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THREE TECHNIQUES FOR RAPID CLONAL PROPAGATION OF WHITE LISBON YAM (*DIOSCOREA ALATA* L.)

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INTRODUCTION

Techniques for rapid multiplication of vegetatively propagated crops are required when it is desirable to obtain a large number of plants from a limited amount of mother plant material within a relatively short space of time. Such situations are frequently encountered in plant introduction and selection work when only limited quantities of material may be available for propagation. Recently, these techniques have also proved invaluable in the production of disease-free planting material e.g. in the production of cassava clones free of Cassava Bacterial Blight (Cock et al., 1976) and a wide range of virus-free horticultural crops (Hollings, 1971).

Efforts are currently being made to free *Dioscorea alata* L. and other food yams of the Eastern Caribbean of the viruses described by Harrison and Roberts (1973), Mohamed and Mantell (1976) and Mohamed (1976) by means of meristem tip culture and heat therapy. Once virus-tested clones are produced there is a need for rapid multiplication of these in order to supply the large amount of tuber planting material required by commercial yam growers. The traditional method of propagation of *Dioscorea* spp. by means of 100-150 gm tuber setts, although satisfactory for full scale crop production, provides a relatively low and slow of multiplication (table 1). Also, this method is not suitable for comparatively small scale greenhouse operations. Therefore, attempts have been made to devise methods of rapid propagation of yam using Barbados clones of *D. alata cv.* White Lisbon. Three of the most promising techniques so far developed are described here.

1 - The split-node vine cutting technique

Several workers have investigated the possibility of using vine cuttings as a means of propagation of *Dioscorea* spp. e.g. *D. floribunda* Mart. and Gal. and *D. composita* Hemst. (Martin and Delpin, 1969), *D. rotundata* Poir. and *D. cayenensis* Lam. (Anon, 1974) and *D. dumentorum* (Kunth) Pax., *D. rotundata* and *D. alata* (Njoku, 1963). Results of these investigations have established that although complete plants can be produced from node cuttings, shoot, root and tuber production from these can be extremely variable. The latter situation has been attributed to differences in the age of the mother plant and original position of nodes on vines. In addition, tubers produced by cuttings are usually small and consequently unsuitable for direct field planting which has led to the

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Table 1 - Levels of multiplication of *D. alata* achieved on a field scale in E. Caribbean using the traditional tuber sett method of propagation.

Mean Sett wt. (gm)	Mean tuber yield per plant (gm)	Mean level of Multiplication	Source
120	1540	13.3x	Table 4
125	2020	16.2x	Degras et al (1975)
142	2180	15.4x	Gooding et al (1968)
Mean 129	1913	15.0x	

suggestion that the nodal cutting method is most unlikely to be of any commercial interest for the growing of edible yams (Ferguson, 1972). However, even with the apparent disadvantages outlined above, it was considered that this method still had distinct potential as a specialised greenhouse method of rapid propagation, especially when it was observed that the number of nodes suitable for rooting on a single *D. alata* plant grown from a 120 gm sett can be in the order of 100-150. Therefore, the nodal cutting method was assessed as a rapid propagation technique for *D. alata*.

