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Gelling behaviour of alternative biogel in tissue culture

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Abstract
Guinea corn (G.C) *Shorghum bicolor* (L.) Moench] and Semovita (S.V) *Triticum turgidum* (powder) were investigated as agar substitutes. The medium supplemented with 0.2mg/L α-naphthalene acetic acid (NAA) and 0.5mg/L 6-benzylaminopurine (BAP). G.C flour (white variety) and S.V were added at 0-100 g/L (0:1 to 2:1). Medium with either G.C or S.V flour, thick slurries of the starch were made by continuously heating the medium at a temperature of 95 ± 5ºC, with vigorous stirring at intervals for 10 - 15 min and allow to cool to study their gelling behavior by turning the vessel after 24 h. Media gelled with 60, 80 and 100g/l of guinea corn and semovita with combination of agar (0:1 to 2:1) formed solid media. Media gelled with G.C and S.V alone at 60g/l formed a runny matrix while G.C and S.V (80 and100 g/l) at 1:0 and G.C (100 g/l) with combination of agar (2:1) formed semi-solid media. Media gelled with 60, 80 and 100g/l of G.C and S.V (0:1 to 2:1) were found to be promising alternate candidate for agar.

Keywords: semovita, guinea corn, tissue culture, alternative, biogel

Introduction
A culture medium may either be a liquid or solid body that supports the growth of *in vitro* propagation. Different media are used for culturing different types of plants which include those derived from plants and other tissue culture media used for growing plants. Agar has been in use for years. Moreover, high cost of rank agar necessitates alternatives. Series of gelling materials such as corn flour, coconut powder and host of others are obtainable. Guinea corn [*Sorghum bicolor* (L.) Moench] also referred to as Sorghum is widely adapted to a range of agro ecological zones. Sorghum varieties range from white, red or brown. Sorghum is an annual plant of about six to ten feet tall (Duffy and Calvert, 2010) [1], and it belongs to the family Poaceae (Plessis, 2008) [3]. Sorghum have C4 photosynthetic pathway which makes it more efficient in water use. Guinea corn has other common names in West Africa such as great millet and kafir corn which has a connection with corn or millet. It is called jowar in India, kaoliang in China and milo in Spain, dura in Sudan, and mtama in Eastern Africa (Purseglove, 1972) [4]. Cultivated sorghum can be grouped as grain sorghum, sweet sorghum, Sudan-grass and bromcom. It reproduces through seeds and vegetative through rhizome. Spontaneous sorghum or shatter cane as the name implies, shatters its seeds as a method of seed dispersal. Shatter cane is a product of an out crossing between *S. bicolor* and *S. halepense* (Sikora and Berenji, 2008) [5]. Guinea corn is one of the most important breakfast cereal crops. Estimates of sorghum between 1999 and 2003 amounts to 57.7 million tonnes annually from 42.6 million hectares of land (FAO, 2008) [2]. Corn [*Shorghum bicolor* (L.) Moench] and, Semovita *Triticum turgidum* is flour ground from dried wheat. The present studies were undertaken to look for additional cheap and easily available substitutes of agar to be used in tissue culture media.

Materials and Methods
1.1 Experimental site
The experiment was performed at the Biotechnology laboratory, Department of Agronomy, University of Ibadan, Ibadan

1.2 Media Preparation
The commercially prepared Murashige and Skoog supplemented with sucrose (30 g/L) using plant growth regulators at 0.2mg/L Naphthalene acetic acid (NAA) and 0.5mg/L Benzylaminopurine (BAP). Guinea corn flour (white variety) and semovita which were procured from the local market were added to the media at 0-100 g/L (Table 1) and each medium was adjusted to pH 5.7 after adding guinea corn and semovita flour. For the medium containing agar either in whole or in part, the agar was added to the media after the pH was adjusted to 5.7 were added to the basal medium. For the medium with either guinea corn or semovita flour, thick slurries of the starch was made by continuously heating the medium at a temperature of 95 ± 5ºC, with vigorous stirring at intervals for 10 - 15 mins (Table 1), dispensed into smaller culture vessels (a 28ml medium was dispensed into a 500ml jar) and then sterilized in an autoclave at 121 ºC for 15 min. The autoclaved medium was then transferred to the laminar flow hood, allowed to cool to study the solidification behavior.

