Conservation of *Dioscorea rotundata*: Effect of Basal Medium Type and Naphthalene Acetic Acid on Growth and Microtuberization

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Abstract

Nodal cuttings of plantlets of *Dioscorea rotundata* cv. Puna were cultured in an NAA-enriched Murashige and Skoog medium and its modified form (T-Medium) in which NH_4NO_3 was omitted. Deposition of phenolic compounds was more in the NH_4^+ omitted medium with increasing concentration of NAA. There were differences in microtuberization response in the two media types used. However, for optimum tuberization, explants have to be pulsed in MS medium prior to culture in the medium. The modified medium supplemented with 25 μM and 50 μM NAA induced 670 mg and 500 mg respectively of microtubers. The sizes of microtubers produced favour the use of *in vitro* techniques for the induction of the propagules for the conservation and production of seed yam.

Introduction

Field genebanks have been the traditional methods for preserving yam germplasm. However, other conservation methods including *in vitro* consevation techniques, particularly the slow-growth method has been receiving attention. In this respect, the culture systems which could be used for *in vitro* conservation of yam including meristem culture, nodal culture, microtuberization, embryo and suspension culture (Ng & Ng, 1997).

Microtuberization, apart from serving the needs of conservation could also be a means of facilitating germplasm exchange as well as for the production of planting materials for farmers. Edible yams (*Dioscorea* sp.), particularly *D. roundata*, constitute one of the major tuber crops in West Africa. The propagation of this crop is by the use of edible (i.e. tuber) portions or small whole tubers as planting materials. Although this method produces genetically uniform materials, the rate of multiplication is low. The rooting of vine cuttings, which are non-edible portions, in sand, however, with difficulty, has been used for the propagation of some yam species (Coursey, 1967, Wilson, 1982). The non-edible plant parts have proven to provide excellent explants for *in vitro* multiplication to produce large numbers pf plantlets, as well as microtubers.

Various factors have been documented to influence microtuberization in yam. Mineral medium strength, nitrogen source in the medium, sucrose concentration, auxins, cytokinins and photoperiod have been shown to be some of the factors that influence *in vitro* tuberization in yam (Mantell & Hugo, 1989; Alhassan, 1991; Jean & Cappadocia, 1991, 1992; Ng and Ng, 1997). Cytokinin and cytokinin/auxin combinations have been in use frequently for *in vitro* tuberization in a number of yam species. Ng (1988) has reported on the use of kinetin for the initiation of microtubers in some *D. rotundata* varieties.

The paper provides preliminary

information on studies carried out to evaluate the effects of differences in nitrogen levels in growth media in combination with NAA on *in vitro* shoot growth and development of microtubers in *D. rotundata* for germplasm conservation and exchange.

Materials and methods

Plant material

Nodal cuttings from stage II plantlets of *D. rotundata* var. Puna were used. This is a stage in which after successfully cutting the explants aseptically, the developed shoots are maintained in a stabilized state. They had been in maintenance medium made up of Murashige & Skoog (MS) medium (Murashige and Skoog, 1962), 2% sucrose and 0.3% phyagel (Sigma™). A single nodal explant was cultured in each Honey Food jar (Sigma™).

Culture media

Two multiplication media were used (Table 1). One was made up of MS basal medium and the other was the T-medium (Mantell & Hugo, 1989). Each was supplemented with 4% sucrose (12.5, 25.0 and 50.0 μ*M*) naphthalene acetic acid (NAA), 10 mgl^{-1} thiamine hydrochoride and solidified with 0.3% phytagel. Media *p*H was adjusted to 5.8 and served into Honey Food jars prior to autoclaving at 121 °C under KPa for 15 min.

Culture conditions

Cultures were incubated at 25 °C and 16 h for 14 days and then transferred into a 18 °C and 8 h facility, all at 4000 lx light intensity.

