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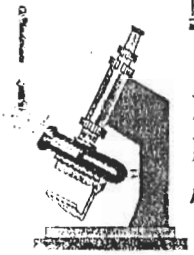

PLANTLET REGENERATION FROM SEED EMBRYO-DERIVED CALLUS CULTURES OF WHITE YAM (*Dioscorea rotundata* POIR)

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ABSTRACT

Mature embryos of ***Dioscorea rotundata*** *Poir (white Guinea yam) were induced to form callus on Gamborg's B5 medium supplemented with a range of 2-10 mgl^{-1} 2,4 - dichlorophenoxyacetic acid and 1-5 mgl^{-1} kinetin for 28 days in vitro. The resultant calli grew profusely and remained undifferentiated when maintained in the callus induction medium. Shoot formation occurred on transferring the calli onto a medium devoid of growth regulators or supplemented with low cytokinin concentrations. An average of three shoots were obtained per explanted callus within 56 days of growth in the absence of growth regulators while up to five shoots were produced within the same period in the presence of 0.2 mgl^{-1} 6-benzylaminopurine. The regenerated shoots rooted within 21 days on transfer to a medium containing varying concentrations of either α-naphthalene acetic acid or indole butyric acid. Thirty three per cent of plantlets were successfully established in the field after hardening.*

Key words: Seed embryo-derived callus cultures of white yam

INTRODUCTION

Problems associated with the use of tuber for yam (*Dioscorea*) propagation (15), which included high labour and cost input, tuber deterioration in storage, tuber dormancy and non-uniform tuber sprouting, have necessitated the search for alternative modes of propagation. One such alternative is the use of tissue culture technique in which different parts of the yam plant such as nodal cuttings (4, 11), excised leaves (18), the meristem (6, 9), zygotic embryo (13, 16) and petioles and stem pieces (12) have been used to raise yam plants.

Regeneration of yams through tissue culture technique often involves callus induction and subsequent reorganization of the callus cells into organs (13, 16). The pattern of organogenesis depends on the basal medium and its hormonal additives. The most frequently used basal medium has been that of Murashige and Skoog (10) but Okezie *et al.* (14) also found Linsmaier and Skoog's (7) medium useful for *in vitro* culture of white yam zygotic embryos. In general these basal media have been found to be slow both in the induction and subsequent callus growth. It is desirable to use media that could quicken callus induction. In the work reported here, Gamborg's B_5 medium (5) supplemented with varying concentrations of growth regulators was used to study callus induction and subsequent plantlet regeneration in white yam zygotic embryo.

MATERIALS AND METHODS

Mature embryos, excised from post-dominant white Guinea yam seeds were used in this study. Prior to embryo excision, the seeds were soaked for 24 h in water, and then

surface-sterilized in 1% sodium hypochlorite solution and three drops of a wetting agent (Tween 20) for 10 min and rinsed in four changes of sterile distilled water. The excised embryos were then inoculated in the appropriate culture media.

The basal medium used consisted of Gamborg's B_5 major and minor salts and vitamins (5). Sucrose was used as the carbon source at 3% (w/v) level and solidified with 0.8% (w/v) Difco-Bacto agar. The pH of each medium was adjusted to 5.5-5.8 before dispensing in 20 ml portions in culture glass tubes stoppered with non-absorbent cotton wool wrapped in aluminium foil. The medium was then autoclaved for 15 min at 121 °C and 103 KN M^{-2} pressure.

For callus induction the medium was supplemented with 0-10 mgl^{-1} 2,4 -dichlorophenoxyacetic acid in combination with 0-5 mgl^{-1} kinetin. Calli were withdrawn from the callus induction medium (a medium supplemented with 2.0 mgl^{-1} 2,4 -D and 2.0 mgl^{-1} kinetin) and subcultured on a basal medium devoid of growth regulators or one supplemented with 0.2-10 mgl^{-1} of either kinetin or 6 -benzylaminopurine for shoot induction. The regenerated shoots were transferred to a cytokinin-free medium or media supplemented with 0-24 mgl^{-1} α-naphthaleneacetic acid (NAA) or indolebutyric acid (IBA) for root initiation.

All inoculations were done in a sterile transfer hood previously exposed to ultraviolet radiation for 30 min. One embryo or callus piece or shoot explant was inoculated per culture tube and there were 20 replicates per treatment. Embryo cultures were maintained in the dark, while callus cultures were maintained either in total darkness or 12 h -light regime at 27 ± 2 °C. Regenerated shoots were induced to form roots under light at 600 *lux* provided by cool white fluorescent tubes for 12 h/day at 27 ± 2 °C.

Treatments were evaluated after 28 days for callus induction in terms of fresh weight and percentage of explants forming callus per treatment. Presence or absence of shoots or roots were scored after 56 days in culture.