For adequate rooting of cuttings it was found that a mist propagation system was necessary. Results of an experiment in which single node cuttings taken from 15 week old vines of *D. alata cv.* White Lisbon (clones B 74 and B 75) were rooted in either tap water, washed coarse quarry sand (mean particle size 0.5-1.0 nm) or washed quarry sand under a wet-leaf operated mist propagator (Table 2), confirmed that mist propagation gave best rooting and that root growth of cuttings was better in coarse sand media than in tap water after 28 days. The number of rooted cuttings from a given quantity of vine material could be doubled by splitting single nodes in half, longituinally, with a sharp razor. Split node cuttings rooted in the same way as complete node cuttings (Table 3). Cuttings which were transplanted into 11 x 18 cm black plastic bags filled with a methyl bromide-sterilised 4 : 4 : 3 soil/sand/peat potting mixture and kept in a greenhouse, developed tubers after 12-14 weeks (Table 3). It was observed that even when little new shoot growth occurred, cuttings still produced tubers. Although these tended to be small, the latter remained viable since more than 70 per cent germinated when planted on moist sand capped with a 5-10 mm laver of moist peast in the following season. After germination, tubers were transplanted into 20 x 30 cm black plastic bags containing the above potting mixture and allowed to grow for 9-10 months in a greenhouse to produce larger tubers. Tuber production following this treatment was as follows : mean yield $\pm$ S.E. per plant was 164.2 $\pm$ 12.7 gm and mean tuber weight $\pm$ S.E. was 90.6 $\pm$ 8.2 gm. Therefore, it was possible to obtain tubers large enough to plant directly in the field after a two year propagation cycle using the split node cutting technique.

2 - The mini sett scoring technique

In order to increase the number of plants obtainable from a given amount of tuber material, setts smaller than those traditionally used could be planted. This type of procedure has been investigated by MATHURIN and DEGRAS (1974) and DEGRAS and MATHURIN (1975) who found that with setts as small as 25 gms, mean multiplication rates of 87 could be achieved. However, the rate of germination of these small setts was found to be slow and problems were experienced with rotting of sett tissues.

Pleliminary investigations using fungicide dips to control rotting were only partially successful (Anon, 1974) which suggested that rotting of 10-20 gm setts was probably enhanced by the length of time which these setts required to germinate. The use of a scoring method to induce bud formation similar in principle to that used in propagation of hyacinth and *Scilla* spp. (Hartman and Kester, 1975), was investigated. Skins of whole *D. alata cv.* White Lisbon (clone B 76/B 001) tubers were scored with a sharp scalpel to a depth of 10 mm into either 10 x 10, 20 or 50 x 50 mm squares at 4 weeks after harvesting. In this way, it was hoped to reduce the effect of apical dominance of the proximal end of tubers before normal bud differentiation occurred without the need for exposing

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Table 2 - Rooting of two *D. alata cv.* White Lisbon clones B 74 and B 75 at 28 days after planting in tap water or sand.

Medium	% cuttings rooted	Mean root length (mm)	
		Clone B 74	Clone B 75
Tap water	[a]29.4a	6.9a	[b]6.2a
Coarse sand	35.4a	34.9b	31.5b
Coarse sand under mist	60.0b	39.8b	37.2b
Residual df	3	6	6
S.E. of mean	2.8	2.6	3.0
C.V. (%)	6.6	9.6	12.0

[a] Mean of 2 replicate batches of 96 cuttings each

[b] Mean of 4 blocks of 24 cuttings each

Means followed by similar letter when compared vertically were not significantly different (P = 0.05) using a Duncan's Multiple Range Test.

Table 3 - Effect of splitting single node cuttings of *D. alata cv.* White Lisbon (clone B 75) on rooting and tuber yield after 4 wks and 12 wks, respectively.

Cutting type	Per cent rooted	Tuber yield (gm)	Germination of tubers (%)
[a]Complete	64.6a	2.4a	77.1
[b]Split	63.7a	1.7a	
S.E. of mean with 3 df	9.4	0.3	
C.V. (%)	14.7	14.8	

[a] Means of 40 complete nodes

[b] Means of 80 split nodes

Means followed by similar letter were not significantly different (P = 0.05) when compared vertically.

