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ALTERNATIVE GELLING AGENTS FOR *IN VITRO* PROPAGATION OF ORCHID (*DENDROBIUM SONIA*)

M. A. Ullah[1,2*], M. I. Uddin[2, 3], A. B. Puteh[4*], M. S. Haque[1] and M. S. Islam[5]

[1]Department of Biotechnology, Bangladesh Agricultural University, Mymensingh-2202
[2]Plant Breeding Division, Bangladesh Institute of Nuclear Agriculture, BAU Campus, Mymensingh-2202
[3]Institute of Systems Biology (INBIOSIS), Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia
[4]Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[5]Agronomy Division, Bangladesh Institute of Nuclear Agriculture, BAU Campus, Mymensingh-2202
[*]Corresponding author's email: adam@upm.edu.my ; himelasad@yahoo.com

ABSTRACT

We investigated the appropriate growth conditions of shoot and root through *in vitro* propagation of orchid (*Dendrobium sonia*) using different gelling agents. Protocorm Like Bodies (PLBs) of orchid were initiated earlier *in vitro* and one month old protocorms were used for this study. Protocorms were cultured on Murashige and Skoog (MS) medium supplemented with different concentrations of starches viz. isubgol (15, 20, 25 and 30gL^{-1}), cassava (15, 20, 25 and 30gL^{-1}), corn (50 and 60gL^{-1}) and potato (50 and 60gL^{-1}) used as alternative gelling agents as compared with 8gL^{-1} agar at different stages at 35, 70 and 105 days. The MS medium supplemented with the increasing dose of isubgol, cassava, corn and potato gelled with 2gL^{-1} agar progressively increased their growth performance. The best performance on growth of shoots leaves and roots were recorded at 30gL^{-1} isubgol when isubgol used as an alternative gelling agent on agar. The use of 30gL^{-1} isubgol (500g costs US$ 10 only) was very much cheaper than conventionally used agar. The present study offers a new possibility of using low cost alternative gelling agent which will reduce the production costs considerably plant tissue culture techniques.

Key words: Alternative gelling agent, *Dendrobium sonia*, Orchid propagation, Isubgol

INTRODUCTION

Orchids belong to the largest and most diverse family Orchidaceae consisting of about 700-800 genera and more than 48,000 cultivars of 25000 species (Begum, 2000). Orchids are abundant in countries like South East Asia, South and Central America and South Africa. Bangladesh has a wide scope of large scale production of orchid through tissue culture to meet the demand of internal market and to earn foreign currency through export. For tissue culture, agar represents one of the most expensive and commonly used media components, contributing about 70% of the total production cost (Prakash, 1993). However, establishment of plant propagation laboratories must be based on cost effectiveness.

In the past, many attempts have been made to identify a suitable cheaper alternative of expensive agar as a gelling agent for microbial and plant tissue culture media. These are isubgol (Babbar and Jain, 1998), gum katira (Jain and Babbar, 2002), guar gum (Babbar *et al*., 2005) and xanthan gum (Jain and Babbar, 2006). Use of isubgol for tissue cultures of Chrysanthemum was reported by Bhattacharya *et al*. (1994). Recently, isubgol or psyllium husk (*Plantago psyllium/Plantago ovata* husk) has been successfully used for the culture of organisms as varied as prokaryotic and eukaryotic microalgae and commercially important plants, such as, turmeric, tobacco, blueberry, woad plant (*Isatis tinctoria L.*) and banana (Agrawal *et al*., 2010). However, despite all these having a distinct cost advantage over agar, none is likely to be used as routinely as agar because of some inherent drawbacks. Isubgol and guar gum remain highly viscous even at high temperature and therefore, pose problem in adjustment of pH and in dispensing of the medium to culture vessels (Jain and Babbar, 2006). Media for *in vitro* cultures can be classified as liquid. Gelling agents are added to culture medium to increase viscosity wherein explants are not submerged in the medium (Prakash *et al*., 2000). Like for other plants, culture media used for orchid tissue propagation are also gelled with agar at a concentration of 0.8% (Gebre and Sathyanarayana, 2001). The wide distribution and cultivation of its source plant, *Plantago ovata* for medicinal purposes in many countries, can prove to be an added advantage. Although the different starches viz. isubgol, cassava, corn, potato etc. has been proven effective to support orchid tissue cultures, different starches will be cheaper alternative gelling agent especially to developing countries having to import agar while isubgol is ubiquitous. Besides an essential and baseline technology for plant biotechnology, plant tissue culture is widely used the world over for commercial mass propagation of many plants. In the present study, different substratum like agar, isubgol, cassava, corn and

potato were compared. Here we evaluated various commercial starches (isubgol, cassava, corn and potato) as an effective substitute of agar which commonly used in tissue culture medium and to select some low cost substitutes replacing agar for protocol development of *in vitro* plant propagation.

