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Full Length Research Paper

Effect of multiple subcultures on *Musa* shoots derived from cassava starch-gelled multiplication medium during micropropagation

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Shoot tip explants excised from *in vitro* plantlets of two *Musa* genotypes (TM3X 15108-6 and TMBX 612-74) were seeded singly into test tubes containing twenty milliliters each of *Musa* multiplication medium gelled differently in 60 and 70 gL^{-1} cassava starch as well as 5 gL^{-1} agar and placed on shelves under 14 h photo period (30-40 µmole m^{-2} S^{-1}) supplied by white fluorescent tubes. Temperatures were maintained at 27 ± 2°C. Monthly subcultures were carried out by separating shoot clusters developed from each explant into individual shoots and each shoot trimmed down and re-cultured as a shoot tip under the same conditions. This process was repeated for four months after which well formed shoots were subsequently transferred into a rooting medium. Cultured shoot tips grew and multiplied in the differently gelled medium. Shoots derived from starch-gelled medium after several subcultures became less robust than those grown on agar medium. This tendency for reduced robustness over time increased with increase in starch concentration in the medium and was more acute in TMBX 612-74 than TM3X 15108-6. Shoot water content after the fourth subculture revealed that shoots derived from agar-gelled medium had significantly (P=0.05) higher water content compared to shoots derived from starch gelled medium. This phenomenon must probably, is related to the availability of absorbable water in the differently gelled medium.

Key words: Cassava starch, *Musa* shoot tips, micro-propagation, subculture.

INTRODUCTION

Plant starches are suitable for gelling tissue culture media, comparing favorably with the more conventional gelling agent such as agar. Plant starches commonly used for plant tissue culture studies include potato, rice, wheat and barley starches (Sovari, 1986a,b), sago and isobugol (Bhattacharya et al., 1994), corn starch (Zimmerman et al., 1995) and cassava starch (Mbanaso, 1999). These starches are sought after as cheaper and more readily available alternatives to agar or gelrite in certain developing countries (Bhattacharya et al., 1994; Mbanaso, 1999). More importantly however, is the need to avoid the inhibitory symptoms in cultures caused by toxic substances contained in agar (Nairn et al., 1995) or to enhance shoot growth in species recalcitrant on media gelled in standard gelling agents (Zimmerman et al., 1995). Apart from conferring solidity to media plant starches elicit other effects, some beneficial to tissues cultured on them (Sovari, 1986 a, b; Henderson and Kinnersley, 1988; Zimmerman et al., 1995). This paper describes the effect of multiple subcultures in *Musa* shoots grown on cassava starch-gelled multiplication medium.

MATERIALS AND METHODS

Source of explants

Shoot tip explants were excised from *in vitro* plantlets of two *Musa* genotypes (TM3X 15108-6 and TMBX 612-74), obtained from an *in vitro* collection housed at the International Institute of Tropical Agriculture (IITA) Outstation at Onne near Port-Harcourt, Rivers State Nigeria. The original cultures were initiated and maintained via monthly passages on multiplication medium gelled in gelrite after Vuylsteke (1989).

Table 1. Multiplication of *Musa* shoots on cassava starch and agar gelled medium during successive subcultures.

Type of gelling agent	No. of subculture	Survival (%)	No. shoots produced/explant/month
Cassava starch (60 gL^{-1})	1	77.66	3.21
	2	95.83	3.24
	3	87.10	3.00
	4	95.79	3.84
Cassava starch (70 gL^{-1})	1	81.25	3.23
	2	97.85	3.68
	3	88.30	3.09
	4	96.77	3.28
Agar (5 gL^{-1})	1	67.71	3.02
	2	59.38	2.51
	3	76.34	3.95
	4	87.36	3.55
$LSD_{(0.05)}$		11.28	1.01

Preparation of cassava starch

Cassava starch was obtained from the roots of NR8082 (Okeke et al., 1989), a cassava genotype bred at the National Root Crops Research Institute, Umudike. Freshly harvested roots were peeled, washed thoroughly and then crushed. The pulp was suspended in excess quantity of water, sieved and the effluent collected in large 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 carried out in a moisture extraction oven. The dried starch was milled into powder using a household milling machine and packaged in sealed polyethylene bags until required.

