academicJournals

Vol. 11(12), pp. 403-407, December 2017
DOI: 10.5897/AJPS2017.1323
Article Number: 829006366659
ISSN 1996-0824

http://www.academicjournals.org/AJPS

African Journal of Plant Science

Full Length Research Paper

Potential of cassava starch from TME 419 as suitable gelling agent in micropropagation of cassava (*Manihot esculenta* Crantz)

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Received 4 June, 2015; Accepted 22 September, 2015

Cassava starch from nine varieties, namely, NR 8082, TMS 97/2205, TMS 97/0162, TMS 92/0057, TMS 98/0505, TMS 92/0326, TMS 30572, TMS 82/0058, and TME 419 were evaluated for their suitability as gelling substitute to conventional gelling agents (gellan gum and agar) in medium using cassava shoot tips and nodal segments as explants. Explants were seeded singly into a 15 ml cassava multiplication medium gelled either in 0.2% gellan gum, 0.7% agar or 7% starch from the nine cassava varieties. Cultures were maintained at 28 ± 2°C, 16 h photoperiod and 30 to 40 $\mu Em^{-2} s^{-1}$ flux intensity supplied by white fluorescent tubes on shelves for four weeks. Percentage survival of explants irrespective of type ranged from 61.5 to 100 with NR 8082 and TMS 97/2205 cassava starch-gelled medium recording the highest score while the mean number of nodes produced per explant ranged between 3.6 ± 1.43 and 5.33 ± 0.87 for shoot tips and 2.73 ± 0.96 and 4.79 ± 0.97 for nodal segments. The nodal segments from TME 419 starch-gelled medium had the highest mean number of nodes though not significantly different (p>0.05) from those from gellan gum and agar media. TME 419 was the most consistent in influencing regeneration of cassava plantlets.

Key words: TME 419, cassava starch, explants, gelling agent, micropropagation.

INTRODUCTION

Micropropagation technology is more expensive than the conventional methods of plant propagation and requires several types of skills. It is a capital-intensive industry and in some cases the unit cost per plant becomes unaffordable. The major reasons are cost of production and know-how. During the early years of the technology, there were difficulties in selling tissue culture products because the conventional planting material was much cheaper. Now this problem has been addressed by inventing reliable and cost effective tissue culture methods without compromising on quality. This requires a constant monitoring of the input costs of chemicals, media, energy, labour and capital. For example, the cost of medium preparation (chemicals, energy and labour)

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Table 1. Functional property of the different gelling agents.

Gelling agent	Properties						
	Bulk density (g/ml)	Gelation capacity (% w/v)	Gelatinazation temperature (°C)	Solubility	Swelling capacity (g/ml)	Water absorption capacity	Multiplication rate
NR 8082	0.69	0.50	69	6.71	2.19	1.85	3.90
TMS 97/2205	0.60	0.50	75	6.26	1.95	2.95	4.00
TMS 97/0162	0.64	0.50	68	8.75	1.80	2.16	4.00
TMS 92/0057	0.66	0.5	70	6.75	1.82	2.17	3.50
TMS 98/0505	0.65	0.5	64	5.80	1.75	2.48	3.71
TMS 92/0326	0.63	1.0	62	5.72	2.12	2.80	3.60
TMS 30572	0.66	0.5	65	8.34	1.86	2.45	4.12
TME 419	0.65	1.0	75	4.70	1.86	2.95	4.44
TMS 82/0058	0.67	1.0	69	8.71	1.92	2.40	3.78
Gellan gum	0.69	0.5	31	3.53	8.10	10.0	5.33
Agar	0.67	0.5	45	3.82	7.52	7.56	5.00

can account for 30 to 35% of the micropropagated plant production (Prakash, 1993). Media chemicals cost less than 15% of micro-plant production. In some cases the cost may be as low as 5%. Of the medium components, the gelling agents such as agar contribute 70% of the costs (Prakash, 1993). Other ingredients in the media: water, salts, and sugar have minimal influence on production cost and are reasonably cheap.

Low cost alternatives are needed to reduce production cost of tissue-cultured plants. Plant starches have been shown to be good gelling alternatives in plant tissue culture medium to conventional gelling agents such as agar, gellan gum and gelrite (Pierik, 1989; Nagamori and Kobayashi, 2001; NRDC, 2002). The substitution of conventional gelling agent with cassava starch is a welcomed development towards low cost micropropagation. This study confirms the gelling potential of starch from TME 419 cassava in medium over other starches from different cassava varieties in the micropropagation of cassava.

MATERIALS AND METHODS

Source of explants

Shoot-tip explants and nodal segments were excised from vigorously growing *in vitro Manihot esculenta* cultivar Egedudu (OY 001) obtained from the gene bank housed at the Biotechnology Unit (Plant Tissue Culture Laboratory) of National Root Crops Research Institute (NRCRI) Umudike, Abia State, Nigeria.

Starch preparation

Starch was obtained from nine cassava varieties, namely: NR 8082, TMS 97/2205, TMS 97/0162, TMS 92/0057, TMS 98/0505, TMS 92/0326, TMS 30572, TMS 82/0058, and TME 419 according to Mbanaso (2008) and Nkere and Mbanaso (2009).