MATERIALS AND METHODS

Plant materials: Protocorm Like Bodies (PLBs) of orchid (*Dendrobium sonia*) were initiated and maintained earlier in the Tissue Culture Laboratory of the Department of Biotechnology, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh. These PLBs were used as plant materials.

Different culture media: As basal medium, full strength MS (Murashige and Skoog, 1962) medium was used to culture the PLBs and plantlets as control treatment ($8gL^{-1}$agar). The medium gelled with $2gL^{-1}$ agar supplemented with different doses of isubgol (15, 20, 25 and $30gL^{-1}$) cassava powder (15, 20, 25 and $30gL^{-1}$), corn flour (50 and $60gL^{-1}$) and boiled potato (50 and $60gL^{-1}$) were used as alternative gelling agent on agar *in vitro* propagation of orchid.

PLBs culture: One month old *in vitro* grown protocorms were used for this study. Four PLBs (0.004g) were cultured on 20ml MS medium supplemented with different concentrations (as mentioned above) of starches viz. isubgol, cassava, corn and potato at different stages at 35, 70 and 105 days to investigate their effects on shoot multiplication and root formation. In each vial, 4 uniform PLBs were cultured for *in vitro* propagation of orchid where four replications were used following Randomized Complete Design (CRD). The vials were marked differently following treatment and replications. The culture vials were maintained in a growth chamber and allowed to grow at $25\pm1^{O}C$ under 16h photoperiod illuminated with florescent tubes of $50\mu molm^{-2}s^{-2}$.

Data recorded and statistical analysis: Data were recorded on number of shoot, length of shoot, fresh weight of shoots, number of leaves per plantlet, length of leaves, width of leaves, number of roots, length of roots, fresh weight of roots. The analysis of variance was performed and means were compared by Duncan's Multiple Range Test (DMRT) at 5% level of probability for interpretation of results (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

In vitro regeneration of plantlets offers a feasible propagation method in orchids and can be utilized for the year round and rapid propagation of orchid plants. The shoot formation and root regeneration were developed from the cultured PLBs on different substratum.

Length of shoots: The length of shoot (cm) showed statistically different due to the effect of different levels of different starch. At all the (35, 70 and 105) days after culture (DAC), shoot length showed better performance on different levels of isubgol in the medium. The longest shoot (2.34cm) was found in MS medium supplemented with $30gL^{-1}$ isubgol and the shortest (0.92cm) was observed in corn ($60gL^{-1}$) at 35 DAC (Table 1). At 70 DAC, the longest (3.92cm) shoot and the shortest (1.85cm) shoot were observed in $30gL^{-1}$isubgol and $60gL^{-1}$ corn starch, respectively. At the same treatment, the longest (4.90cm) and shortest (2.30cm) were observed at 105 DAC (Table 1, Fig 1 and Fig1.2). Whereas, the $8gL^{-1}$ agar (control) showed statistically more or less similar results (2.12, 3.64 and 4.38cm) at 35, 70 and 105 days, respectively that with $30gL^{-1}$isubgol. A similar finding was reported earlier by Priadi *et al*. (2008). They found the highest shoot length on genotype Iding (17.20mm) obtained from maize, meanwhile on Gebang obtained from that *Sinar kencana* 2% (8.95mm). It might be due to the increased starch levels significantly enhanced the shoot growth of orchid.

