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Interaction among cultural factors in microtuberization of white yam (*Dioscorea rotundata*)

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Abstract *Single node cuttings from pathogen-tested in vitro plantlets of white guinea yam (*D. rotundata*), cultivar TDr 93–23, were cultured in tuberization and half- and full-strength Murashige and Skoog media with 5% or 8% sucrose, with or without agar, in light or darkness and at 25 or 18°C. Microtuberization is influenced by interactions among the factors, but 25°C and daylight are critical. Copyright © 2006 John Wiley & Sons, Ltd*

Key words: yam, microtuberization, culture medium, cultural conditions, *Dioscorea*

Introduction

The need to develop more efficient, cost-effective means of propagating yams have resulted in efforts being directed at microtuber production from pathogen-tested, *in vitro* plantlets (Ng and Ng 1997). This is because microtubers are less vulnerable to transportation hazards, less bulky and more easily established in the soil without the need for acclimatization and transplanting as for *in vitro* plantlets (Ng and Mantell 1998). Previous investigators of microtuberization (MTZ) in a number of *Dioscorea* species have reported different cultural conditions as being optimum, such as the level of mineral nutrients in the medium, carbon sources and concentrations, medium matrix and incubation conditions. An understanding of the interactions among the basal medium formulations and cultural conditions will help to identify the optimum conditions for MTZ. This paper describes the responses of nodal explants from pathogen-tested *in vitro* plantlets of *D. rotundata* (white yam) to light and temperature, sucrose and agar concentration and their interactions with different basal media.

Materials and methods

Single-node cuttings from pathogen-tested *in vitro* plantlets of *D. rotundata* (TDr 93–23), multiplied as described by Ng (1992), were obtained from the tissue culture unit of the IITA and grown under different cultural conditions. In a preliminary experiment, microtuberization

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in two genotypes each of *D. alata* and *D. rotundata* was investigated using three media that differed in sucrose and agar concentrations, and TDr 93–23 had the lowest microtuberization rate, 3.33%. It was therefore considered that increasing microtuberization in this poor tuberizing genotype would identify the optimum combination of cultural factors. The basal medium formulations used were Murashige and Skoog (1962) medium (MS), half-strength MS (HMS) and tuberization (T) medium (Mantell and Hugo 1989). Each formulation was prepared with one of the following: 5% sucrose; 8% sucrose; 5% sucrose and 0.7% agar; and 8% sucrose and 0.7% agar. For the first two treatments filter paper bridges were used to provide support for the explant materials. The pH of each medium was adjusted to 5.7 ± 0.1. Media were dispensed into test tubes (125 × 16 mm) in 5 ml quantities each, capped and autoclaved for 15 min at 121°C and 103.4 Kpa. A single-node cutting 1 cm long was inoculated onto each tube. For each basal medium, cultures of each treatment were divided into two sets: one was incubated under 12 h light with 4000 lux intensity and the other was kept in complete darkness. Cultures at each light regime were divided into two, one incubated at 25°C and the other at 18°C.

In the experimental design, replicates were made of basal medium formulation (3), and ½ fractional replication of four factors, each at two levels (2^4) as treatments (Plan 6A.1, Cochran and Cox 1957). There were 10 test tubes (units) per treatment combination per replicate. After 5 months, data were taken on the number of cultures of each treatment which formed microtubers, as a percentage of the total number of cultures (% MTZ) and means were separated at 5% probability level using the generalized linear model procedure of SAS (SAS, 1999–2000).

Results and discussion

Differences in % MTZ among the three basal media, MS, HMS and T were, on average, not significant (Table 1). Under 12 h photoperiod, the % MTZ was significantly higher than in darkness, but in T the difference was not significant. This suggests that probably NH_4^+ (present in HMS and MS but not in T) enhances the response of plantlets to light. This is in contrast with the findings using vine cuttings from screenhouse-grown plants which produced MTs under dark incubation (Chang and Hayashi 1995; Balogun et al. 2004), and which was attributed to their higher food reserves in the explant than in those from *in vitro* plantlets (Balogun et al. 2004). Our result, however, does agree with Ng (1988), who reported optimum tuberization at 12 h photoperiod for *in vitro* plantlets of *D. rotundata*. Under both light and dark incubation, plantlets derive their energy from sucrose in the medium before becoming autotropic, then photosynthesis continues in light-incubated plantlets in contrast to those in the dark. In addition, respiration proceeds at a higher rate than photosynthesis under dark incubation, which may lead to CO_2 accumulation and stoppage of respiration (Desjardins 1995).