tuber ground tissues. Scored tubers were kept in a dark chamber held at 90 per cent relative humidity for 10 weeks after which time scored tubers had developed as many buds as unscored tubers (fig. 1). At 12 weeks after scoring, tubers were broken up along the scored tissues into mini-setts. The latter were dipped in 8 per cent (v/v) aqueous solution of ethylene chlorohydrin for 5 min. before being planted in moist sand. After 28 days, as many of the mini-setts had germinated as 20 gm setts which had been cut from unscored tubers and treated in the same way (fig. 1). A further experiment was set up to determine the tuber yields which could be produced from mini-setts produced by the scoring method. *D. alata cv.* White Lisbon (clone B 76/B 001) tubers were scored at either 10 x 10, 20 x 20, 30 x 30 or 40 x 40 mm levels and after 10 weeks incubation broken into either 10, 20, 30 or 40 gm mini-setts, respectively. After germination in moist sand, mini-setts were planted directly into the field at Bullens Agricultural Research Station, St. James. The experiment was laid out in a randomised block design and setts planted on ridges at 0.5 x 0.7 M spacing. Plants were left unstaked and tuber yields assessed nine months after planting. Yield increased with increasing sett size but smaller mini-setts gave better multiplication rates than larger ones (table 4). These results which were consistent with those of MATHURIN and DEGRAS (1974), showed that a high multiplication rate was possible using mini-setts and that the rate of germination of mini-setts could be increased using the scoring technique.

3 - The micropropagation technique

The application of tissue culture methods in the clonal multiplication of plant species is termed micropropagation and many plant species have already been propagated using the technique (Murashige, 1974). Recently, a micropagation technique was developed for *D. alata* and *D. rotundata* using nodal vine segments taken from plants previously exposed to 16 hr photoperiods for four months as source material (Mantell et al., 1977). Nodal segments, 10-15 mm in length, were immersed briefly in 70 per cent (v/v) ethanol before surface sterilising in 0.52 per cent (v/v) sodium hypochlorite solution for 5 minutes. Sterilised nodal segments were washed in two changes of sterilised distilled water before placing on a culture medium. The medium (dispensed at 5 ml per McCartney bottle) consisted of 0.6 per cent agar, 2 per cent sucrose, 10 mg/1 myo-inositol 1 mg/1 thiamine and the mineral salts of Murashige and Skoog (1962). The pH of the medium was adjusted to 5.8-6.0 with HC1 or NaOH before autoclaving for 15 minutes at 1.25 kg cm^{-2} pressure. Mother plants and cultures were kept under light banks fitted with tropical daylight fluorescent and 40 W incandescent lamps producing and intensity of 1200-1400 Lux at plant level. The daily light period was 16 hr and day and night temperatures were kept at 27 $\pm$ 2°C. Under these conditions complete plantlets developed 3-5 weeks after excision. At this stage, plantlets could be successfully established in a steam-sterilised 4 : 4 : 3 peat/vermiculite/soil mixture. However, shoots produced by plantlets in culture were frequently multibranched bearing numerous leafy nodes separated by short internodes. Further multiplication of clones by dissection of cultures was, therefore, possible. Shoots of plantlets were split into 3-5 separate nodal segments (each bearing at least one leaflet) and transplanted onto fresh basal medium. After 14-20 days new multibranched plantlets developed and these were used to produce further generations of plantlets in a regular 14-20 day cycle (fig.2). Established plants were transferred to an insect-proof greenhouse and these produced 3-5 gm tubers per plant after three months. Tuber production was completed using methods similar to those described for the split-node cutting technique.