Results and discussion

The effects of MS and T-media as well as NAA on shoot growth, browning (deposition of phenolic compounds) of cultures and microtuberization were evaluated. The presence of NAA and ammonium nitrate in the media promoted browning and this was more pronounced with increase in NAA concentration in both T and MS media (Fig. 1). The intensity of browning at a specified concentration of the auxin was deeper in the T-medium, in which shoot growth was inhibited, than in the MS medium (Fig. 2). There is a possible correlation between ammonium ion level (which is the main difference between the two types of media) and the release of the phenolic compounds

TABLE 1

Composition of inorganic constituents of culture media used (mg l^{-1})

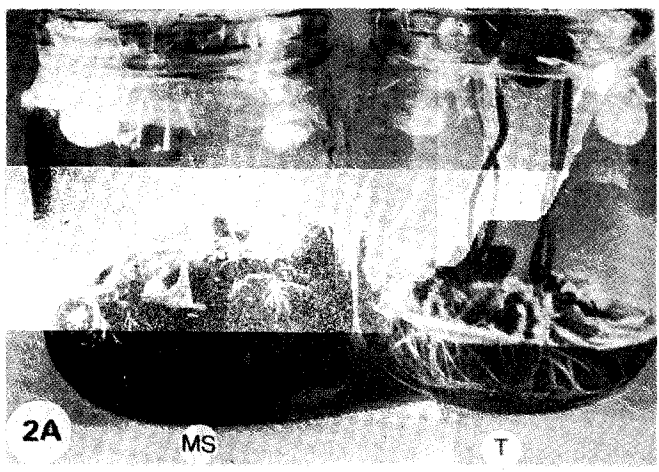
Component	*Murashige & Skoog medium*	*T-medium*
Macroelements		
$CaCl_2.2H_2O$	440.00	-
$Ca(NO_3)_2.4H_2O$	-	300.00
KCl	-	65.00
KNO_3	1900.00	80.00
KH_2PO_4	170.00	-
$Na_2EDTA.2H_2O$	36.70	-
$NaH_2PO_4.2H_2O$	-	16.50
Na_2SO_4	-	200.00
NH_4NO_3	1650.00	-
$MgSO_4.7H_2O$	370.00	720.00
$Fe(SO_4).7H_2O$	27.80	25.00
Microelements		
$AlCl_3$	-	0.03
$CoCl_2.6H_2O$	0.025	-
$CuSO_4.5H_2O$	0.025	0.03
H_3BO_3	6.20	1.24
KI	0.83	0.01
$MnSO_4.4H_2O$	22.30	0.01
$Na_2MoO_4.2H_2O$	0.25	-
$NiCl_2.6H_2O$	-	0.03
$ZnSO_4.7H_2O$	8.60	1.00

Fig. 1. Shoot growth and deposition of phenolic compounds in (1A) MS medium and (1B) T-medium supplemented with various concentrations of naphthalene acid (NNA).

into the medium. This has been corroborated by George & Sherrington (1984) in their review in which the natural rate of formation of phenolic compounds has been correlated with the morphogenesis capacity of tissues and the auxin/cytokinin levels in the medium.

The formation of microtubers is influenced by various factors including sucrose concentration, auxin, cytokinins, nitrogen source in the medium as well as photoperiod with species and varietal differences in response (Ng & Ng, 1997). The main factors under consideration in this experiment are auxin and nitrogen levels. Nitrogen source in the medium can affect the capacity of a culture to produce propagules. The nitrogen in the form of nitrate must be supplemented with a source of reduced nitrogen such as NH_4^+ in order to be effective (Dougal, 1981). The inorganic nitrogen in the media used was supplied as potassium nitrate, ammonium nitrate and calcium nitrate. Apart from potassium nitrate, which is common to the MS and T-media, calcium nitrate is available only in the MS cultures while ammo-nium nitrate is found in T-medium alone. Stimu-lation in microtuber formation associated with reduced levels of shoot growth has been recorded in *D. batatas* and *D. alata* cultures in which media did not contain ammonium ions. Additionally, it has been observed that T-medium supported high microtuber induction frequencies in *D. alata* shoot cultures. The MS medium has been reported to have some level of inhibitory effect on the tuberization process (Mantell & Hugo, 1989). Auxins have been reported to reverse this inhibitory effect of MS medium on microtuberizaion (Asahira & Yazawa, 1979). These have been exhibited in this experiment.

