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The Influence of Liquid Media Support, Gelling Agents and Liquid overlays on performance of in vitro Cultures of Ginger (*Zingiber officinale*)

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Abstract- This study was designed to examine the effects of liquid media supports, gelling agents and liquid overlays on growth and multiplication of ginger. The use of luffa coir as physical support for explants in liquid media enhanced growth compared to other types of supports. Guar gum revealed a promising gelling potential as an alternative for agar. In concentration of 30 g/l, guar as a sole gelling agent, increased shoot and leaf numbers significantly. Enhanced multiplication was obtained upon supply of a 10 ml liquid overlay aliquot per vessel composed of MS salts plus 4 mg/l BA onto the solid multiplication medium. The use of luffa coir and guar gum propose effective alternatives to the high cost agar and may help in reducing cost of tissue culture protocols especially at commercial level.

Index Terms- in vitro, ginger, physical form, solidifying agents, medium overlay

I. INTRODUCTION

Ginger (Zingiber officinale Rosc.) belongs to the family Zingiberaceae. It is one of the most highly consumed dietary substances in the world (Surh, 2003). Its rhizome, which is also known as "ginger root", has been used in Asia for thousands of years in ethno-medicine to relief various pains and treat many infectious diseases (College, 1985). It has also been studied for its efficacy for acute chemotherapy-induced nausea and vomiting (Zick et al., 2009). The ginger rhizome has been shown to contain many bioactive compounds which possess many pharmacological and physiological activities (Surh et al., 1998). Studies on the major pungent principle compound such as 6-gingerol, showed anti-oxidant, anti-inflammation and anti-tumor activities (Jagtap et al., 2009).

In plant tissue culture, the components of medium like mineral salts, organic supplements, growth regulators and gelling agents affect culture responses (Gamborg and Phillips, 1995). Based on physical form, media are classified as solid, semi-solid, semi liquid and liquid. Gelling agents are added to culture media to increase viscosity wherein explants are not submerged in the medium (Prakash *et al.*, 2000). The *in vitro* growth of shoots or roots is strongly influenced by the physical consistency of culture media. Agar is the most popular gelling agent for *in vitro* cultures due to its inert nature that prevents its intervention with plant metabolism (Ozel *et al.*, 2008). There are different types and grades of gelling agents such as agar, agarose, phytagel and gerlite (Prakash *et al.*, 2000). These agents may constitute about 70% of tissue culture production costs (Mohamed *et al.*, 2009). Due to the high price of pure grade agar and the assumption that the exclusive use of agar may result in over exploitation of its resources, trials on alternatives seem valid (Deb and Pongener, 2010). Different materials had been tried as agar alternatives with various degrees of success (Babbar and Jain, 2006; Dabai and Muhammad, 2005; Jain *et al.*, 2005).

In-vitro multiplication of plant species is a promising tool to improve quality of planting materials, but the expenses of the technique might limit this potential in under-developed countries. The objective of this study was to investigate the influence of liquid media supports, gelling agents and liquid overlays on growth and multiplication of *in vitro* cultures of ginger.

II. MATERIALS AND METHODS

In this study, shoots were excised from stocks of *in vitro* cultures of ginger raised in the Tissue Culture Laboratory of Sudan University of Sciences and Technology. The process of explant preparation was done under aseptic conditions where the explants were cut into convenient sizes (1.5–2 cm) after removal of leaf sheaths and roots.

Whatever the physical form, Murashige and Skoog MS (1962) basal medium supplemented with 0.2 mg/l NAA and 5mg/l BA was used according to Idris *et al.*,(2010). The pH of the medium was adjusted to 5.7± 0.1 for solid media prior to addition of the gelling agent, whereas, the pH for liquid media was 5.0 ± 0.1. Cultures were incubated at 25 ±1 º C and 16 hrs day length under 1000 lux illumination from cool white fluorescent lamps.

Test of gelling agents:

Single and combined gelling agents were tested in the following order: (7g/l) agar; (2g/l) phytagel; (30g/l) guar; (20g guar+ 2g agar) /l ; (20g guar + 0.5g phytagel) /l and (20g guar + 2g agar + 0.5 g phytagel) /l.

Test of physical form of media

Explants were cultured onto one of the following form of media: Agar solidified medium (7g/L); Agar solidified medium mounted by 10 ml liquid medium; liquid medium with filter paper support; liquid medium with cotton balls support and liquid medium with luffa coir (*Luffa aegyptiaca*) support.