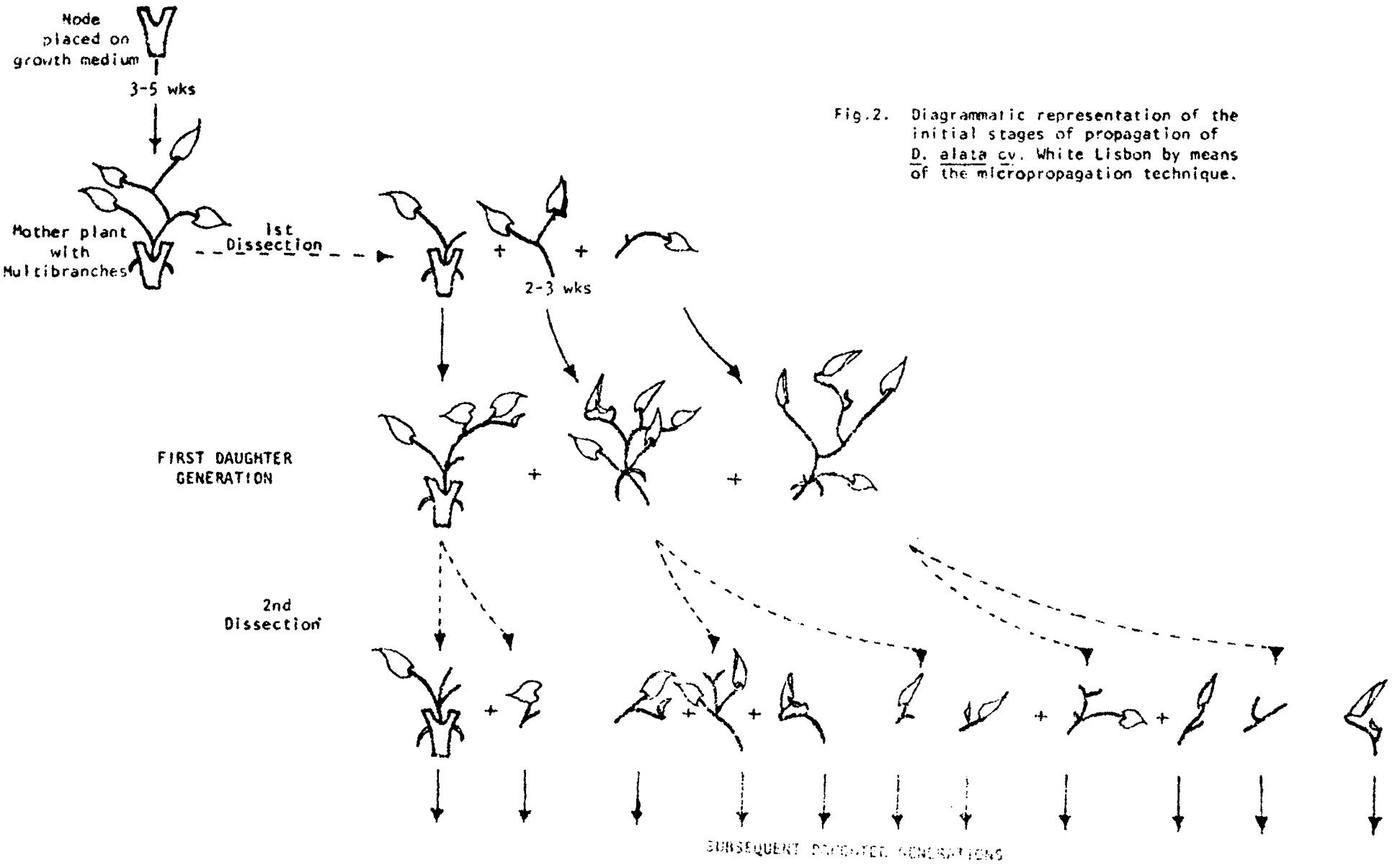


Fig.2. Diagrammatic representation of the initial stages of propagation of D. alata cv. White Lisbon by means of the micropropagation technique.

Table 4 - Tuber yield and multiplication levels of *D. alata cv.* White Lisbon (Clone B 76/B 001) using the minisett scoring method.

Wt. of sett (gm)	% Sett Germination	Mean Tuber yield per plant (gm)	Mean Multiplication level
10	[a]42.9	[b]636.8a	63.7x
20	50.0	745.9b	37.3x
30	43.6	837.8c	34.9x
40	43.4	822.7c	24.2x
120	40.0	1540.0d	13.3x

[a]Percentage sett germination at 4 wks after planting in moist sand.

