EFFECTS OF SUCROSE ON DEVELOPMENT OF CULTURES OF SOME ACCESSIONS OF *DIOSCOREA SPP.*

A.T. Asare[1], E. Acheampong[2], I.K.A. Galyuon[1], L.M. Aboagye[3] and J.F. Takrama[4]
[1]Department of Molecular Biology and Biotechnology, School of Biological Sciences, University of Cape Coast, Cape Coast, Ghana
[2]Department of Botany, Faculty of Science, University of Ghana, Legon – Accra
[3]Plant Genetic Resources Research Institute, P. O. Box 7, Bunso, Ghana
[4]Cocoa Research Institute of Ghana, P. O. Box 8, Tafo-Akim, Ghana

ABSTRACT
The method of micropropagation was used to establish nodal cultures of D. rotundata, D. alata, D. praehensilis, D. dumetorum, D. esculenta and D. cayenensis on 6 ml or 8 ml MS medium supplemented with 2.5 µM kinetin. The cultures were incubated at 28ºC and 16 h photoperiod at 4500 Lux, on 3%, 5%, 7%, and 10% sucrose, with a sucrose-free medium as control in a CRD design. Generally, increase in sucrose concentration significantly (P≤0.05) enhanced the rate of survival and growth of the Dioscorea cultures. Mean shoot height and root formation were highest in D. rotundata cultures on full-strength MS medium containing 10% sucrose compared to half-strength MS medium. However, 7% sucrose stimulated the highest rate of vigorous growth of healthy cultures, leaf formation and plantlet production among the Dioscorea spp. It also enhanced development of bud primordia to regenerate plantlets in D. rotundata meristem cultures. There was a positive correlation (r=0.983) between the rate of leaf production and shoot height. It was evident that 5% and 7% sucrose were more effective for culture development towards multiple bud, lateral and terminal bud differentiation in most of the Dioscorea spp as well as reduced the duration for plantlet development and the demand for regular sub-culturing since the growth phase of cultures could be prolonged. However, D. esculenta cultures appeared to grow better on 3% sucrose than the other Dioscorea spp tested, suggesting that the optimum sucrose concentration required to initiate and establish culture differs significantly among various Dioscorea species.

INTRODUCTION
Six species of *Dioscorea* (*D. rotundata, D. alata*, *D. esculenta*, *D. dumentorum*, *D. cayenensis and D. bulbifera*) are cultivated worldwide due to their economic importance (Ayensu, 1972). Cultivated yams are rich sources of carbohydrate in the diet of West Africans. They are used domestically and some are also exported to earn foreign exchange. Conventionally, yam production is mainly by vegetative propagation involving the use of perennating organs, tubers and bulbils, which directly influence the crop yield (Gurnah, 1994). The implication is that large quantities of yam sett, which otherwise may serve as food, must be reserved for propagation of the crop. This accounts for the highest percentage of the non-labour cost of production (Dorosh,

1988). In addition, yam production has been constrained over the years by inadequate planting materials (Okoli *et al.,* 1991). The long annual cycle, coupled with tuber dormancy especially makes it virtually impossible to cultivate yam more than once in a year.

A more novel approach involving the techniques of micropropagation could be explored and integrated with the conventional method for sustainable production of yams. Several workers have successfully used nodal and meristematic tissue explants to propagate different genotypes of *Dioscorea in vitro* (Uduebo, 1971; Ng, 1988; Jean and Cappadocia, 1991; Krikorian, 1994).

Micropropagation of yam requires Murashige and Skoog (1962) (MS) basic salt supplemented with vitamins, hormones and sucrose. Indeed, Ng (1988) found 3% sucrose suitable for establishment and maintenance of *Dioscorea* cultures and this has been used by many workers ever since. However, different genotypes of *Dioscorea* spp may respond differently to sucrose concentrations *in vitro* and this may warrant alteration of the growth medium to establish cultures of other species of *Dioscorea*. The objective of this work was to screen and establish optimum levels of sucrose for the initiation and early development of plantlets in six species of *Dioscorea* in Ghana. Such findings would facilitate the generation of adequate planting material and improve the conservation of genetic resources of the crop.

MATERIALS AND METHODS

Germplasm collection and culturing of *Dioscorea* spp.