Test of liquid overlays with MS constituents

Explants were cultured onto solid media mounted by 10 ml liquid aliquots composed of MS constituents in the following order: Control (No overlay); MS salts + sucrose; MS salts + inositol; MS salts + 4mg/l BA and MS salts + 8 mg/l Kinetin.

Design and analysis:

The tests were arranged in completely randomized design. Treatments were replicated 10 times where each Magenta GA3 culture vessel containing 50 ml medium aliquots accommodating two explants was considered a replicate. Data were collected 6 weeks after culturing for the following parameters: Survival %, number of shoots, shoot length, leaf number, root number and length. Data were subjected to analysis of variance and means separation by Duncan's multiple range test using MStat-C computer program.

III. RESULTS

Significant differences were obtained as a result of gelling agents and their combinations. The shoot and leaf numbers were increased significantly by the 30g/l guar treatment compared to the other treatments. Cultures in media solidified with sole agar or phytagel performed alike for almost all measured parameters except for root number where phytagel induced significant increase over agar. No significant difference was observed between treatments for root length. Guar combined with agar plus phytagel and phytagel alone resulted in significant increase in number of roots per explant (Table 1, Fig. 1).

The growth of explants responded differently to the different media physical forms. Luffa coir support onto liquid media resulted in 100% survival, while cotton or filter paper supports resulted in 50% survival. Luffa and filter paper supports significantly enhanced shoot and leaf formation compared to other treatments. The longest shoots were obtained in liquid media with either luffa or cotton supports (Table 2).

In the liquid overlay experiment, shoot multiplication was enhanced when the agar-gelled media was mounted by a liquid supplement composed of MS salts + 4 mg/l BA. Supplements of liquid MS salts combined with either sucrose or vitamins ranked second with no significant difference from the control or MS + inositol. MS + kinetin reduced most parameters. The best shoot length was recorded for the control, MS + sucrose and MS + vitamins. Leaf number was statistically equal for all treatments except the MS + kinetin treatment that resulted in significantly lower number of leaves (Table 3, Fig. 2).

IV. DISSCUSSION

Agar is of popular use as a solidifier for most *in vitro* cultures. This may be attributed to its high clarity and inert nature that limits its intervention with metabolism (Jain and Babbar 2002; Ozel *et al.,* 2008). Scientific interest in agar alternatives to reduce cost of tissue culture protocols had been well documented (Babbar and Jain, 2006; Dabai and Muhammad, 2005; Jain *et al.,* 2005; Prakash *et al.,* 2002). In the same line, this study investigated the potential of single guar and phytagel and their combinations with low concentrations of agar as means to replace or reduce agar as ingredient of solid media. The significantly high numbers of shoots and leaves obtained from the use of guar as a sole gelling agent proposes its potential as agar substitute. The guar enhancive effects might be attributed to its richness in nutrients and/or to the probability of some growth regulators in its constituents, as guar gum is of endospermic origin. However, guar chemical constituents may impose changes in chemically defined media. For scientific trials, where agar would do best, guar should be avoided. The enhancive effect of phytagel on rooting parameters coupled with shoot retardation might be attributed to its high osmotic potential which may restrict diffusion of nutrients through the medium and cause lesser availability of water to explants, as suggested by (Bhat *et al.,* 2001). Nevertheless, the *in-vitro* development is dependent on the explants and the medium interaction. Agar, the conventional gelling agent has been reported to have a number of drawbacks that negatively affect culture growth and differentiation. Kuria *et al*., (2008) reported lower rate of development of plantlets in solid media compared to liquid media and attributed that to lower uptake of nutrients in solid cultures. Besides, Preece (2011) reported reduced growth in many plant species with the increase of agar levels and concluded that eliminating agar or other gelling agent can improve micro-shoot proliferation and growth. From the results of this study, the liquid medium with supporting material (luffa, filter paper and double phase) gave more shoots and more leaves per plantlet than the solid medium or cotton support in liquid medium. This finding is in agreement with that of Kuria *et al.,* (2008) who reported higher biomass accumulation in liquid media than in solid media. More-over, use of liquid media had been frequently reported to enhance shoot and root growth in many plant species (Preece 2011; Sandal *et al*., 2001; Ziv 1989). However, plants in liquid media or in a media having low concentration of the gelling agent suffer hyper-hydricity (Pierik, 1997). Nevertheless, this study proposed luffa coir support for explants in liquid media as an alternative to other supports conventionally used in liquid cultures.