The Culture medium

The culture medium was based on Murashige and Skoog's (1968) medium modified by Vuylsteke (1989) for *Musa* shoot multiplication. The pH of the medium was adjusted to 5.8 before autoclaving. Twenty ml aliquots were dispensed into test-tubes and sterilized by autoclaving at 121°C, 1.05 kg/cm^2 (103.4 KPa), for 15 min and cooled before use.

Gelling of culture medium

For each concentration (60 and 70 gL^{-1}), the dry cassava starch powder was first made into a thick slurry with part of the medium to be gelled. The remaining medium was heated to a temperature of 78±1°C and the corresponding cold slurry stirred vigorously into it. The medium was dispensed into culture vessels and autoclaved as previously stated. For agar, 5 gL^{-1} was dissolved in the medium by heating and distributed into culture vessels prior to autoclaving.

Culture of shoot tips under controlled conditions

For development *in vitro*, shoot tips were rid of all superfluous tissues and seeded singly into culture vessel and placed on shelves under 14 h photo period (30-40 µmole m^{-2} S^{-1}) supplied by white fluorescent tubes. Temperatures were maintained at 27±2°C by air conditioning units

Monthly subcultures

Monthly subcultures were carried out by separating shoot clusters developed from explants into individual shoots which were then trimmed down to shoot tips and re-cultured under the same conditions. This process was repeated for four months after which well formed shoots were subsequently transferred into a rooting medium.

Collection of data

Shoot clusters from both gelling agents were examined visually monthly up to the fourth month and each time, proliferation and any physical changes that had occurred were noted. Percentage moisture content of shoots was determined at the fourth month using the following formula:

$[(X_1 - X_2) / X_1] \times 100$

Where X_1 = fresh weight of shoot and X_2 = dry weight of shoot (shoots dried to constant weight in moisture extraction oven).

Data management and statistical analysis

Computer programmes for the statistical analysis of data generated during the study were constructed using PC-SAS (SAS Institute, 1992) programming language. The General Linear Model (GLM) procedure of SAS was used for the analysis of variance (Crompton, 1994). Where a significant F-statistic was indicated, the Least Significant Difference (LSD) served as the procedure for mean separation. Probability (P) was always at 0.05 level of significance.

RESULTS AND DISCUSSION

Cultured shoot tips grew and proliferated in the differently gelled medium as shown in Table 1. However visual observations showed variability in the degree of robustness between shoots derived from starch and agar-gelled medium after repeated subculture as illustrated on