Culture medium

The culture medium was Murashige and Skoog (1962) basal medium with 3% sucrose. Medium was solidified with gellan gum, agar or starch at 0.22, 0.7 and 7%, respectively. The pH was adjusted to 5.8. Gellan gum and agar were dissolved by heating while the starches were incorporated as described (Mbanaso, 2008; Nkere and Mbanaso, 2009). 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 78 ± 2°C and the corresponding cold slurry stirred vigorously into it. A 15 ml aliquot each of the different media was then dispensed into culture tubes and autoclaved at 121°C for 15 min.

Explants culture/Parameters assessed

A total of 275 cultures representing 11 treatments of 25 tubes each (15 shoot tips and 10 nodal segments) were used. Explants were seeded singly into culture tubes containing the prepared medium. Cultures were maintained at 28°C ± 2, 16 h photoperiod and 30 to 40 $\mu Em^{-2}\ s^{-1}$ flux intensity supplied by white fluorescent tubes on culture shelves for four weeks. The number of shoot tips and nodal segments were assessed after two and four weeks in culture. The experiment was repeated twice.

Statistical analysis

Data were analysed using analysis of variance (ANOVA) and multiple comparison–least significant difference (LSD) of the GenStat (DE3) ver. 7.2.

RESULTS AND DISCUSSION

The functional properties of the different gelling agents are shown in Table 1. Like the conventional gelling agents, starch from TME 419 cassava variety exhibits low solubility at lower temperatures. However, as with the former, solubility increased as temperature increased. This apparently favoured diffusion and availability of

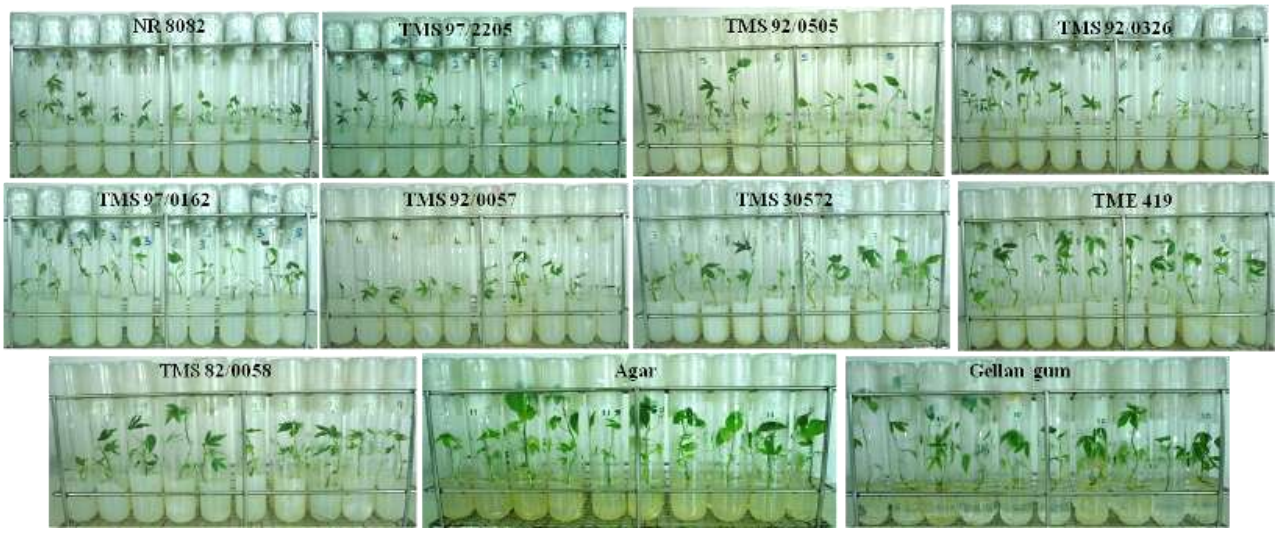

Figure 1. Picture of plantlets after 4 weeks in culture in the different gelling agents.

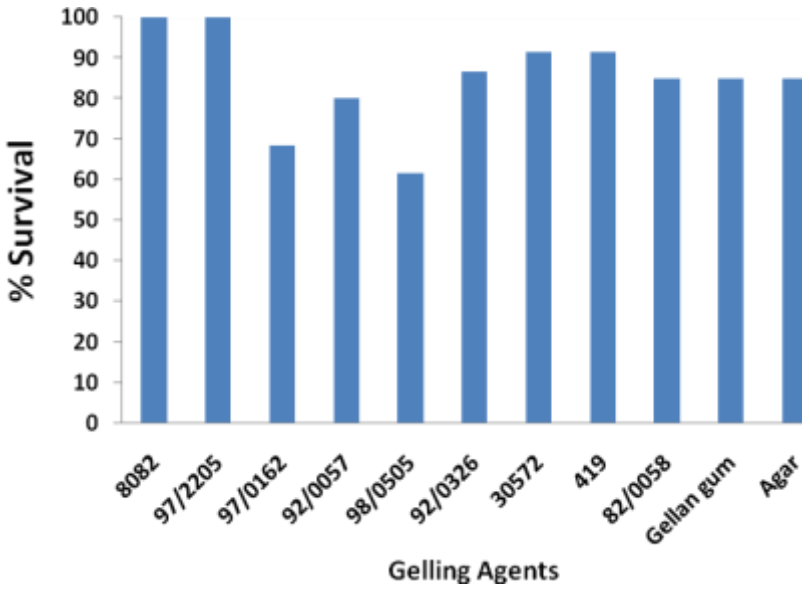

Figure 2. Survival of explants in differently gelled medium after 4 weeks in culture.

medium constituents to the plantlets. In addition, TME 419 cassava starch had a relatively higher water absorption capacity compared to other starches from the different cassava varieties (Table 1).