Fresh weight of shoot: A highly significant variation was found in fresh weight of shoots at different levels of starches at different days after culture. In the control treatment (agar) showed the fresh weight of shoot (0.11, 0.21 and 0.31g) at 35, 70 and 105 DAC, respectively (Table 1). Whereas, the highest (0.13g) shoot weight was recorded in $30gL^{-1}$isubgol and the lowest (0.011g) was found from $50gL^{-1}$corn starch at 35 DAC. The highest (0.23g) fresh weight of shoot and the lowest (0.12g) were also observed in $30gL^{-1}$isubgol and $50gL^{-1}$ corn, respectively at 70 DAC. At the same treatments, the highest (0.32g) and the lowest (0.21g) fresh weight of shoot were also observed at 105 DAC. This results also supported by Zhe *et al*. (2005). Plantlets developed on media with $40gL^{-1}$ of corn and potato starch had higher shoot fresh and dry weight ($p < 0.1$) compared to those in the control one. Media with $50gL^{-1}$and $60gL^{-1}$of potato starch or $60gL^{-1}$ of corn starch and $50gL^{-1}$of corn starch + agar at $1gL^{-1}$significantly enhanced the percentage of dry weight reported by Mohamed *et al*. (2009). In the above results, isubgol showed the better performance on the entire above shoot characters than agar whereas, isubgol was used here as an alternative gelling agent on agar supplemented with MS medium. However, $8gL^{-1}$agar (control) showed more or less similar result with isubgol on shoot length and fresh weight of shoot. Here we found that the shoot formation of orchid progressively increases when the starches level significantly increased by the individual treatments of isubgol, cassava, corn and potato. This might be due to increased starch levels providing good conditions for growth of orchid by maintaining proper biochemical and physiological processes.

Number of leaves plant^{-1}: The number of leaves plant^{-1} was highly significant with different levels of starches gelled with 2gL^{-1}agar in medium. At 35 DAC, number of leaves (3.00) was found in agar 8gL^{-1}(control) whereas the maximum (3.75) number of leaves was recorded in isubgol 30gL^{-1} at 35 DAC. At 70 and 105 DAC, the maximum (6.75 and 8.75, respectively) number of leaves were also found in isubgol 30gL^{-1}followed by (6.00 and 8.00) were observed with 8gL^{-1}agar. The minimum (0.75, 3.75 and 5.75) number of leaves were found with the both treatment combinations of corn starch at 35, 70 and 105 DAC, respectively (Table 2, Fig 2.1 and 2.2). It was observed that the 8gL^{-1}agar and 30gL^{-1} isubgol showed statistically more or less similar results. A similar phenomenon was also observed by Naik and Sarker (2001). They reported that sago starch provided a firm gelling surface throughout the entire culture period, and fostered optimum plantlet growth in terms of shoot height, number of nodes per plant, number of leaves and fresh mass. Likewise, isubgol also provided similar firm gelling conditions for orchid growth resulting higher number of leaves.

Length of leaves: A significantly variation was found due to the effect of different levels of isubgol, cassava, corn and potato starch gelled with 2gL^{-1}agar in the medium on the number of leaves plant^{-1}. The longest (1.35cm) leaves was recorded in 30g L^{-1} isubgol and the shortest (0.49cm) was observed in 50gL^{-1} corn at 35 days after culture. At 70 DAC, the longest (2.19cm) was also found in 30gL^{-1} isubgol and the shortest (1.05cm) leaves found in 50gL^{-1} corn starch (Table 2). At the same treatments, the longest (2.79cm) and the shortest (1.64cm) were found from 105 DAC (Table 2 and Fig 2.2). However, 8gL^{-1}agar showed the length of leaves (1.26, 2.10 and 2.76cm) which were significantly differed to that of 30gL^{-1} isubgol at different days (35, 70 and 105, respectively) which gave longest length of leaves. A similar experiment was found that the best substrates for *Megalechis picta* were vermiculite + charcoal and vermiculite + carbonized rice husks by Faria *et al.* (2001).