Accessions of *D. rotundata, D. alata, D. dumetorum, D. esculenta D. praehensilis* and *D. cayenensis* obtained from the Plant Genetic Resources Research Institute, Bunso were potted and raised in a screen house. Nodal explants were excised from young growing vines. These were then sterilized in 10% sodium hypochlorite for 10 minutes, 70% ethanol for 2 minutes and 5% sodium hypochlorite for 5 minutes. The sterilants were washed off with three changes of sterile distilled water. The explants were trimmed (1-1.5 cm size) under sterile conditions of a laminar-flow hood, and inoculated onto agar solidified MS medium (6 ml) in 16 x 125 mm test- tubes. The medium was amended with 2.5 µM kinetin and 3% sucrose. The cultures were incubated under a light intensity of 4500 Lux and 16 h photoperiod at 28ºC, and maintained by sub-culturing at regular intervals of six weeks for one year to generate adequate material for use in the experiments.

Screening for optimum levels of sucrose for growth and plantlet development in *D. rotundata* (BA/97/001)

D. rotundata (BA/97/001) cultured and multiplied on 3% sucrose supplemented MS medium was used in an experiment to optimize sucrose concentrations for the *Dioscorea* cultures. Single nodal cuttings, 1.5-2.0 cm long, were incubated on 8 ml agar-gelled MS medium in each of 25 x 250 mm test-tubes. The full-strength and half-strength MS media were supplemented with 2.5 µM kinetin and varied concentrations of sucrose - 0%, 3%, 5%, 7%, and 10%, with the sucrose-free medium (0%) as control. The experimental set-up was arranged in 2 x 5 factorial outlay based on CRD design comprising 15 replicates per accession. Data were collected on developing leaves, roots and plantlets, as well as shoot heights. The data were analysed by ANOVA using the Minitab statistical software, version 15. The differences in means were distinguished by the least significant difference (LSD) test.

Comparison of effects of 3% and 7% sucrose on growth of *Dioscorea* cultures

The effects of 3% and 7% sucrose concentrations on different developmental stages of cultures of six species of *Dioscorea* to ascertain the responses of the different genotypes to sucrose in agar-gelled MS medium amended with 2.5 µM Kinetin were assessed. The nodal cuttings were isolated from cultures that were maintained for a year on 3% sucrose but appeared slow growing with less vigour. There were 10 replicates for each accession. Growth

parameters were measured at 10 weeks of incubation. The experiment was repeated to initiate cultures from nodal explants of *D. alata, D. rotundata* and *D. cayenensis* that were difficult to grow. Furthermore, 7% sucrose-supplemented MS medium was used to regenerate plantlets from bud primordia of meristems of *D. rotundata* (AMA/97/011), which were initiated on agar-gelled MS medium amended with 5 µM BA, 0.5 µM NAA, 0.5 µM GA_3 and 80 mg/L Adinosine disulphate and 3% sucrose.

RESULTS

Generally, there was a significant ($P \leq 0.05$) increase in growth rate of shoots in *D. rotundata* cultures with increasing levels of sucrose concentrations up to 10% sucrose on full-strength MS medium and 7% sucrose on half-strength MS medium (Table 1). Mean shoot height was greatest for 10% sucrose, which also caused the highest rate of root formation.

There was a positive correlation (r= 0.983) between the rate of leaf formation and shoot height for full-strength MS and half-strength MS media, with a significant ($P \leq 0.05$) interaction between sucrose concentration and strength of MS medium. The highest rate of leaf formation was recorded for 7% sucrose (at 9.0 and 20.0 leaves per culture) on full-strength MS medium, and for 5% sucrose, (at 9.0 and 15.0 leaves per culture) were observed on half-strength MS medium at 10 and 24 weeks of continuous incubation, respectively (Table 1 and 2). The absence of sucrose in the culture medium (0% sucrose) retarded growth of cultures; there was no plantlet production since rooting was completely inhibited and leaf formation was highly reduced at 2.0 per culture in both full-strength and half-strength MS media. Lack of sucrose and 10% sucrose caused the lowest survival of cultures, in both full-strength and half-strength MS media. With the exception of half-strength MS containing 7% sucrose, all other cultures on media containing 3%, 5% and 7% sucrose showed 100% survival at 24 weeks. There was a corresponding leaf production and tuberization as sucrose levels increased up to 7%, which supported both basal and aerial tuber formation in the full-strength MS medium (Table 2).