RESULTS

Both 2,4-D and kinetin induced callus formation in the explanted embryos within 28 days at 2 to 10 mgl^{-1} and 1 to 5 mgl^{-1}, respectively, either alone or in combination. The greatest amount of callus was formed when 2,4 -D was used at 2 mgl^{-1} in combination with 2 mgl^{-1} kinetin in the medium (Tables 1 and 2). The calli grew profusely especially with increase in the frequency of subculturing and more callus was produced in the dark than under light (Fig. 1).

As listed in Table 3, an average of three shoots were produced per callus piece in the absence of growth regulators while up to five shoots were produced in the presence of 0.2 mgl^{-1} BAP.

As indicated in Fig. 2 auxin was found essential for root induction by the regenerated shoots with 8 and 12 mgl^{-1} being the optimal concentrations for NAA and IBA respectively.

On transfer of plantlets from culture tubes to the field, 33% of the shoot explants

survived hardening.

TABLE 1: **Effect of 2,4-D and Kinetin on Callus Production (mg ± S.E., Fresh Weight) from *Dioscorea rotundata* Embryos after 28 Days in Culture on Gamborg B_5 Basal Medium**

2,4 –D (mg/1)	Kinetin (mg/1)			
	0.0	1.0	2.0	5.0
0.0	0±0	7.9±1.4	8.0±1.8	4.4±0.5
2.0	4.6±1.1	12.8±1.9	33.7±3.9	10.2±1.9
5.0	6.3±1.3	8.3±1.0	23.9±3.3	6.7±0.9
10.0	2.6±0.7	3.8±0.5	9.1±1.1	3.9±0.6

All cultures were maintained in the dark for 28 days. Each treatment consisted of 20 replicate tubes.

TABLE 2: **Effect of 2,4-D and Kinetin on Percentage of *D. rotundata* Embryo Explants per Treatment Producing Callus after 28 Days in Culture on Gamborg B_5 Basal Medium**

2,4 –D (mg/1)	Kinetin (mg/1)			
	0.0	1.0	2.0	5.0
0.0	0±0	15.0±6.9	18.6±7.9	10.0±4.6
2.0	10.6±3.8	19.6±5.8	68.2±8.3	18.0±6.2
5.0	15.0±6.2	18.1±7.6	63.6±11.4	13.3±5.9
10.0	6.6±2.9	7.5±3.6	17.5±7.4	5.9±1.8

All cultures were maintained in the dark for 28 days. Each treatment consisted of 20 replicate tubes.

TABLE 3: **Mean Number of Shoots Produced When *Dioscorea rotundata* Embryo-Derived Callus was Cultured for 56 Days on a Gamborg B_5 Basal Medium Supplemented with 0.2-10 Mg/1 Kinetin or BAP**

Hormone Concentration (mg/1)	Kinetin	BAP
0.0	3.0±1.0	3.0±1.0
0.2	2.8±1.0	5.0±0.9
0.5	1.9±0.8	3.5±0.7
2.0	1.2±0.6	2.1±0.8
5.0	0.9±0.2	1.2±0.5
10.0	0±0	0.4±0.2

BAP = Benzyl aminopurine. All cultures were maintained in the dark for 56 days. Each treatment consisted of 20 replicate tubes.