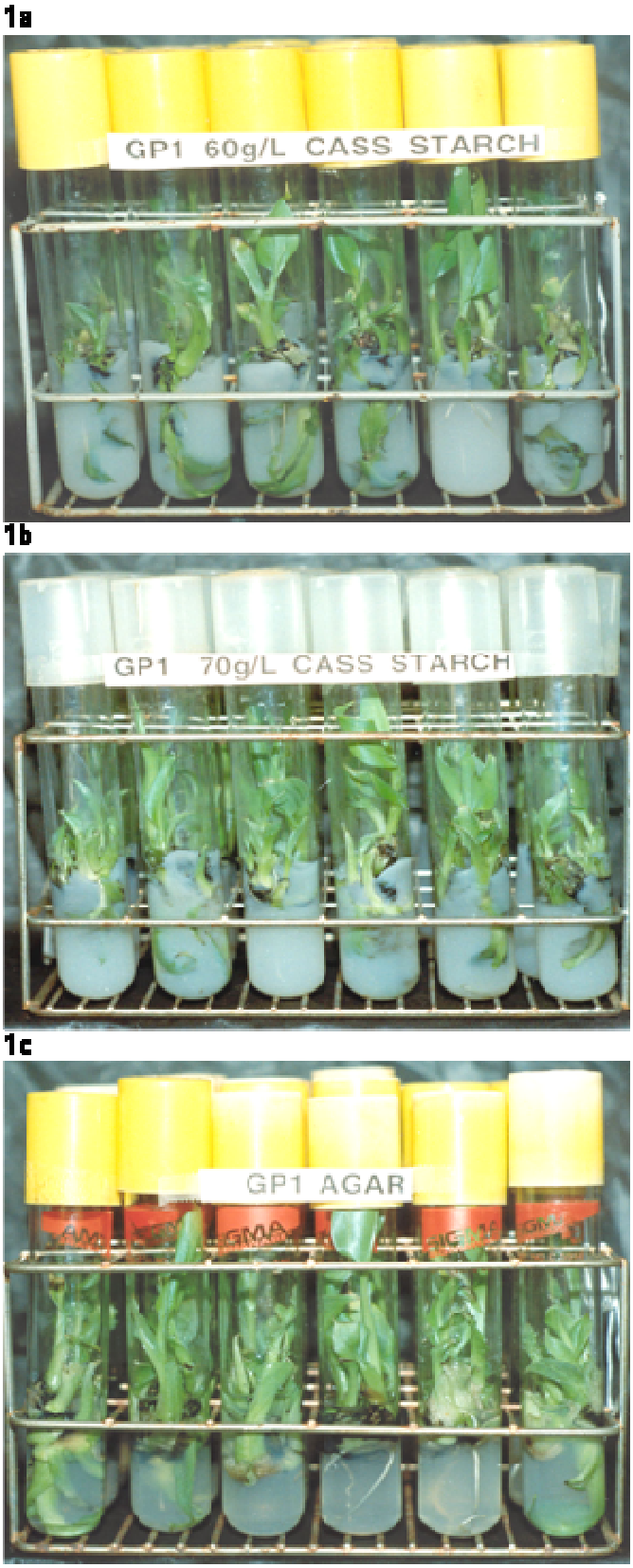

Figure 1. *In vitro* derived shoots of TM3X 15108-6 after four successive subcultures on starch (a and b) and agar (c) gelled multiplication medium.