Width of leaves: Width of leaves was significantly influenced due to the effect of different levels of starches. After 35 days of culture, the highest (0.24cm) width of leaves was found at 30gL^{-1} isubgol whereas the agar showed (0.23cm). The lowest (0.11cm) width of leaves was observed in 50gL^{-1} corn. The highest (0.64cm) and the lowest (0.51cm) were recorded in 30gL^{-1} isubgol and 50gL^{-1} corn starch, respectively at 70 DAC. Isubgol with 30gL^{-1} and corn with 50gL^{-1} showed the highest (0.84cm) and the lowest (0.71cm) width of leaves, respectively at 105 DAC (Table 2). At 70 and 105 DAC, the width of leaves (0.63 and 0.83cm) was recorded in 8gL^{-1} agar (control) which was statistically similar with isubgol. Similar results are also obtained by Faria *et al*. (2001). They found that the best substrates for *M. picta* were vermiculite + charcoal and vermiculite + carbonized rice husks. In these substrates, *M. picta* recorded highest leaf width (1.84 and 1.83 cm).

Number of roots plantlet^{-1}: The different levels of starches (isubgol, cassava, corn and potato) showed significant number of root plant^{-1}. The highest (3.25) number of roots plant^{-1} was found in 30gL^{-1} isubgol at 35 DAC. At the same days, 8gL^{-1} agar (control) showed (3.00) and the lowest (1.00) was observed in 50gL^{-1}corn (Table 3). At 70 DAC, the highest (6.25 and 6.00) were recorded in 30gL^{-1}isubgol and 8gL^{-1}agar (control), respectively whereas, the lowest (4.00) was found at 50gL^{-1} corn. In 30gL^{-1} isubgol showed the highest (8.25) when 8gL^{-1} agar showed the number of root plant^{-1}(8.00) which was statistically similar with isubgol and the lowest (6.00) was found in 50gL^{-1} corn at 105 DAC (Table 3 and Fig 3.1). Ozel *et al*. (2008) reported that the highest number of roots was also recorded on MSD4X2 medium gelled with 7gL^{-1} isubgol which was similar with my study. The different level of starches supported root growth differently. Number of root plant^{-1} progressively increases by the increasing different levels of starches individually. Beside this, the isubgol showed better performance on root growth than other concentrations of another starches.

Length of root: The different levels of starches (isubgol, cassava, corn and potato) showed significant variations on length of roots. At 35 DAC, the agar showed of 0.75cm of root whereas, the longest (0.85cm) was recorded in 30gL^{-1} isubgol when isubgol used as an alternative gelling agent on agar and supplemented with MS medium. The lowest (0.09cm) was observed in 50gL^{-1} corn. At 70 DAC, the longest (1.45) and the lowest (0.69cm) were recorded also in 30gL^{-1} isubgol and 50gL^{-1}corn, respectively (Table 3). However, the length (1.35cm) was recorded in agar which was statistically identical with that of isubgol. The longest (1.85cm) and the shortest (1.09cm) were also found in 30gL^{-1}isubgol and 50gL^{-1} corn, respectively at 105 DAC (Table 3 and Fig. 3.2). Finally, at 105 DAC, the length of root (1.75cm) was recorded in 8gL^{-1} agar. Isubgol showed better performance in all the parameters at different stages of days when isubgol used as an alternative gelling agent on agar. From the above, length of leaves was also increased by the increasing level of isubgol. This study concerns the efficacy of partial agar substitution by galactomannans as support in plant regeneration media for *Nicotiana tabacum* (Lucyszyn *et al*., 2008). The *in vitro* performance allowed to conclude that the length of roots and the size of leaves were significantly higher in the media solidified with agar/guar galactomannans (GMs) extracted from seeds of a native Brazilian specie designated *Cassia fastuosa* (cassia) and from *Cyamopsis tetragonolobus* (guar gum–a commercial GM) were

mixed with agar (Jain and Babbar, 2005), in the proportion of 3/3gL^{-1} (w/w), and used as a gelling agent in Marubakaido apple rootstock (*Malus prunifolia* Borkh) micropropagation.

Table 1. Effects of different treatments of starches supplemented with MS medium gelled with 2gL^{-1}agar on number of shoots per PLB and fresh weight of shoot of *Dendrobium sonia* as compared with control 8gL^{-1} agar.