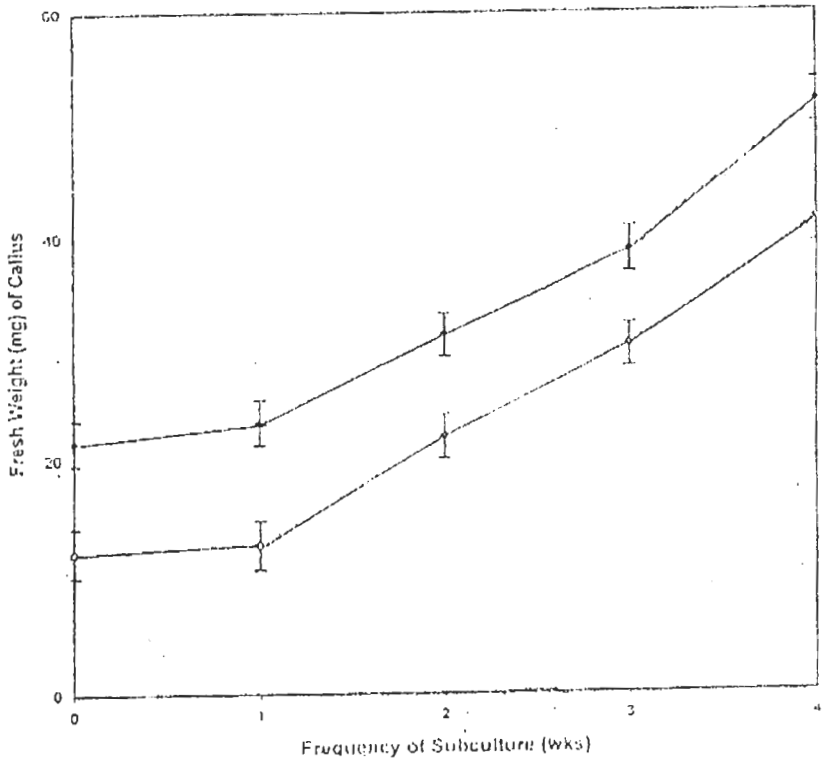

Figure 1: Effect of Frequency of Subculture on Callus Production (Mg Fresh Weight) in The Dark (●—●) or 12h Light/12h Dark (0-0) Regimes

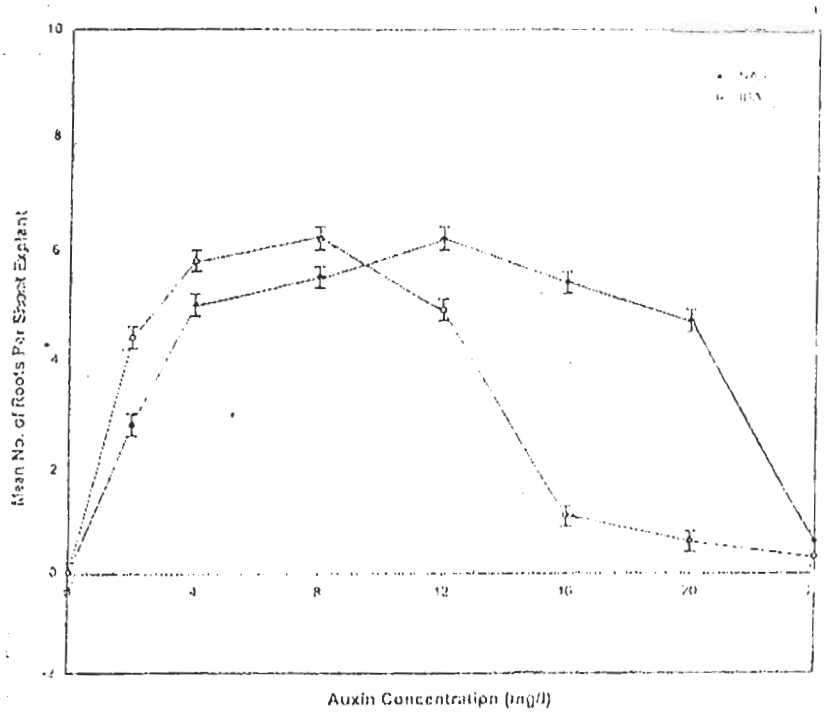

Figure 2: Effect of Auxin Concentration on Rooting of Shoot Explants Arising from *D. rotundata* Callus Cultures in Gamborg's Basal Media

DISCUSSION

Most reports in which non-tuber explants have been employed in yam propagation have involved prior induction of callus from such explants (2, 8, 19). In most of these reports either Murashige and Skoog (10) or Linsmaier and Skoog (7) basal media had been used and it took at least 40 days to produce reasonable quantities of callus in culture. The medium of Gamborg *et al.* (5) has substantially shortened the period (about 28 days in this study) required for callus induction in mature *D. rotundata* embryos. This is encouraging although in an earlier report (16) Murashige and Skoog's (10) medium was used to induce callus within 12 days and planlets within 18 days in dormant *D. rotundata* embryo cultures.

Profuse callus growth as a result of frequent subculturing is consistent with earlier studies (1, 3). Yam culture *in vitro* is characterized by production of phenolic compounds which tend to slow down growth if explants are maintained in the same medium for a long time. Frequent subculturing tends to lessen the amount of these phenolics in the culture medium and decreases the chances of subsequent death of the callus tissue. There is also the tendency for an increase in the concentration of growth regulators available to the callus tissue as a result of subcuturing and therefore can lead to increase in growth of tissue otherwise deficient in endogenous growth regulators.

Organogenesis in callus cultures is usually influenced by the types and levels of auxins and cytokinins in the medium. While shoot formation occurred in callus tissues when a low auxin to a high cytokinin ratio was used, root formation was generally observed with a high auxin and low cytokinin ratio (20). Although this has been shown to be the general organogenetic trend in dicots, there are very few reports of such trend in monocots. In fact Pierik (17) reported that dicots generally respond better to *in vitro* stimulus to a particular morphogenetic pattern than monocots. The regeneration of shoots from embryo callus is significant not only from the point of view of generating shoot buds from the tissues of monocots, but also the applicability in edible yam improvement.

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