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In-vitro Ginger Multiplication: Screening of Starch from Different Cassava Varieties as Gelling Agent in Medium

C.K. Nkere, I.C. Umezurumba and E.N.A. Mbanaso
Laboratory of Plant Tissue Culture, Biotechnology Unit, Institute of National Root Crops Research, Umudike, P.M.B. 7006, Umuahia, Abia State, Nigeria

Abstract: Shoot tip explants excised from vigorously growing *in vitro* shoot culture of ginger (UG1) were seeded singly into culture tubes containing 15 mL of ginger multiplication medium gelled differently in 7% starch from eight different cassava varieties (TMS 92/0057, 97/0162, 97/2205, 82/0058, 92/0323, 92/0505 TME 419 and NR 8082) and in 0.7% agar (control). Cultures were maintained at 28°C ±2 and 16 h photoperiod (30-40 μmole m^{-2} S^{-1}) supplied by white fluorescent tubes on shelves for 6 weeks. Percentage shoot survival ranged from 10-80%, with shoots from TME 419 starch gelled medium having the highest (80%) as against agar (60%). The multiplication rate of shoots from the different medium ranged from 1.0-7.0. Of the eight starches tested, shoot multiplication in three namely TMS 97/0162, TMS 98/0505 and TME 419 were not significantly different ($p<0.05$) from agar. In fact, shoots from TMS 98/0505 starch gelled medium recorded the highest multiplication rate of 7.0. These results provide the rationalization for the adoption of cassava starches especially, from these varieties in the order (TME 419, TMS 97/0162 and TMS 98/0505) as substitute for agar as a gelling agent for *in vitro* multiplication of ginger.

Key words: Ginger, cassava starch, shoot tips, explants, *in vitro*, micropropagation, multiplication

INTRODUCTION

Ginger (*Zingiber officinale*) is a spice, which is used for cooking and is also consumed whole as a delicacy or medicine (Jamaica ginger). The characteristic odor and flavor of ginger root is caused by a mixture of zingerone, shogaols and gingerols, volatile oils that compose about one to three percent of the weight of fresh ginger. In laboratory animals, the gingerols increase the motility of the gastrointestinal tract and have analgesic, sedative, antipyretic and antibacterial properties. It has a sialagogue action, stimulating the production of saliva, which makes swallowing easier (O' Hara *et al*., 1998).

Plant starches are suitable for gelling agents in tissue culture, comparing favourably with the conventional gelling agents such as agar. Plant starches commonly used for plant tissue culture studies include potato, rice, wheat and barley starches (Sovari, 1986a, b) and sago and isobugol (Bhattacharya *et al*., 1994).

Ginger has been found amenable to rapid multiplication *in vitro* using gelled medium. The conventional gelling agents (agar and gelrite) for *in vitro* micropropagation are presently imported, expensive and not readily available. Therefore, there is need for a cheaper, easily available local alternative for generating clean propagules of ginger. Thus, this informed the use of starch as a gelling substitute.

MATERIALS AND METHODS

Source of explants: The shoot tip explants used in the study were excised from *in vitro* ginger cultivar (UG1) plantlets obtained from in vitro gene bank housed in the Biotechnology unit (Plant tissue culture lab) of National Root Crops Research Institute (NRCRI) Umudike, Abia State Nigeria.

Starch preparation: The cassava varieties used (TMS 92/0057, 97/0162, 97/2205, 82/0058, 92/0323, 92/0505 TME 419 and NR 8082) were obtained from NRCRI, Umudike. Freshly harvested roots of cassava varieties were peeled, washed and then crushed separately. The pulp was suspended in excess quantity of water, sieved and the effluent collected in large labeled bowls. The effluent was left overnight for the starch to sediment and the supernatant decanted. The surface of the starch was rinsed with clean water and the starch scooped into trays for sun drying at ambient temperature to remove excess moisture. Further drying to constant weight was in moisture extraction oven. The dried starch was milled into

Corresponding Author: C.K. Nkere, Laboratory of Plant Tissue Culture, Biotechnology Unit, Institute of National Root Crops Research, Umudike, P.M.B. 7006, Umuahia, Abia State, Nigeria

powder using a household milling machine, packaged in sealed polyethylene bags and stored under room temperature until required (Mbanaso, 2008).

Culture medium: The basic culture medium (Murashige and Skoog, 1962) with 3% sucrose was used. It was supplemented with 100 mg L^{-1} myo-inositol, 10 mg L^{-1} L-cysteine, 5 mg L^{-1} BAP and 0.1 mg L^{-1} NAA and was solidified with agar and the starches at 0.7 and 7%, respectively. The pH was adjusted to 5.8.

For agar, the medium was dissolved by heating, while for the starches; the dried cassava starch powder was first made into thick slurry with a part of the medium to be gelled. The remaining part was heated to a temperature of 78±1°C and the corresponding cold slurry stirred vigorously into it. A 15 mL aliquot each of the different medium were then dispensed into culture tubes and autoclaved for 15 min at 121°C, 1.05 kg cm^{-2}.

Shoot tip culture: The shoot tip explants from vigorously growing *in vitro* shoot culture of ginger (UG1) were excised and all superfluous tissues remove under aseptic conditions. Explants were seeded singly into culture tubes containing the prepared medium. Cultures were maintained at 28°C ±2, 16 h photoperiod supplied by white fluorescent tubes on shelves for 6 weeks. Shoot survival and proliferation was then assessed.