Starches (gL^{-1})	No. of shoots PLB^{-1}			Fresh weight of shoot (g)			Length of shoot (cm)		
	35 DAC	70 DAC	105 DAC	35 DAC	70 DAC	105 DAC	35 DAC	70 DAC	105 DAC
Agar (Control)	3.50 ^{ab}	4.25 ^{bc}	5.75 ^{bc}	0.11 ^{b}	0.21 ^{b}	0.31 ^{a}	2.12 ^{b}	3.64^{b}	4.38^{b}
Isubgol 15	2.75 ^{bcd}	3.25 ^{de}	4.75 ^{cd}	0.03 ^{h}	0.13 ^{h}	0.23 ^{e}	1.09^{h}	2.59^{h}	3.50^{g}
Isubgol 20	3.25 ^{bc}	3.75 ^{cd}	5.50 ^{c}	0.06 ^{f}	0.16 ^{f}	0.26 ^{c}	1.56^{f}	2.72^{g}	4.00^{d}
Isubgol 25	3.50 ^{b}	4.75 ^{b}	6.50 ^{b}	0.09 ^{d}	0.19 ^{d}	0.29 ^{b}	1.80^{e}	3.48^{c}	4.14^{c}
Isubgol 30	4.25 ^{a}	6.25 ^{a}	8.50 ^{a}	0.13 ^{a}	0.23 ^{a}	0.32 ^{a}	2.34^{a}	3.92^{a}	4.90^{a}
Cassava 15	1.75 ^{ef}	2.75 ^{ef}	4.00 ^{de}	0.02 ^{i}	0.12 ^{i}	0.22 ^{f}	0.98^{i}	2.11^{j}	3.13^{i}
Cassava 20	2.25 ^{def}	3.25 ^{de}	4.75 ^{cd}	0.03 ^{g}	0.13 ^{g}	0.23 ^{d}	1.45^{g}	2.24^{i}	3.29^{h}
Cassava 25	2.25 ^{def}	3.50 ^{cde}	5.25 ^{c}	0.06 ^{f}	0.16 ^{f}	0.26 ^{c}	1.60^{f}	2.63^{h}	3.52^{g}
Cassava 30	2.50 ^{cde}	4.25 ^{bc}	5.75 ^{bc}	0.08 ^{de}	0.18 ^{de}	0.28 ^{b}	1.85^{d}	2.94^{f}	3.63^{f}
Corn 50	1.50 ^{f}	2.25 ^{fg}	3.50 ^{e}	0.01 ^{i}	0.11 ^{i}	0.21 ^{f}	1.00^{i}	1.98^{k}	2.54^{j}
Corn 60	1.50 ^{f}	1.75 ^{g}	3.25 ^{e}	0.01 ^{i}	0.11 ^{i}	0.21 ^{f}	0.92^{j}	1.85^{l}	2.30^{k}
Potato 50	2.50 ^{cde}	3.50 ^{cde}	5.25 ^{c}	0.08 ^{e}	0.18 ^{e}	0.18 ^{h}	1.82^{de}	3.13^{e}	3.90^{e}
Potato 60	3.25 ^{bc}	4.25 ^{bc}	5.50 ^{c}	0.10 ^{c}	0.20 ^{c}	0.20 ^{g}	1.94^{c}	3.43^{d}	4.11^{c}
CV (%)	20.72	14.04	11.76	5.60	2.12	1.91	2.20	1.20	1.05
Level of significance	**	**	**	**	**	**	**	**	**

** = 1% level of significance; PLB= Protocorm like bodies; DAC=Days after culture
Same letter (s) in a column didn't differ significantly.

Table 2. Effects of different treatments of starches supplemented with MS medium gelled with 2gL^{-1}agar on number of leaves per plant and width of leaves of *Dendrobium sonia* as compared with control 8gL^{-1} agar