Table 1: Effects of different levels of sucrose concentrations on growth parameters of *D. rotundata* (BA/97/001) cultures at 10 weeks

Culture Medium	Sucrose concentration (%)	Mean number of leaves	Mean number of roots	Mean shoot length (cm)
Full-strength MS	0	2.0 c	0.0 e	2.4 c
	3	7.0 b	4.0 d	4.1 d
	5	8.0 ab	7.0 c	5.3a
	7	9.0 a	10.0 b	5.4 a
	10	9.0 a	12.0 a	5.6 a
Half-strength MS	0	2.0 d	0.0 e	2.3 c
	3	6.0 b	3.0 d	4.1 d
	5	9.0 a	7.0 c	5.3 a
	7	8.0 ab	9.0 b	5.4 a
	10	4.0 c	11.0 a	4.3 a
LSD ($P \leq 0.05$):		**1.38**	**1.60**	**1.41**

Values in the same column followed by different letters are significantly different ($P \leq 0.05$)

Table 2: Effects of various sucrose concentrations on development of *D. rotundata* BA/97/001 cultures at 24 weeks

Culture Medium	Sucrose concentration (%)	Mean number of leaves	Surviving cultures (%)	Micro-tuber formation (%)
Full-strength MS	0	2.0 d	73.3 b	0.0 d
	3	13.0c	100.0 a	13.3 c
	5	19.0 a	100.0 a	66.7c
	7	20.0 a	100.0 a	73.1 b
	10	19.0 b	80.7 b	53.3 a
Half-strength MS	0	2.0 d	80.0 b	0.0 d
	3	12.0 c	100.0 a	6.7 c
	5	15.0 a	100.0 a	6.5 c
	7	15.0 a	80.0 b	13.3 b
	10	6.0 b	50.0 c	20.0 a
LSD (P≤0.05):		**2.12**	**10.41**	**6.12**

Values in the same column followed by different letters are significantly different (P ≤ 0.05)

Table 3: Effects of 3% and 7% sucrose on nodal explants of three species of *Dioscorea* at 6 weeks

Dioscorea sp./ Accession number	Sucrose concentration (%)	Developing organs		
		Buds (%)	Leaves (%)	Roots (%)
D. rotundata	3	30	30	20
AGA/97/007	7	80	80	90
	3	60	30	0
BA/97/003	7	100	80	60
D. alata AGA/97/203	3	50	50	30
	7	100	100	100
AGA/97/115	3	30	30	20
	7	40	90	90
D. cayenensis				
AGA/97/099	3	10	0	0
	7	80	20	60
IF/89/030	3	20	0	0
	7	60	30	20

The growth performance of six *Dioscorea* species in culture, on 7% sucrose treatments was significantly ($P \leq 0.05$) higher than those of 3% sucrose, except for *D. esculenta* that had better growth on 3% sucrose (Fig.1). The highest number of leaves produced (20.0 per culture) at 10 weeks was observed in *D. rotundata* (BA/97/004), which was cultured on media containing 7% sucrose and was significantly ($P \leq 0.05$) higher than the 5.0 leaves per culture obtained with media containing 3% sucrose at the same time. Similarly, *D. praehensilis, D. dumetorum* and *D. alata* produced 12.0, 11.0 and 9.0 leaves per culture, respectively, in media containing 7% sucrose compared with 8.0 and 4.0 leaves per culture, respectively, which developed on media containing 3% sucrose (Fig.1). There was 100% plantlet production among all the six species of *Dioscorea* on 7% sucrose in culture, compared with 10 - 14 % on 3% sucrose for *D. rotundata, D. alata* and *D. dumetorum* (Fig. 2).

Indeed, 7% sucrose promoted shoot and root growth and enhanced the formation of more vigorous and robust plantlets (Plates 1 and 2). Furthermore, it stimulated early bud development, leaf and root formation from nodal explants of *D. rotundata, D. alata,* and *D. cayenensis* initiated at 6 weeks. The growth performance of initiated cultures on 7% sucrose far exceeded that for the corresponding cultures on 3% sucrose (Table 3). In addition, 7% sucrose also facilitated early plantlet development of bud primordia initiated from meristematic tissue of *D. rotundata* (Plate 3A-C).

DISCUSSION

The growth rate of *D. rotudata* (BA/97/001) cultures under increasing sucrose levels was significantly ($P \leq 0.05$) high. Lateral shoot elongation, leaf production, rooting and tuberization increased with increasing concentrations of sucrose up to 7% in MS medium. The fact that there was no linear correlation between size of explants and plantlets indicated that the observed increase in size of plantlets could be a direct growth response to increase doses of sucrose. It has been reported by Danso (1997)