Incubation at 25°C yielded more microtubers than at 18°C in MS and T, but in HMS the difference was not significant. For HMS and T, MTZ was greater in the agar-free, liquid medium than in the agar-solidified form. This agrees with the report of Watanabe et al. (1993)

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that in HMS, nodal growth was enhanced four times in non-rotated liquid compared with in the agar solidified medium, attributed to the relatively easier absorption of nutrients in the former. In contrast in MS, the agar-solidified medium gave a higher % MTZ than agar-free medium. It appears that with the high salt content of MS the yield is better with agar addition, whereas in the low salt media HMS and T it is better without agar.

The main effect of the sucrose concentration was not significant, but there was a significant interaction with the basal medium formulation. Only HMS gave significantly more MTZ with 5% sucrose than with 8%. Light by sucrose interaction was significant. With light, 5% sucrose gave significantly more MTZ than 8%, while in the dark the difference was not significant (Table 2). The interactions among light, sucrose concentration and basal medium were also significant. In MS there was no significant difference between the two sucrose concentrations either in darkness or daylight, while for HMS-only, cultures incubated in light

Table 1. Microtuberization (%) in *D. rotundata* plantlets cultured in different regimes

Factor		Medium			
		MS	HMS	T	Mean
Photoperiod	Light	41.7^{a}	47.5^{a}	22.9^{a}	37.3^{a}
	Dark	16.7^{b}	0.0^{b}	28.6^{a}	15.1^{b}
Temperature	25°C	45.8^{a}	20.0^{a}	38.7^{a}	34.8^{a}
	18°C	12.5^{b}	27.5^{a}	12.7^{b}	17.6^{b}
Matrix	Liquid	16.7^{b}	32.5^{a}	47.3^{a}	32.1^{a}
	Agar	41.7^{a}	15.0^{a}	4.2^{b}	20.3^{b}
Sucrose	8%	29.2^{a}	12.5^{b}	30.0^{a}	23.9^{a}
	5%	29.2^{a}	35.0^{a}	21.4^{a}	28.5^{a}
Mean		29.2^{A}	23.8^{A}	25.7^{A}	
Standard error		6.71	6.15	6.60	3.73

Values of each factor in each column with the same letter are not significantly different at $p = 0.05$. MS: Murashige and Skoog (1962) medium; HMS: Half strength MS; T: Tuberization medium (Mantell and Hugo 1989).

Table 2. Microtuberization (%) in *D. rotundata* plantlets cultured in different regimes of sucrose and light

Medium	Incubation condition			
	Light		Dark	
	8% S*	5% S	8% S	5% S
MS	50.0^{ab}	33.3^{bc}	8.3^{cd}	25.0^{bcd}
HMS	25.0^{bcd}	70.0^{a}	0.0^{d}	0.0^{d}
T	10.0^{cd}	35.7^{bc}	50.0^{ab}	7.1^{cd}
Mean	28.3^{B}	46.4^{A}	19.4^{BC}	10.7^{C}
Standard error	3.73			

Notes as Table 1. *S: Sucrose.

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Table 3. Microtuberization (%) of *D. rotundata* under different cultural conditions

Treatment	Medium		
	MS	HMS	T
5% sucrose / 0.7% agar / 12 h light / 18°C	33[b]	60a	0[b]
5% sucrose / 0.7% agar / dark / 25°C	33[b]	0[b]	0[b]
5% sucrose / liquid / dark / 18°C	17[b]	0[b]	14[b]
5% sucrose / liquid / 12 h light / 25°C	33[b]	80[a]	71[a]
8% sucrose / 0.7% agar / dark / 18°C	0[b]	0[b]	17[b]
8% sucrose / 0.7% agar / 12 h light / 25°C	100[a]	0[b]	0[b]
8% sucrose / liquid / 12 h light / 18°C	0[b]	50[a]	20[b]
8% sucrose / liquid / dark / 25°C	17[b]	0[b]	83[a]
Standard error	6.7	6.2	6.6

Notes as Table 1.

with 5% sucrose gave higher % MTZ values. Cultures in T responded significantly to sucrose concentration only in the dark incubation, 8% giving the highest % MTZ.

Interactions among light, agar and basal medium were also significant. In dark incubation, cultures in MS and HMS did not respond significantly to agar but T gave significantly more MTZ (49%), while it was 8% in the agar-solidified medium. In light, MTZ was 65 and 46% in the agar-free HMS and T respectively, significantly higher than 30 (HMS) and 0% (T) in agar-solidified media. In MS medium, however, MTZ was higher in the agar-solidified medium (67%) than in liquid (17%).

Table 3 shows the optimum combination of factors for each basal medium. In MS, MTZ was 100% with agar, 8% sucrose incubated under light at 25°C. In HMS the highest was with liquid, 5% sucrose under 12 h photoperiod and 25°C. In T there was no significant difference between the two highest values.

Thus *in vitro* tuberization is affected by many factors and adequate combination of basal medium formulation with sucrose, agar, temperature and light regimes gave up to 80% microtuber formation. A temperature of 25°C and light are critical for MT production from *in vitro* plantlets.

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