Starches (gL^{-1})	No. of leaves plant^{-1}			Width of leaves (cm)			Length of leaves (cm)		
	35 DAC	70 DAC	105 DAC	35 DAC	70 DAC	105 DAC	35 DAC	70 DAC	105 DAC
Agar (Control)	3.00 ^{ab}	6.00 ^{ab}	8.00 ^{ab}	0.23 ^{c}	0.63 ^{a}	0.83 ^{ab}	1.26^{b}	2.10^{b}	2.76^{b}
Isubgol 15	1.50 ^{cde}	5.00 ^{bc}	7.00 ^{bc}	0.16 ^{h}	0.56 ^{bc}	0.76 ^{e}	0.54^{i}	1.25^{i}	1.87^{g}
Isubgol 20	2.00 ^{bcd}	5.00 ^{bc}	7.00 ^{bc}	0.19 ^{g}	0.59 ^{abc}	0.79 ^{d}	0.70^{g}	1.46^{g}	2.05^{f}
Isubgol 25	2.75 ^{ab}	5.75 ^{ab}	7.75 ^{ab}	0.24 ^{b}	0.64 ^{a}	0.84 ^{a}	0.99^{d}	1.85^{d}	2.55^{d}
Isubgol 30	3.75 ^{a}	6.75 ^{a}	8.75 ^{a}	0.24 ^{a}	0.64 ^{a}	0.84 ^{a}	1.35^{a}	2.19^{a}	2.88^{a}
Cassava 15	1.25 ^{de}	4.25 ^{cd}	6.25 ^{cd}	0.15 ^{j}	0.55 ^{cd}	0.75 ^{e}	0.50^{ij}	1.19^{j}	1.69^{i}
Cassava 20	2.00 ^{bcd}	5.00 ^{bc}	7.00 ^{bc}	0.20 ^{f}	0.60 ^{ab}	0.80 ^{d}	0.61^{h}	1.31^{h}	1.89^{g}
Cassava 25	2.25 ^{bcd}	5.25 ^{bc}	7.25 ^{bc}	0.22 ^{d}	0.62 ^{a}	0.82 ^{bc}	0.84^{f}	1.55^{f}	2.08^{f}
Cassava 30	2.50 ^{bc}	5.50 ^{b}	7.50 ^{b}	0.23 ^{c}	0.63 ^{a}	0.83 ^{ab}	0.93^{e}	1.68^{e}	2.28^{e}
Corn 50	0.75 ^{e}	3.75 ^{d}	5.75 ^{d}	0.11 ^{k}	0.51 ^{d}	0.71 ^{f}	0.49^{j}	1.05^{k}	1.64^{j}
Corn 60	0.75 ^{e}	3.75 ^{d}	5.75 ^{d}	0.16 ^{i}	0.56 ^{bc}	0.76 ^{e}	0.52^{ij}	1.16^{j}	1.74^{h}
Potato 50	2.25 ^{bcd}	5.25 ^{bc}	7.25 ^{bc}	0.20 ^{fg}	0.60 ^{abc}	0.80 ^{d}	0.68^{g}	1.24^{i}	1.88^{g}
Potato 60	2.75 ^{ab}	5.75 ^{ab}	7.75 ^{ab}	0.21 ^{e}	0.61 ^{a}	0.81 ^{c}	1.15^{c}	1.95^{c}	2.65^{c}
CV (%)	32.78	14.43	10.40	8.77	2.87	214	3.24	1.76	1.41
Level of Significance	**	**	**	**	**	**	**	**	**

** = 1% level of significance; DAC= Days after culture
Same letter (s) in a column didn't differ significantly.

Fresh weight of roots: On fresh weight of roots (g) showed significant variations due to the effect of different levels of starches (Table 3). Isubgol 30gL^{-1} had highest weight 0.07g at 35 DAC and the lowest weight 0.01g was observed in 50gL^{-1} corn. At 70 DAC, the highest weight (0.13g) and the lowest weight (0.06g) were recorded in 30gL^{-1} isubgol and 50gL^{-1}corn, respectively. In 30gL^{-1} isubgol also showed the highest weight (0.16g) and in 50gL^{-1}corn showed the lowest weight (0.09g) at 105 DAC (Table 3). Similar results are also obtained by Zhe

et al. (2005) and Zapata (2001). The reasons for attaining lower fresh weight in $50gL^{-1}$corn as it didn't give firm gelling conditions for root growth and development as compared to $30gL^{-1}$ isubgol.

