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Micropropagation of Yam (*Dioscorea rotundata)*: Assessment of Performance in Cassava Starch-gelled Medium

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Abstract: Cassava starches from nine different cassava varieties (TMS 97/2205, 97/0162, 92/0057, 98/0505, 92/0326, 30572, 82/0058 TME 419 and NR 8082) were evaluated as gelling agents substitute for in *vitro* propagation of yam (*Dioscorea rotndata*). *In vitro* yam explants (nodal segments) were seeded singly into culture tubes containing 1 mL of yam multiplication medium with no gelling agent (liquid medium-control I) and 15 mL of yam multiplication medium gelled differently in 0.2% gelrite (control II), 0.7% agar (control III) and in 7% starch from the nine different cassava varieties. Cultures were maintained at 28°C±2 illumination and 16 h photoperiod (30-40 μmole m^{-2} sec^{-1}) supplied by white fluorescent tubes on shelves for four weeks. The overall percentage survival ranged from 86.67-100% after 60 days in culture. Explants in TMS 97/2205 performed relatively better in terms of height, number of leaves and nodes compared to those in liquid, gelrite or agar-gelled medium. This is significant because survival and multiplication is a key factor in the choice of a gelling agent.

Key words: *Dioscorea rotndata,* cassava starches, micropropagation, multiplication, gelling agent

INTRODUCTION

Starches (corn, potato and cassava) have been shown to be good gelling agent substitute in media to conventional gelling agents like agar or gelrite in plant tissue propagation (Prakash, 1993; Nkere and Mbanaso, 2009; Nkere *et al*., 2009). The use of starches has drastically reduced the cost of micropropagation. For example, 1 kg of agar cost about $200 as against $1.2 for 1 kg of cassava starch. However, the adoption of a particular starch for a plant depends on proper screening as it has been observed that different plants respond differently to starches.

Yam is not easily amenable to tissue culture procedure, hence, the need to develop protocol aimed towards low-cost propagation. Therefore, the suitability of some cassava starch-gelled media was assessed for in *vitro* regeneration and multiplication of yam.

MATERIALS AND METHODS

Source of explants: Shoot-tip explants and nodal segments were excised from vigorously growing *in vitro Dioscorea rotundata* cultivar (Obiaturugo) obtained from the *in vitro* gene bank housed at the Biotechnology Programme (Plant Tissue Culture Lab) of National Root Crops Research Institute (NRCRI) Umudike, Abia State Nigeria.

Starch preparation: Starch was obtained from nine cassava varieties (NR 8082, TMS 97/2205, TMS 97/0162, TMS 92/0057, TMS 98/0505, TMS 92/0326, TMS 30572, TME 419 and TMS 82/0058) as was stated in earlier reports (Mbanaso, 2008; Nkere and Mbanaso, 2009).

Culture medium: Murashige and Skoog (1962) basal medium, with 3% sucrose was used. Medium was supplemented with myo-inositol (0.1 g L^{-1}), L-cysteine (0.02 g L^{-1}), kinetin (0.5 mg L^{-1}) and solidified with gelrite, agar or starch at 0.22, 0.7 and 7%, respectively. The liquid media contained no gelling agent. The pH was adjusted to 5.8. Gelrite and agar were dissolved in medium by heating while the starches were incorporated into the medium accordingly as has been reported earlier (Mbanaso, 2008; Nkere and Mbanaso, 2009). Fifteen milliliter aliquot each of the different medium were then dispensed into culture tubes and autoclaved for 15 min at 121°C.

Explants culture: Explants were seeded singly into culture tubes containing the prepared medium. Cultures were maintained at 28°C±2, 16 h photoperiod (30-40 μmol m^{-1} sec^{-1}) supplied by white fluorescent

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tubes on culture shelves for four weeks. The plant height, number of leaves and nodes were assessed after two and four weeks in culture.

Statistical analysis: All the data was analyzed using DMRT test considering 5% level of significance.

RESULTS AND DISCUSSION

The growth and proliferation of explants in the differently gelled medium is shown in Fig. 1. The overall percentage survival (nodal segment) ranged from 86.67-100% (Fig. 2). The performance of the yam plantlets from the differently gelled-medium is shown on Table 1. Generally, plantlets from TMS 97/2205 starch gelled medium gave the best result after 60 days in culture with respect to the parameters accessed. However, the performance did not differ significantly ($p<0.05$) from those of the conventional gelling agents (gelrite and agar) and of the liquid media.

Survival and multiplication is a key factor in the choice of a gelling agent. Therefore, this relatively high performance of explants from TME 97/2205 starch gelled medium over that of liquid and gelrite or agar gelled medium is significant because starch from this cassava genotype could conveniently serve as a gelling substitute. Although, liquid media has been shown to favour yam micropropagation, this low performance may be attributed to the drying of the media as it was observed that most cultures in liquid media dried up before the 60 days.