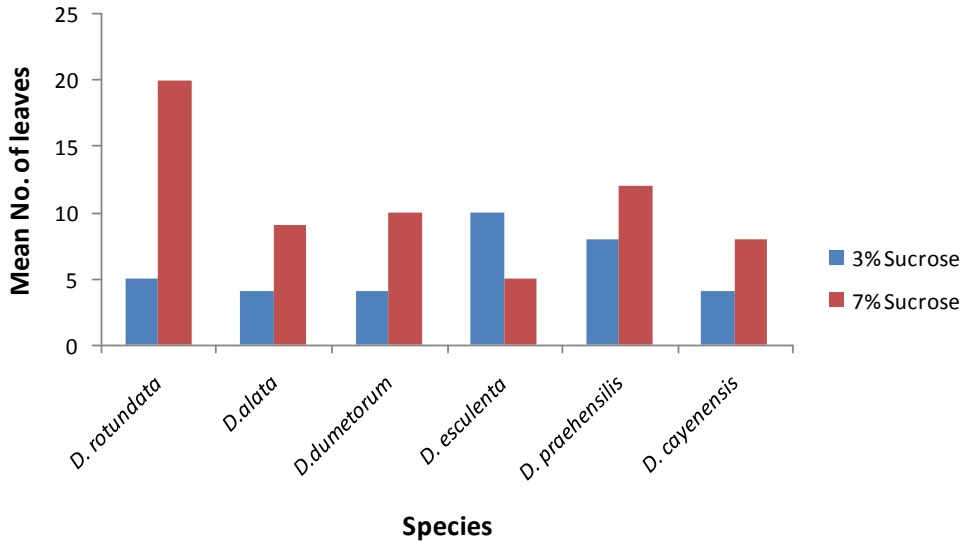

Fig. 1: Effects of 3% and 7% sucrose on leaf production in some *Dioscorea* cultures at 10 weeks

that increasing sucrose concentration increased lateral shoot growth and size of plantlets in cassava cultures. The implication is that sucrose could influence the endogenous hormone levels, particularly synthesis of auxin, could increase with increasing sucrose concentration. This could have accounted for the large plantlet size and high rooting of both nodal and meristem cultures observed in this study. According to George (1993), auxin induces root formation in culture and antagonizes the effects of cytokinin. The average shoot height and root forma-

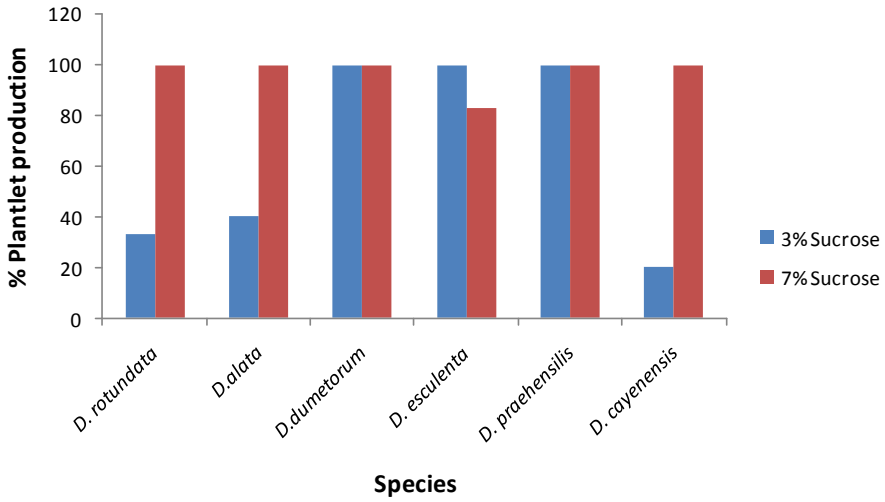

Fig. 2: Effects of 3% and 7% sucrose on plantlet production in some *Dioscorea* cultures at 10 weeks

Plate 1: Cultures of *D. rotundata* (BA/97/004) on MS medium with 2.5 µM kinetin (8ml), showing slow growth on 3% sucrose (A) and vigorous growth on 7% sucrose (B) (under 16 h photoperiod) at 8 weeks

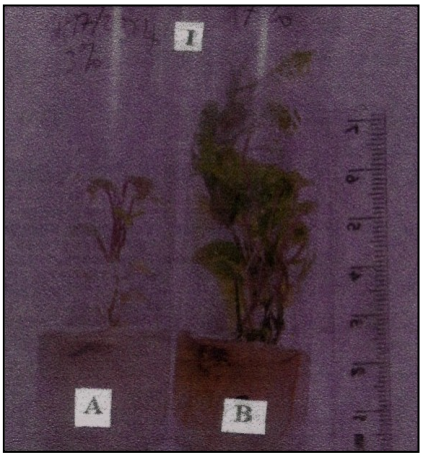

Plate 2: Cultures of *D. alata* (SO/89/77) on MS medium with 2.5 µM Kinetin (8ml) showing slow growth on 3% sucrose (A) and vigorous growth on 7% sucrose(B), under 16 h photoperiod) at 8 weeks.

