unexpected. This view is also supported by more recent analysis of wild and cultivated *Dioscorea* species, where variations in chromosome numbers in different individuals of the same species or even within cells of the same individual, have been reported, and cells with 38, 42 and other numbers have indeed been observed in the same individual (Baquar 1980). Previous investigations of *D. mangenotiana* reported $2n = 72$ and $2n = 80$ (cf. Martin & Ortiz 1963). In the present study all the individuals showed $2n = 40$, and were karyologically stable. Since all plants originated from seeds, the karyological stability might well be related to the method of propagation. This study further supports the results of Baquar (1980) who found karyological stability in all but one of the wild species, all raised from seeds, but cytochimerism and a high degree of polyploidy in the cultivated species, all propagated from tubers.

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References

Ammirato PV (1982) Growth and morphogenesis in cultures of the monocot yam *Dioscorea*. In: Fujiwara A (Ed) Plant Tissue Culture (pp 169–170). Maruzen Co, Tokyo

Ammirato PV (1985) Yams. In: Ammirato PV, Evans DA, Sharp WR & Yamada Y (Eds) Crop Species, Vol 3 (pp 327–354). MacMillan, New York

Ayensu ES (1972) Anatomy of the Monocotyledons VI Dioscoreales (p 182). Oxford University Press, Oxford

Baquar SR (1980) Chromosome behaviour in Nigerian yams (*Dioscorea*). Genetica 54: 1–9

Chaturvedi HC, Sharma AK, Sharma M & Prasad RN (1982) Morphogenesis, micropropagation and germplasm preservation of some economic plants by tissue culture. In: Fujiwara A (Ed) Plant Tissue Culture (pp 687–688). Maruzen Co, Tokyo

Coursey DG (1967) Yams (p 230). Longman, Green and Co, London

Coursey DG (1976) Yams, *Dioscorea* spp. (*Dioscoreaceae*). In: Simmonds ED (Ed) Evolution of Crop Plants (pp 70–74). Longman, London

Degras L (1986) L'igname. Techniques Agricoles et Productions Tropicales. Tome XXXVI (p 408). Maisonneuve et Larose, Paris

Essad S (1984) Variation géographique des nombres chromosomiques de base et polyploïdie dans le genre *Dioscorea* à propos du dénombrement des espèces *transversa* Brown, *pilosiuscula* Bert. et *trifida* L. Agronomie 4: 611–617

Forsyth C & Van Staden J (1984) Tuberization of *Dioscorea bulbifera* stem nodes in culture. J. Plant Physiol. 115: 79–83

Grewal S, Koul S, Sachdeva U & Atal CK (1977) Regeneration of plants of *Dioscorea deltoidea* Wall. by apical meristem cultures. Ind. J. Exp. Biol. 15: 201–302

Lakshmi Sita G, Bammis RK & Randhawa GS (1976) Clonal propagation of *Dioscorea floribunda* by tisue culture. J. Hort. Sci. 51: 551–554

Mantell SH, Haque SQ & Whitehall AP (1978) Clonal multiplication of *Dioscorea alata* L, and *Dioscorea rotundata* Poir. yams by tissue culture. J. Hort. Sci. 53: 95–98

Mantell SH & Hugo SA (1989) Effect of photoperiod, mineral medium strength, inorganic ammonium, succrose and cytokinin on root, shoot and microtuber development in shoot cultures of *Dioscorea alata* L. and *D. bulbifera* L. yams. Plant Cell Tiss. Org. Cult. 16: 23–27

Martin FW & Ortiz S (1963) Chromosome numbers and behaviours in some species of *Dioscorea*. Cytologia 28: 96–101

Miège J (1958) Deux ignames nouvelles d'Afrique occidentale à tubercules vivaces. Bull de l'IFAN 20: 39–55

Murashige T & Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473–497

Nie NH, Hull C, Jenkins JG, Steinbrenner K & Bent DH (1975) SPSS: Statistical Package for the Social Sciences. McGraw-Hill Book Co, New-York

Ng SYC (1988) *In vitro* tuberization in white yam (*Dioscorea rotundata* Poir.). Plant Cell Tiss. Org. Cult. 3: 325–332