[b]Mean tuber yield of 5 blocks of 10 plants each ; means followed of similar letter were not significantly different (P = 0.05) using a Duncan's Multiple Range Test ; S.E. = 100.1 (16 df) ; C.V. (%) = 10.92.

DISCUSSION

The three techniques described fulfil the functions of rapid clonal propagation since the number of individual plants produced by each method was substantially greater than that produced by the traditional propagation method (table 5). In addition, daughter plants appeared to have the same clonal characteristics as the mother plants.

It was considered that the use of a particular yam propagation technique is likely to be dependent upon several factors : the quantity and type of mother plant material available, the type of propagation facilities available, the risk of reinfection of disease-free daughter plants and finalyy, whether tuber material suitable for direct field planting can be produced after a particular propagation phase. For example, Methods 1 and 3 would produce high multiplication levels but would require a two year propagation cycle to produce sizeable tubers whereas Method 2, which produced the lowest multiplication level, would only require a one year propagation cycle.

Where propagation of plantlets produced by meristem tip culture is required then the micropropagation method would probably be the most suitable since a large number of plantlets could be produced from a single multibranched culture within a relatively short space of time. Method 3 also has other advantages over advantages over Method 1 and 2 i.e. more than two plants can be produced from a single vine node during one growing season, because it was possible to get more than one daughter generation from a single culture (fig. 2) and the space required for the multiplication stage of Method 3 was minimal compared to the mist propagation and sand bed facilities required for Methods 1 and 2, respectively.

Both the split-node cutting and micropropagation methods could probably be used for the propagation of nematode-free *D. alata* planting material as a means for controlling certain nematode diseases of *D. alata* which are serious in some islands *e.g.* Jamaica (Hickling, 1974). These methods could only be successfully applied if sand used in mist propagation beds and soil used in potting mixtures was adequately sterilised.

Methods 1 and 3 are currently being used for propagation of virus-tested clones of *D. alata cv.* White Lisbon in Barbados since the multiplication stages of both methods can be carried out under protected laboratory and greenhouse conditions where the risk of reinfection by viruses is much reduced. It is proposed that once tubers are produced from daughter plants, further multiplication of tuber stocks could be carried out using the scoring technique (Method 2).