The activating overlay treatments were ineffective in multiplication, except the BA containing treatment. This double phase treatment might have provided extra water, nutrients, and multiplication promoting hormone. Explants seemed to take up nutrients and growth regulators from both the lower and upper layers, without being submerged. The results also forward a question about the degree of BA availability to explants in the solid medium two weeks after culture establishment. Further research on the topic seems needed. The enhanced multiplication rate caused by the BA containing overlay treatment might be due to better availability of this regulator to explants. The potency of BA as an enhancer of multiplication had been well documented for *in vitro* culture of versatile plant species (Tornero *et al.,* 2010; Nirmal *et al.,* 2005).

In conclusion, as *Luffa aegyptiaca* is a local plant, the use of its coir as explant support in liquid tissue culture media might be adopted in commercial laboratories after further confirmatory research. Besides, guar might suit commercial tissue culture protocols, but further research is needed to elucidate its exact contribution to media nutritional and hormonal composition.

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REFERENCES

[1] Babbar SB, Jain R. 2006. Xanthan gum: an economical partial substitute for agar in microbial culture media. Curr. Microbiol., 52: 287-292.

[2] Bhat DM, Murli KS, Ravindranath NH 200. Formation of secondary forests in Southern India. .J. Trop. For. Sci., 13: 601–620.

[3] College, J.N.M. (1985).The Dictionary of Traditional Chinese Medicine, Shanghai Sci-Tech Press, Shanghai.

[4] Dabai YU, Muhammad S 2005. Cassava starch as an alternative to agar-agar in microbiological media. Afr. J. Biotechnol., 4: 573-574.

[5] Deb CR and Pongener A 2010. Search of alternative substratum for agar in plant tissue culture. Curr. Sci., 98: 99-102.

[6] Gamborg OL, Phillips GC 1995. Plant cell, tissue and organ culture. Springer- Verlag Berlin Heidelberg, pp 21-22.

[7] Jagtap S, Meganathan K, Wagh V, Winkler J, Hescheler J, Sachinidis A, 2009. Chemoprotective mechanism of the natural compounds, epigallocatechin-3 O-gallate, quercetin and curcumin against cancer and cardiovascular diseases. Curr. Med. Chem., 16: 1451-62.

[8] Jain N, Babbar SH 2002. Gum katira - a cheap gelling agent for plant tissue culture media. Plant Cell Tissue Organ Cult. 71: 223-229.

[9] Jain R, Anjaiah V, Babbar SB, 2005. Guar gum: a cheap substitute for agar in microbial culture media. Lett. Appl. Microbiol., 41: 345-349.

[10] Kuria P, DemoP, Nyende A and Kahangi E, 2008.Cassava starch as an alternative cheap gelling agent for the in vitro micro-propagation of potato (Solanumtuberosum L.). African Journal of Biotechnology, 7(3): 301-307.

[11] Mohamed MAH, Alsadon AA and Al Mohaidi MS, 2009. Corn and potato starch as an agar alternative for Solanum tuberosum micro-propagation. African Journal of Biotechnology, 8 (19).

[12] Murashige T, Skoog F, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473-97.

[13] Nirmal B, Samsudeen K, Minoo D, Geetha S, Ravindran P, 2005. Tissue Culture and Biotechnology of Ginger. In: Ginger, the genus Giniber. (eds) Ravindran P, Nirmal B. pp181- 211.

[14] Nitsch JP, Nitsch C, 1969. Haploid plants from pollen grains. Science. 163: 85-87.

[15] Ozel CA, Khawar, KM, Arslan O, 2008. A comparison of the gelling of isubgol, agar and gelrite on in vitro shoot regeneration and rooting of variety Samsun of tobacco (Nicotiana tabacum L.). Sci. Hort.117: 174–181.

[16] Pierik R.LM, 1997. In vitro Cultures of higher plants. Dordrecht, the Netherlands: Martinus Nijhoff Publishers.

[17] Prakash S, Hoque MI, Brinks T, 2000. Culture media and containers. Biotechnology and Eco- development Research Foundation, Bangalore India. pp. 29- 30.

[18] Preece JE 2011. Micropropagation in stationary liquid media. Propagation of Ornamental Plants. 10(4), 183-187.

[19] Sandal I, Bhattacharya A, Ahuja PS 200. An efficient liquid culture system for tea shoots proliferation. Plant Cell Tissue. Organ. Cult. 65, 75-80.

[20] Surh YJ, 2003. Cancer chemo-prevention with dietary phyto-chemicals. Nat. Rev. 3: 768-780.

[21] Surh YJ, Lee E, Lee JM, 1998. Chemo-protective properties of some pungent ingredients present in red pepper and ginger. Mutat. Res., 402: 259-267.

[22] Tagelsir IMI, Sara AMU, EL Fatih MM, 2010. Disinfection potential of some chemicals and local herbs and proliferation studies on the in vitro culture of ginger (Zingiber officinale Rosc). Journal of Science and Technology. 11(2):90-102.