The relatively low performance of explants in TMS 30572 and TME 419 starch gelled medium as against the high survival rate (100%) of the explants is not unusual as it has been reported that some gelling agents contain inhibitory substances that hinder morphogenesis and reduce the growth rate of cultures (Powell and Uhrig, 1987). Early reports (Nkere *et al*., 2009; Nkere and Mbanaso, 2009) have shown that TME 419 starch gelled medium favoured micropropagtion of cassava and ginger whereas TME 97/2205 did not. This once again brings to the fore that the adoption of a starch as a gelling agent for the propagation of any crop would depend on proper screening.

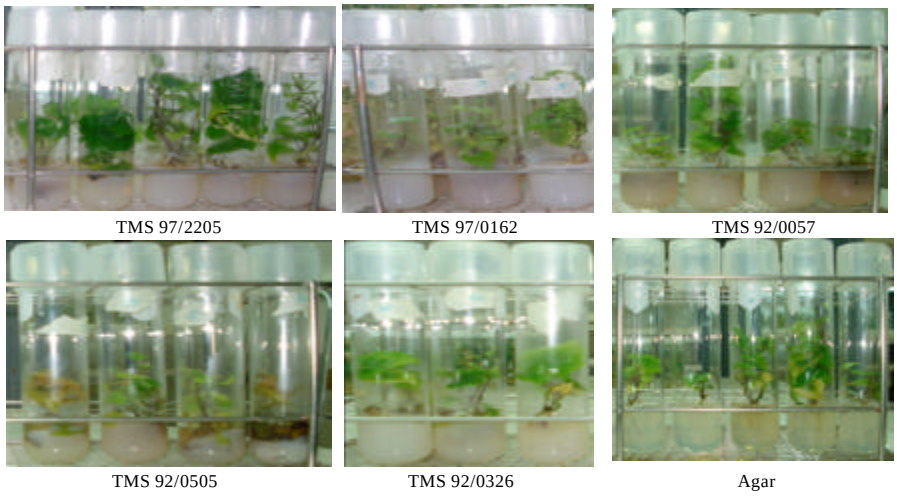

Fig. 1: Yam cultures growing in differently gelled media

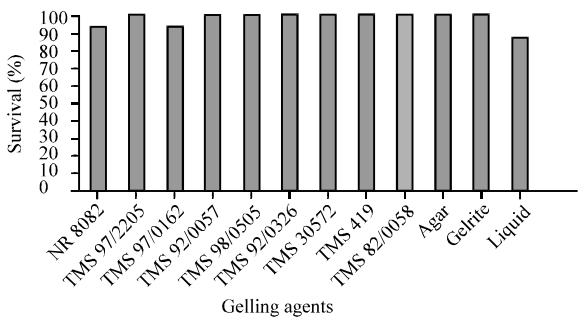

Fig. 2: Survival of explants in differently gelled medium after 60 days in culture

Table 1: Explants performance in the differently gelled media

	Mean values of parameters accessed			
Gelling agent	No. of leaves	No. of nodes	No. of shoots	Height
NR 8082	1.36^{e}	1.55^{cd}	1.0^{b}	2.34^{f}
TMS 97/2205	6.33^{a}	4.07^{a}	1.47^{ab}	6.89^{a}
TMS 97/0162	3.57^{b}	2.07^{c}	1.21^{b}	5.72^{b}
TMS 92/0057	2.46^{bc}	1.85^{cd}	1.23^{b}	5.22^{bc}
TMS 98/0505	2.40^{c}	1.47^{d}	1.93^{a}	3.77^{de}
TMS 92/0326	2.67^{b}	1.53^{cd}	1.13^{b}	4.36^{cd}
TMS 30572	1.60^{e}	1.13^{d}	1.13^{b}	2.98^{f}
TME 419	2.20^{c}	1.40^{d}	1.07^{b}	3.13^{ef}
TMS 82/0058	2.92^{b}	2.08^{c}	1.23^{b}	2.74^{f}
Agar	2.64^{b}	1.86^{cd}	1.07^{b}	3.61^{de}
Gelrite	2.93^{b}	2.33^{bc}	1.0^{b}	2.39^{f}
Liquid	3.44^{b}	3.11^{b}	1.55^{ab}	4.84^{bc}

Mean in a column with the same superscript letter (s) do not differ significantly according to DMRT at 5% level of probability

CONCLUSION

The result from this study has shown that cassava starch from the genotype TMS 97/2205 could serve as a good alternative to agar or gelrite as a gelling agent for *in vitro* multiplication of yam. Further study is needed to confirm this result.

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