Statistical analysis: Tests of significance were calculated using Analysis of Variance (ANOVA) and multiple comparison-Least Significant Difference (LSD) of the GenStat (DE3) ver. 7.2.

RESULTS

The growth and proliferation of cultured shoot tips in the differently gelled medium is shown in Fig. 1. Percentage survival of the shoots ranged from 10-80%, with shoots from TME 419 starch gelled medium having highest (80%) as against those from agar (60%) (Fig. 2).

The multiplication rate of shoots from the different medium ranged from 1.0-7.0. Of the eight starches tested for shoot multiplication, three from (TMS 97/0162, TMS 98/0505 and TME 419) were not significantly different ($p<0.05$, LSD 2.02) from agar. In fact, shoots from TMS 98/0505 starch gelled medium recorded the highest multiplication rate of 7.0 (Table 1).

Table 1: Mean shoot proliferation in the different gelling agents

Gelling agents	Multiplication rate
Agar	6.89
92/0057	2.33
97/0162	6.50
97/2205	1.00
419	6.31
8082	4.00
82/0058	3.67
92/0323	3.33
92/0505	7.00
$LSD_{(005)}$	2.02

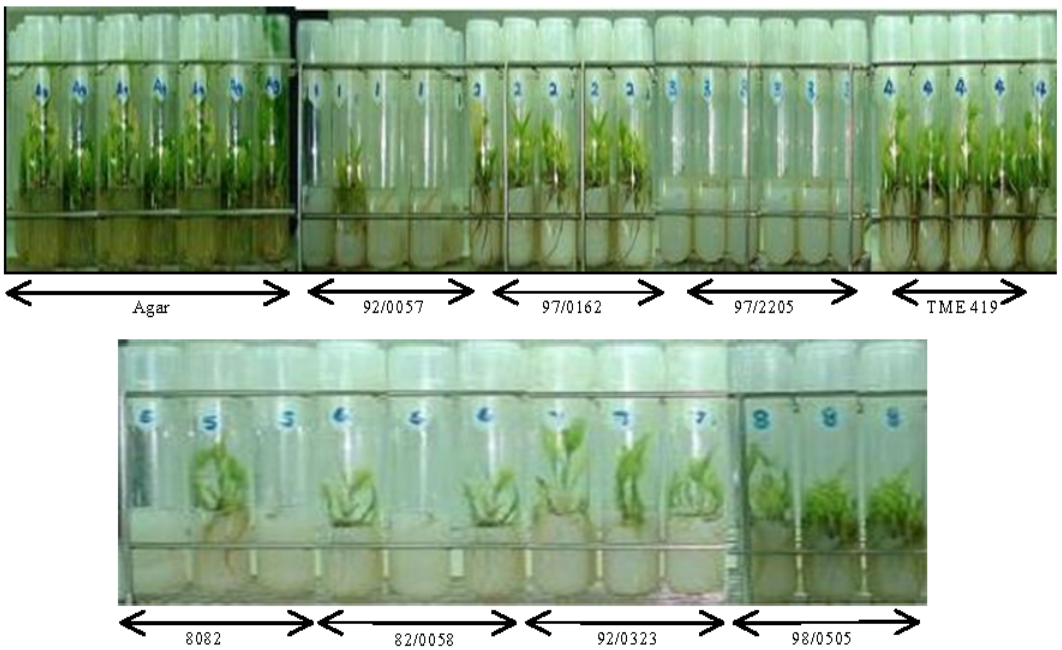

Fig. 1: Ginger shoots proliferation in the differently gelled medium

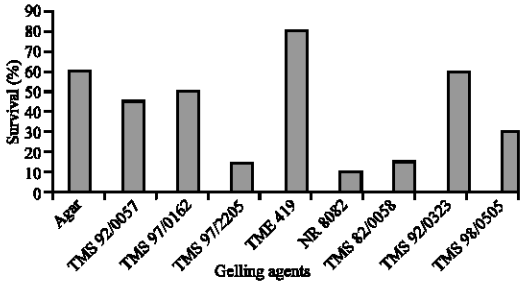

Fig. 2: Shoot survival in the different gelling agents

DISCUSSION

The survival of shoots in the different starch gelled medium was relatively low, with the exception of those from (TMS 97/0162, TMS 920323 and TME 419) that gave 50, 60 and 80%, respectively, others were below 50%, while shoots from agar gelled medium were not quite different as it recorded 60% survival. This high survival rate of shoots from TME 419 starch gelled medium over that of agar gelled medium is significant because survival is a major key factor in the choice of a gelling agent. It has been shown that addition of 8.0% tapioca starch to the MS medium severed as a good substitute for 'Bacto-agar' for potato shoot-culture (Getrudis and Wattimena, 1994).

The relatively high survival (60%) of shoots in TMS 92/0323 starch gelled medium dose not transcend to high multiplication rate (3.33). This result 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). Although, it has been reported in an earlier study that TMS 92/0323 starch gelled medium gave better result after agar in shoot proliferation of cocoyam with a mean multiplication rate of 7.4 after the second passage (Mbanaso, 2008). Thus, the efficiency of a particular starch as a gelling agent in shoot proliferation of a particular plant, dose not transcend to all the plants. Therefore, the adoption of a starch as a gelling agent would depend on proper screening.

CONCLUSION

The study has shown that cassava starches from these varieties in the order TME 419, TMS 97/0162 and TMS 98/0505 could conveniently serve as a substitute for agar as a gelling agent for *in vitro* multiplication of ginger. Further study in the area of characterization of the starches would be needed in other to ascertain the principal active agents.

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