Differences were observed in microtuber response in the two types of media used. *D. rotundata* cultures in T-medium supplemented with 50 μM NAA developed microtubers weighing ca. 500 mg (Fig. 3). Supplementation with a lower concentration of 25 μM NAA led to the induction of microtubers weighing ca. 670 mg after 12 weeks of incubation. Jean & Cappadocia (1991, 1992) also recorded increases in



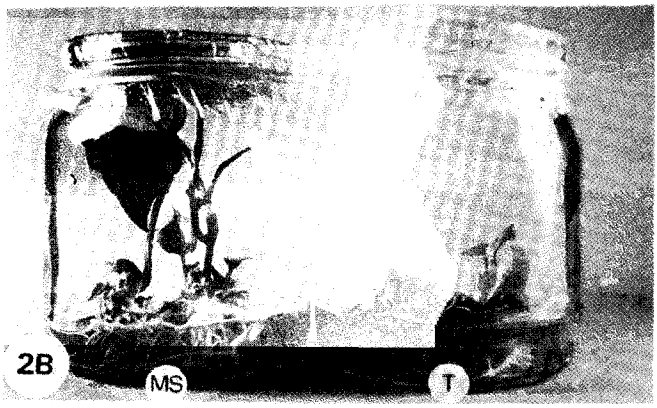


Fig. 2. Effect of Murashige and Skoog (MS) and T-media (2A) as well as 25 μM NAA on shoot growth and deposition of phenolic compounds.

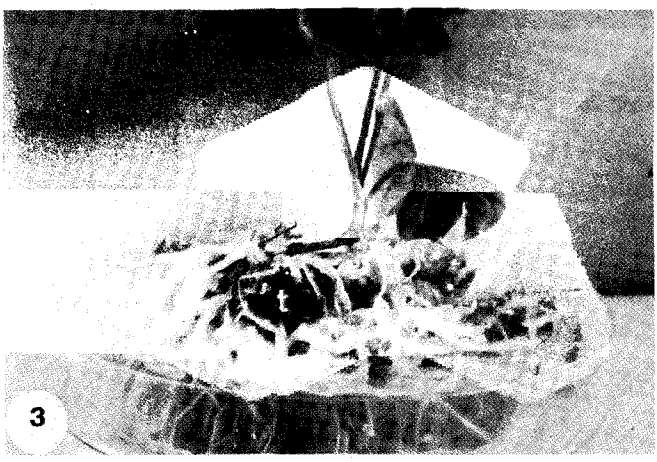


Fig. 3. Microtuber (t) induced in nodal cultures of *D. rotundata* in MS medium supplemented with 25 μ*M* NAA.

microtuber sizes in a *D. alata* culture in which NH_4NO_3 was omitted but enriched with NAA. In this experiment, using a medium without inorganic ammonium ions reduced shoot growth; this has been recorded by Mantell & Hugo (1989). Some level of NAA is required in a medium to stimulate microtuberization; increased concentrations may not be advantageous for microtuber formation. In another experiment (unpublished) it has been observed that the pulsing of the explants, i.e. treatment for a brief period in an NAA supplemented medium may be required prior to culture in a tuberization medium devoid of the auxin. This is necessary to avoid callus formation from prolonged exposure to NAA treatment as well as to high auxin treatment.

Conclusion and recommendation

Levels of nitrogen and NAA in the media used influenced tuberization in *D. rotundata.* The sizes of microtubers induced are larger than those encountered in the literature for *D. rotundata.* The increased sizes favour the use of *in vitro* induction of microtubers for the production of seed yam as well as for conservation. Microtubers have longer dormancy periods

than tubers cultivated under natural conditions (Ng & Ng, 1997) and, therefore, could serve as a means of germplasm conservation and exchange. On the basis of the results obtained, the yam nodal explants require pulse treatment of NH_4^+ as obtainable in MS medium as well as a temporary treatment in NAA prior to culture in T-medium for optimum tuberization if all other factors needed for the process are provided. Experiments are planned, taking into consideration all the factors, particularly the effect of pulsing. The experiments will be carried out further to study the dormancy of microtubers in order to ascertain their role for conservation.

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