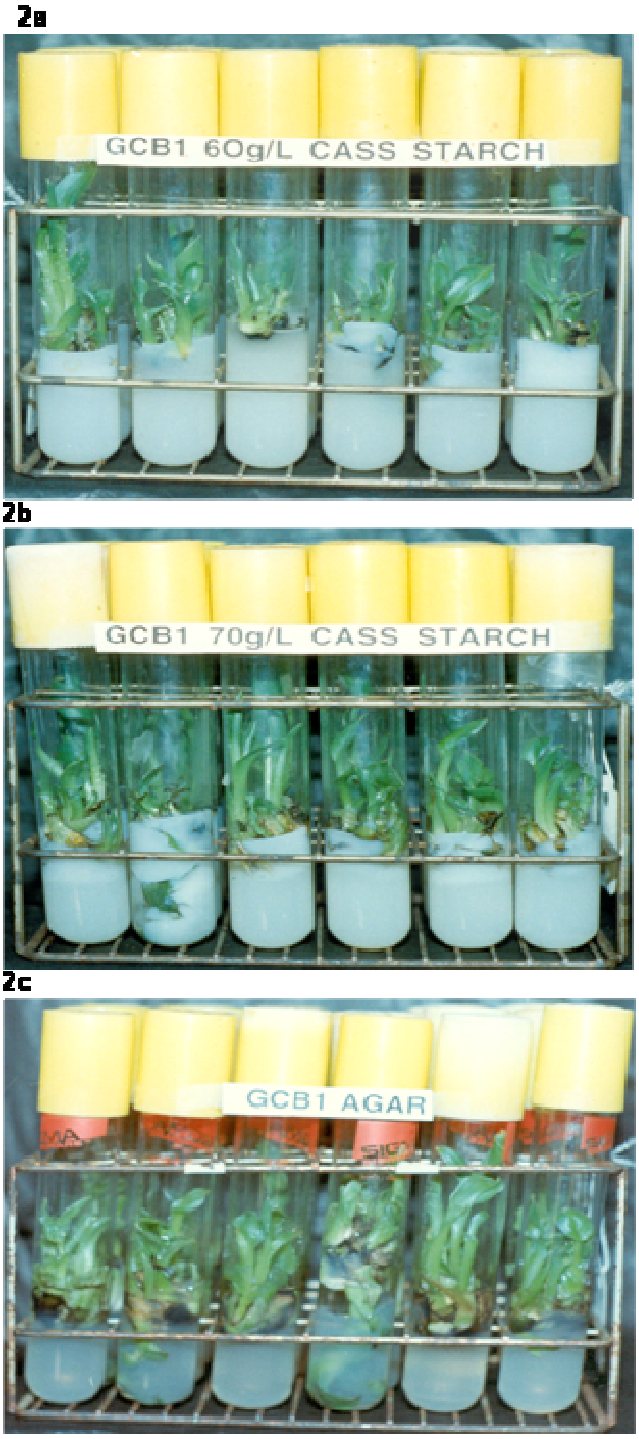

Figure 2. *In vitro* derived shoots of TMBX 614-74 after four successive subcultures on starch (a and b) and agar (c) gelled multiplication medium.

Figures 1a, b and c and 2a, b and c for TM3X 15108-6 and TMBX 612-74, respectively. Shoots derived from starch-gelled medium after several subcultures became less robust than those grown on agar medium. This tendency for reduced robustness increased with increase in

Table 2. Percentage water content of *Musa* shoots after four successive subcultures on cassava starch and agar- gelled multiplication medium.

Genotype	Type of gelling agent	Water content of *in vitro* shoots (%)
TM3X15108-6	60 gL^{-1} cassava starch	93.68
	70 gL^{-1} cassava starch	94.17
	5 gL^{-1} agar	95.11
TMBX612-74	60 gL^{-1} cassava starch	91.97
	70 gL^{-1} cassava starch	91.59
	5 gL^{-1} agar	94.64
$LSD_{(0.05)}$		1.32

starch concentration in the medium over time and was more acute in TMBX 612-74 than TM3X 15108-6. The assessment of shoot water content after the fourth sub-culture revealed that shoots derived from agar-gelled medium had significantly higher water content compared to shoots derived from starch medium (Table 2), corroborating the visual observations.

Henderson and Kinnersley (1988) reported that fresh weights of wild carrot cells cultures grown on agar medium were twenty five times the dry weight whereas on corn starch-gelled medium, it was only 10 times the dry weight. By implication therefore, it follows that cells cultured on agar-gelled medium had more water content compared to those cultured on starch-gelled medium. This confirms the findings from this study which manifested as differential robustness in shoots multiplied on the different gelling agents. This feature was genotype independent but the degree of expression was genotype dependent. Plant tissue culture media are composed largely of water. The amount of unbound or available water in the culture medium for up take by cultured material is dependent on the chemical composition of the media and the concentration of gelling agent used to solidify the medium. The findings indicate a relationship between the availability of absorbable water in the media and the water content of tissues grown on them. The capacity for water release as a result of explant expansion and contortion described as gel expressibility (Owens and Wozniak, 1991) is higher in the agar matrix than in the starch matrix.

The resultant effect would be differential water potential in both systems which would in turn affect water availability. Spomer and Smith (1996) have suggested that *in vitro* plants are either extremely sensitive to subtle shifts in water status or to other physiochemical factors that also change with gelling agent concentration, thus contributing to various effects on plant growth and development. The implications of this finding need to be investigated further if cassava starch is to be used for the micro-propagation of *Musa* species.

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