[23] Tornero OP, Tallon CI, Porras L 2010. An efficient protocol for micro-propagation of lemon (Citrus limon) from mature nodal segments. Plant Cell tissue and Organ Culture. 100: 263- 271.

[24] Ziv M, 1989. Enhanced shoot and cormlet proliferation in liquid cultured gladiolus buds by growth retardants. Plant Cell Tissue Organ Cult. 17, 101-110.

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Table 1: *In vitro* growth of ginger as affected by various gelling agents

Gelling agent	Shoot number	Shoot Length (cm)	Leaf number	Root Length (cm)	Root Number
Agar 7g/l	3.81d	2.78c	10.94d	5.06a	20.83b
Phytagel 2g/l	3.81d	3.00c	11.19d	5.23a	23.83a
Guar 30g/l	8.87a	3.23bc	17.06a	5.11a	19.33b
(Guar 20g + Agar 2g)/l	4.68bc	3.66ab	13.25bc	5.06a	20.33b
(Guar 20g + phytagel 0.5g)/l	3.87cd	3.69ab	12.13cd	4.70a	18.67b
(Guar20+Agar 2+ phytagel 0.5g)/l	5.31b	4.16a	14.88b	5.14a	25.75a

Means followed by the same letter(s) within the same columns are not significantly different at p= 0.05, according to DMRT

Table 2: *In vitro* growth of ginger as affected by the physical form of media

Physical form of media	Survival %	Shoot number	Shoot length (cm)	Leaves number
Agar	81.30	2.40b	3.89bc	06.80b
Agar solidified medium mounted by liquid medium (double - phase)	68.80	6.80a	3.30c	12.50a
Liquid medium with filter paper support.	50.00	5.60a	3.66bc	11.60a
Liquid medium with cotton support.	50.00	2.70b	4.38ab	08.00b
Liquid media with *Luffa* support.	100	5.90a	5.03a	11.90a

Means followed by the same letter(s) within the same columns are not significantly different at p= 0.05, according to DMRT

Table 3: Effect of MS media components as liquid overlay onto agar solidified multiplication medium

Treatments	Shoots number	Shoot length (cm)	Leaves number
Control (No overlay)	6.92bc	6.18a	22.83a
MSsalts+ sugar	7.17b	6.25a	22.00a
MSsalts+ vitamin	7.17b	6.38a	22.08a
MSsalts+ inositol	6.58bc	6.06ab	22.00a
MSsalts+ Kin	5.42c	5.56c	16.83b
MSsalts+ BA	8.83a	5.74bc	26.25a

Means followed by the same letter(s) within the same columns are not significantly different at p= 0.05, according to DMRT