Table 1: Concentrations of Combination of Guinea Corn (GC) flour, Semovita (S.V) and or Agar (g/l)

Quantity	Gc (100ml)	Agar (100ml)	SV (100ml)	Agar (100ml)
		60g		
1:0	6.00	0	6.0	0
0:1	0	0.7	0	0.7
1:1	3.00	0.35	3.0	0.35

1:2	2.00	0.47	2.0	0.47
2:1	4:00	0.23	4.00	0.23
80g				
1:0	8.0	0	8.0	0
0:1	0	0.7	0	0.7
1:1	4.0	0.35	4.0	0.35
1:2	2.67	0.47	2.67	0.47
2:1	5.33	2.23	5.33	0.2
100g				
1:0	10.0	0	10.0	0
0:1	0	0.7	0	0.7
1:1	5.0	0.35	5.0	0.35
1:2	3.33	0.47	3.33	0.47
2:1	6.67	0.23	6.67	0.23

Table 2: Degree of solidification of Guinea Corn (GC), Wheat (semovita) SV) compared with Agar

Quantity	Ratio of combination of Guinea corn (GC) Agar per litre			Degree of solidification	
Guinea corn (GC)	solidification	Remark	Wheat(Semovita) (SV)	Remark	Solidification
60g	1:0	– –	Running	– –	Running
	0:1	+ +	Solid	+ +	Solid
60g	1:1	+ +	Solid	+ +	Solid
60g	1:2	+ +	Solid	+ +	Solid
	2:1	+ +	Solid	+ +	Solid
80g	1:0	+ –	Semi-solid	+ –	Semi-solid
	0:1	+ +	Solid	+ +	Solid
	1:1	+ +	Solid	+ +	Solid
	1:2	+ +	Solid	+ +	Solid
80g	2:1	+ +	Solid	+ +	Solid
100g	1:0	+ –	Semi-solid	+ –	Semi-solid
	0:1	+ +	Solid	+ +	Solid
	1:1	+ –	Semi-solid	+ +	Solid
100g	1:2	+ +	Solid	+ +	Solid
100g	2:1	+ +	Solid	+ +	Solid

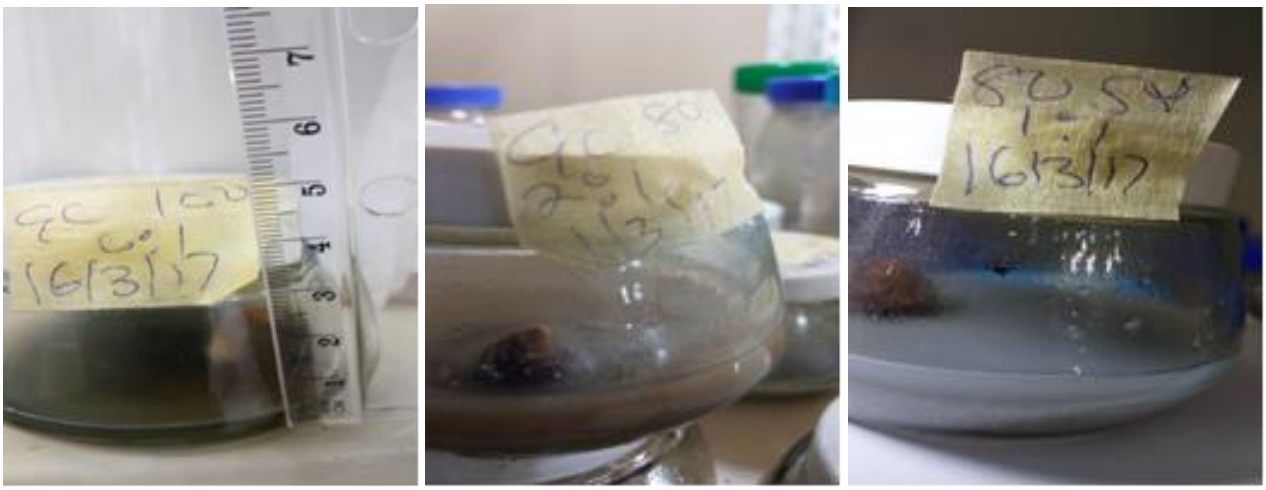
+ Solidification; -, lack of solidification

Results and Discussion

Agar constitutes70% of the total cost of media production during *in vitro* propagation (Prakash, 1993) and there is a need to develop a cheaper alternative that will significantly lower the cost of this technology. Many alternatives to agar such as cassava and maize starch have been suggested to reduce cost (Zimmerman, 1995) with limitations. However, media gelled with guinea corn alone at 60g/l formed a runny matrix, 80g/l of guinea corn alone formed a semi-solid medium

While 60, 80 and 100g/l of guinea corn and semovita with combination of agar (0:1 to 2:1) formed solid media. Other concentration of guinea corn and semovita (80 and100 g/l) alone with no combination of agar (1:0) and guinea corn (100 g/l) with combination of agar (2:1) formed semi-solid media (Table 2). This result is similar to the report of Sorvari (1986).

Media gelled with 60, 80 and 100g/l of guinea corn G.C and semovita S.V (0:1 to 2:1) (Plate1) were found to be promising alternate candidate for agar.



G.C (100) 0:1 G.C (80)2:1 SV (80)1:1

Fig 1: Guinea corn and Semovita as Gelling agent

Conclusion

The addition of agar to guinea corn in certain proportions improves the semi-solid nature of less gelled medium. Guinea corn alone (at 60 and 80g/L concentration) gels less firmly when compared to agar which may be of advantage possibly due to a better uptake of nutrients. Media gelled with 60, 80 and 100g/l of guinea corn G.C and semovita S.V (0:1 to 2:1) (Plate1) were found to be promising alternate candidate for agar.

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