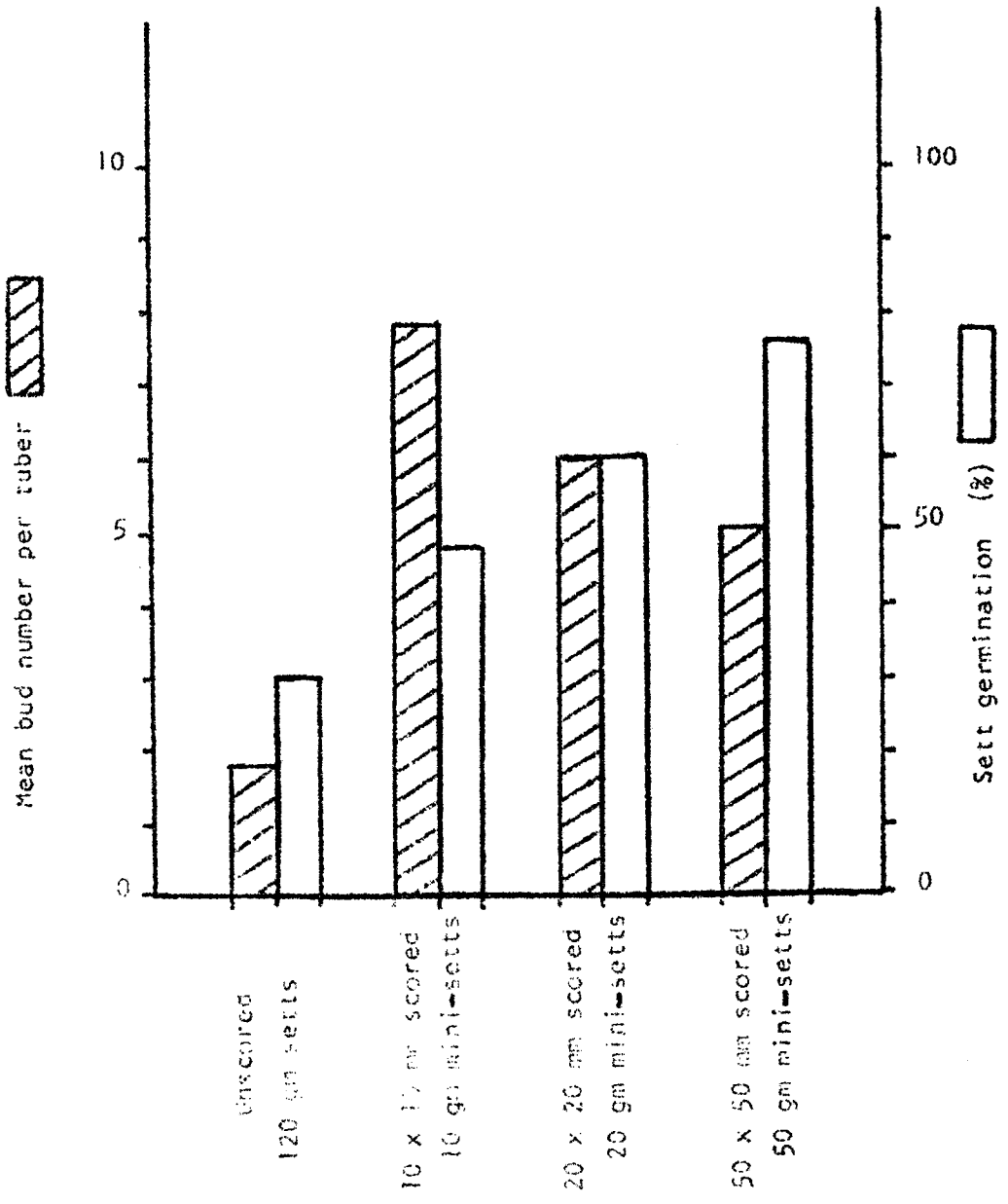
Table 5 - Predicted multiplication levels which could be achieved for *D. alata cv.* White Lisbon using rapid propagation techniques.

Method No.	Propagation technique	Multiplication Level	Seasons required to produce tubers for field planting
	Traditional (120 gm)	15 x	1
1	Split-node cutting	[a]160 x	2
2	Mini-setts (10 gm)	64 x	1
3	Micropropagation	[b]8,100x	2

[a]200 split nodes per mother plant, 60 per cent rooting of cuttings and 75 per cent germination of small tubers assumed.

[b]100 complete nodes per mother plant and four daughter generations assumed.

Fig.1. Bud formation and germination of mini setts of D. alata cv. White Lisbon.



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SUMMARY

Three techniques for the rapid clonal propagation of White Lisbon yam *(Dioscorea alata* L.) are described according to the type of source material used *i.e.* split-node cuttings of vines, mini-setts of tubers and nodal segments of vines. The first two methods were carried out in a greenhouse whilst the third method required specialised tissue culture facilities. The relative merits of each technique, as comared to the traditional method of propagation, are discussed.

RESUME

TROIS TECHNIQUES POUR LA PROPAGATION CLONALE RAPIDE DE L'IGNAME "WHITE LISBON" *(DIOSCOREA ALATA* L.*)*

On décrit trois techniques pour la propagation clonale rapide de l'Igname "White Lisbon" *(Dioscorea alata* L.*)* selon le type de matériel utilisé c'est-à-dire des moitiés longitudinales de nœuds, des petits fragments de tubercules et des segments nodaux de tiges.

Les deux premiers méthodes ont été conduites sous serre tandis que la troisième a nécessité l'emploi de technique de culture de tisssus.

On discute les avantages relatifs de chaque technique en les comparant à la méthode de propagation traditionnelle.