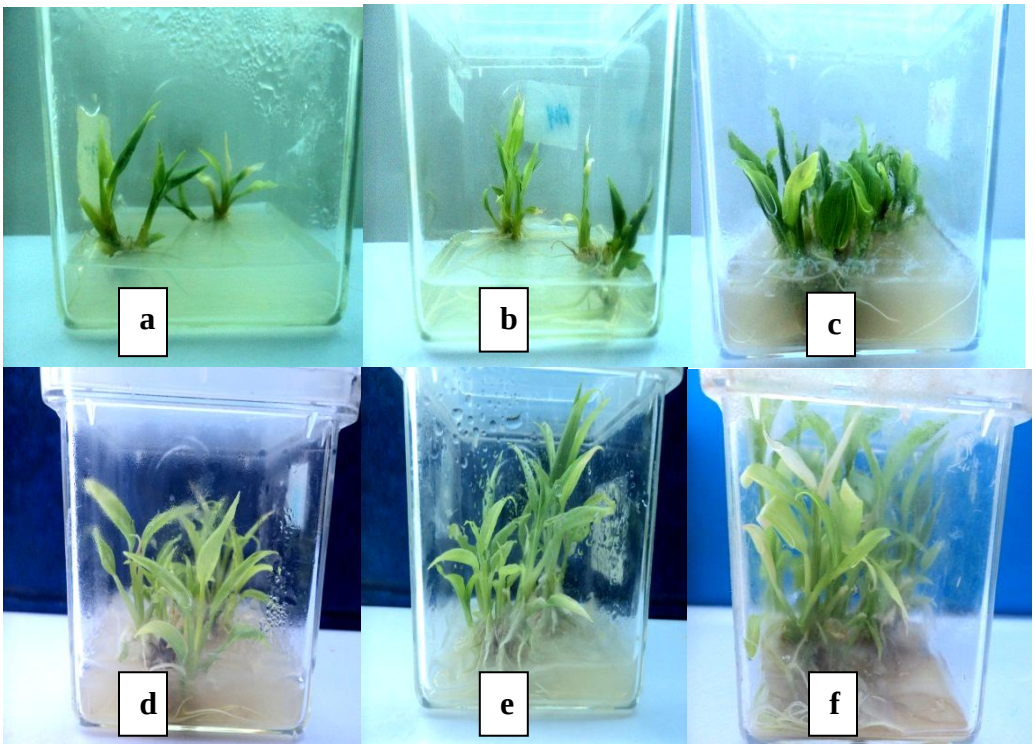

Figure. 1. *In vitro* growth of ginger as affected by media gelling agents **(a)** Agar 7g/l; **(b)** Phytagel 2g/l; **(c)** Guar 30g/l**; (d)** Guar20g +Agar2g /l; **(e)** Guar 20g+ Phytagel 0.5g/l**; (f)** Guar 20+Agar 2+Phytagel 0.5g/l

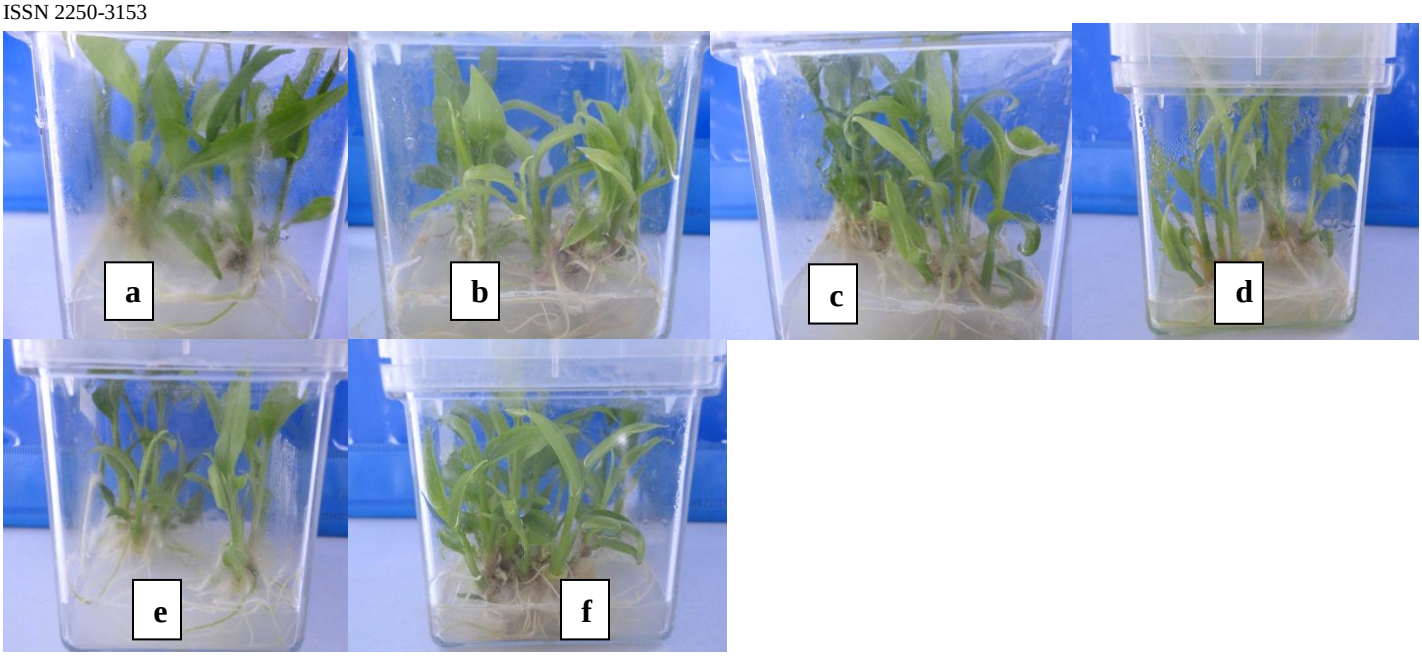

Figure. 2. Effect of MS medium components as liquid overlay onto agar solidified multiplication medium;
(a) control (No overlay); **(b)** Overlay with MS salts+ 30g/l sucrose**; (c)** Overlay with MS salts + vitamins; **(d)** Overlay with MS salts + 100mg/l inositol; **(e)** Overlay with MS salts + 8.0 mg/l Kin**; (f)** Overlay with MS salts +4.0mg/l BA