Table 3. Effects of different treatments of starches supplemented with MS medium gelled with $2gL^{-1}$agar (supplemented with $1.5mLL^{-1}$ IAA) on number of roots plant^{-1} and fresh weight of root of *Dendrobium sonia* as compared with control $8gL^{-1}$ agar

Starches (gL^{-1})	No. of roots plant^{-1}			Fresh weight of root (g)			Length of root (cm)		
	35 DAC	70 DAC	105 DAC	35 DAC	70 DAC	105 DAC	35 DAC	70 DAC	105 DAC
Agar (Control)	3.00 ^{ab}	6.00 ^{ab}	8.00 ^{ab}	0.06 ^{b}	0.12 ^{ab}	0.15 ^{b}	0.75^{b}	1.35^{b}	1.75^{ab}
Isubgol 15	1.75 ^{de}	4.75 ^{cde}	6.75 ^{bc}	0.02 ^{f}	0.07 ^{efg}	0.10 ^{g}	0.24^{i}	0.84^{i}	1.24^{ejg}
Isubgol 20	2.25 ^{cd}	5.25 ^{bcd}	7.25 ^{abc}	0.04 ^{d}	0.09 ^{cde}	0.12 ^{e}	0.41^{g}	1.01^{g}	1.41^{de}
Isubgol 25	2.50 ^{bc}	5.50 ^{bc}	7.50 ^{abc}	0.04 ^{d}	0.09 ^{cde}	0.12 ^{f}	0.65^{d}	1.25^{d}	1.65^{abc}
Isubgol 30	3.25 ^{a}	6.25 ^{a}	8.25 ^{a}	0.07 ^{a}	0.13 ^{a}	0.16 ^{a}	0.85^{a}	1.45^{a}	1.85^{a}
Cassava 15	1.50 ^{d}	4.50 ^{de}	6.50 ^{cd}	0.02 ^{f}	0.07 ^{efg}	0.10 ^{g}	0.15^{j}	0.75^{j}	1.15^{fg}
Cassava 20	1.75 ^{de}	4.75 ^{cde}	6.75 ^{bc}	0.03 ^{e}	0.08 ^{def}	0.11 ^{f}	0.35^{h}	0.95^{h}	1.35^{def}
Cassava 25	2.00 ^{cde}	5.00 ^{cd}	7.00^{abcd}	0.04 ^{d}	0.09 ^{de}	0.12 ^{e}	0.46^{f}	1.06^{f}	1.46^{cde}
Cassava 30	2.75 ^{b}	5.75 ^{abc}	7.75 ^{abc}	0.05 ^{c}	0.10 ^{cd}	0.13 ^{d}	0.57^{e}	1.17^{e}	1.57^{bcd}
Corn 50	1.00 ^{e}	4.00 ^{e}	6.00 ^{d}	0.01 ^{g}	0.06 ^{g}	0.09 ^{h}	0.09^{k}	0.69^{k}	1.09^{g}
Corn 60	1.50 ^{d}	4.50^{cdef}	6.50 ^{cd}	0.02 ^{f}	0.07 ^{fg}	0.10 ^{g}	0.11^{jk}	0.71^{jk}	1.11^{h}
Potato 50	1.75 ^{de}	4.75 ^{cde}	6.75 ^{bc}	0.05 ^{c}	0.10 ^{cd}	0.13 ^{d}	0.58^{e}	1.18^{e}	1.58^{bcd}
Potato 60	2.75 ^{b}	5.75 ^{abc}	7.75 ^{abc}	0.06 ^{b}	0.11 ^{bc}	0.14 ^{c}	0.70^{c}	1.30^{c}	1.70^{ab}
CV (%)	39.61	16.47	11.85	4.63	2.03	1.52	2.61	2.51	10.09
Level of significance	**	**	**	**	**	**	**	**	**

** = 1% level of significance; DAC= Days after culture
Same letter (s) in a column didn't differ significantly.

Conclusion: From the above study, the shoot, leaf and root characters showed better growth and vigor in $30gL^{-1}$isubgol; and $50gL^{-1}$ corn showed the inferior performance. Murashige and Skoog (MS) medium supplemented with the increasing dose of isubgol, cassava, corn and potato progressively increased their growth performance. Actually, the alternative gelling agents show better results with the addition of a little amount of agar ($2gL^{-1}$) which is required for strengthening of semi-solid medium. Gelling agent like agar which is an important component in plant tissue culture media is considered to be expensive, which causes high cost of plant micropropagation. Here we observed that the isubgol is better than the other starches of low cost alternative gelling agent and more effective for plant tissue culture. So, the present study suggests the use of isubgol (500g costs US$ 10 only) as an alternative gelling agent to agar reducing the production cost substantially.

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