The growth and proliferation of explants in the differently gelled medium are as shown in Figure 1. The overall percentage survival irrespective of the explant type ranged from 61.5 to 100 (Figure 2). Worthy of note is zero mortality among the explants cultures in medium gelled in NR 8082 and TMS 97/2205 unlike the conventional gelling agent (Figure 2).

The mean number of nodes produced by the plantlets regenerated from the explants cultured in the differently gelled medium is shown in Table 2. After two weeks in culture, plantlets from shoot tips generally produced more nodes than those from nodal segments. At the fourth week in culture by which time the plantlets were ready for subculture, mean number of nodes from shoot tips had exceeded 5 in both conventional gelling agents although this did not differ significantly ($p>0.05$) from the mean number produced by plantlets gelled in starch from TME 419 only. For nodal segments more nodes were produced in plantlets from the later but did not differ significantly ($p>0.05$) from gellan gum, agar, TMS 82/0058, TMS 98/0505 and TMS 30572. Starch from TME 419 was most consistent in influencing regeneration

Table 2. Number of nodes after 2 and 4 weeks in culture.

Gelling agent	2 weeks in culture		4 weeks in culture	
	Shoot tip	Nodal segment	Shoot tip	Nodal segment
NR 8082	2.80 ± 0.42	2.33 ± 0.96	3.90 ± 1.2	3.40 ± 0.99
TMS 97/2205	2.50 ± 0.53	2.07 ± 0.26	4.00 ± 0.94	2.73 ± 0.96
TMS 97/0162	2.83 ± 0.41	2.46 ± 0.91	4.00 ± 0.71	3.46 ± 1.13
TMS 92/0057	2.75 ± 0.46	2.14 ± 0.66	3.50 ± 0.76	3.17 ± 1.47
TMS 98/0505	2.57 ± 0.79	2.25 ± 0.71	3.71 ± 1.50	4.25 ± 0.71
TMS 92/0326	2.50 ± 0.71	2.31 ± 0.75	3.60 ± 1.43	3.00 ± 1.05
TMS 30572	3.00 ± 0.71	2.47 ± 0.51	4.12 ± 1.58	4.21 ± 1.05
TME 419	3.10 ± 0.74	2.93 ± 0.48	4.44 ± 1.88	4.79 ± 0.97
TMS 82/0058	3.00 ± 0.47	2.75 ± 0.75	3.78 ± 1.30	4.33 ± 1.07
Gellan gum	3.33 ±0.50	2.93 ± 0.92	5.33 ± 0.87	4.67 ± 2.07
Agar	4.00 ± 0.58	2.73 ± 0.80	5.00 ± 1.63	4.42 ± 1.59
$LSD_{(0.05)}$	0.52	0.51	1.12	0.82

of cassava plantlets, generating more nodes if subcultured. This result confirms an earlier evaluation reports on the better performance of TME 419 cassava starch as gelling agent in medium for ginger micropropagation (Nkere et al., 2009).

Several agar alternatives (wheat flour corn starch, laundry starch, potato powder, rice powder and semolina) have been shown to be good substitutes for the micropropagation of various plants (Prakash, 1993). Corn-starch (CS) along with low concentration of Gelrite (0.5 g 'Gelrite' + 50.0 g CS/l) has been used for the propagation of fruit trees, such as apple, pear and raspberry, banana, sugarcane, ginger and turmeric with better shoot proliferation than in agar (Zimmerman, 1995). She found that, corn starch was relatively less expensive ($1.8 kg^{-1}) compared with $200 kg^{-1} of agar. "Isubgol" (a colloidal mucilaginous husk derived from the seeds of *Plantago ovate*), at 3% in MS medium has been used for the propagation of chrysanthemum (Babbar and Jain, 1998; Bhattacharya et al., 1994). The cost of 'Isubgol' is about $4 kg^{-1}. It has also 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 low performance of explants (Shoot tip and nodal segment) in NR 8082 and TMS 97/2205 starch gelled medium as against the high survival rate 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). This once again brings to the fore that the adoption of a starch as a gelling agent would depend on proper screening and evaluation.

Conclusion

The result from this study has shown that cassava starch from the genotype TME 419, could serve as a good gelling agent alternative to agar or gellan gum for *in vitro* multiplication of cassava. This is a welcomed development in cost reduction especially in resource poor laboratories where the price of conventional gelling agents is significant in micropropagation.

CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests.

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