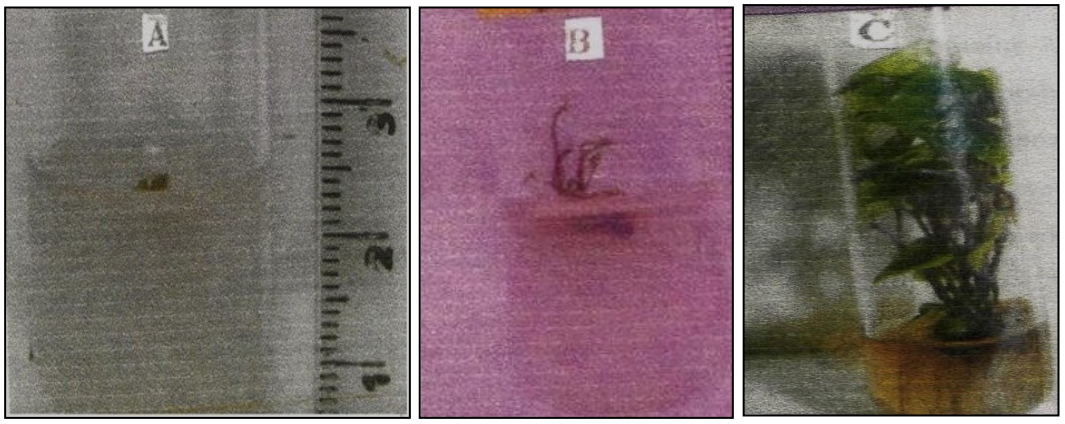

Plate 3: A. Developing meristem culture of *D. rotundata* (AMA/97/011); bud primordia on MS medium (8 ml) with 5µM BA, 0.5 µM NAA, 0.5 µM GA_3, 80 mg/L $AdSO_4$ and 3% sucrose, at week 12

B. Multiple shoots of *D. rotundata* (AMA/97/011) on MS medium (8 ml) with 2.5 µM Kinetin 20 mg/L, L – Cysteine with 7% sucrose at week 12.

C. Whole plantlet of *D. rotundata* (AMA/97/011) with multiple shoots on MS medium with 7% sucrose, 2.5 µM Kinetin, under 16 h photoperiod at week 18

tion was enhanced by 10% sucrose in *D. rotundata* cultures on full-strength MS medium compared to half-strength MS medium. This implies that the available macronutrients and micronutrients could interact with sucrose to influence growth of the cultures.

The retardation of growth of *D. rotundata* cultures on sucrose-free medium underscores the importance of sucrose as the principal source of carbon for growth and metabolism in plant tissues and organs *in vitro*. Indeed sucrose has always been employed as the carbon source for providing the energy required to drive the metabolic processes that lead to plant growth *in vitro* (Ammirato, 1982; Okezie *et al.,* 1984; Osifo, 1988). The combination of 7% sucrose with 2.5µM kinetin in MS medium promoted significant ($P \leq 0.05$) growth rates and plantlet production to establish cultures of *D. rotundata* (BA/97/004), *D. alata* (SO/8977), *D. dumetorum* (AGA/97/165) and *D. praehensilis* (SO/89/74) with enhanced leaf, root and lateral shoot production compared to 3% sucrose, except for *D. esculenta*. This confirms the requirement of high sucrose concentrations by some clones of *Dioscorea* cultures to stimulate growth. However, the varied growth response of cultures on 7% sucrose among the species of the various *Dioscorea* might be due to genetic differences that exist among the accessions of *Dioscorea* spp. In fact, George (1993) has argued that the optimum concentration of sucrose to induce morphogenesis or growth differs between different species. However, sucrose concentration above 7% suppressed shoot growth in half-strength MS medium and rather influenced rooting at the expense of shoot proliferation in *D. rotundata* cultures. This corroborates the finding by Ng (1988) that high sucrose concentration retards shoot growth but stimulates massive rooting in *D. rotundata*.

CONCLUSION

On the whole, there were varied growth responses of the genotypes of Dioscorea to increasing sucrose concentrations in ms medium. Dioscorea cultures that could not grow well on 3% sucrose were established on 7% sucrose in ms medium supplemented with 2.5µm kinetin.

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