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Potential of Alternative Gelling Agents in Media for the *in vitro* Micro-propagation of *Celosia* sp.

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Abstract: Plant tissue culture techniques often require optimization for cost reducing by substitute the culture medium with kitchen necessity. This study conducted to investigate various commercial starches namely; cassava flour, rice flour, corn flour and potato starch to be alternatives gelling agents in culture medium preparation. MS basal medium was prepared without plant regulator supplemented with different combinations of alternative gelling agent with Oxoid technical agar. After 8 weeks in culture, the highest of shoot regeneration were obtained on cultured in MS medium containing 40 g L^{-1} corn flour (10.20±9.17), 60 g L^{-1} cassava flour (40.00±6.73), 40 g L^{-1} rice flour (16:00±12.45) and the combination of 40 g L^{-1} potato starch plus 2 g L^{-1} agar (27.20±8.59). ANOVA test shows there were significant differences interactions effects ($p<0.05$) in terms of number of shoot regenerated between the gelling agents used (40, 50 and 100 g L^{-1}) in combination of agar. On the other hand, no significant different interaction effects at combination of 60 g L^{-1} gelling agent. The results showed that the combination of alternative gelling agent with agar or gelling agent alone successfully regenerated shoots from the stem segment explants of *Celosia* sp. No physiological effects were observed on shoots regeneration on *Celosia* sp. Applications of these alternative gelling agents in tissue culture media should be considered to achieve the optimum benefit for *in vitro* shoots regeneration. These options are cheaper than control one and could be used for low cost alternatives.

Key words: Alternative gelling agent, cassava flour, rice flour, corn flour, potato starch, shoot regeneration

INTRODUCTION

The commercial use of plant tissue culture involves the production of large number of plants with minimum expenses. Due to large cost needed in governing plant tissue culture techniques, many researchers tried to conduct low cost alternatives for micropropagation. For example (Raghu *et al*., 2007) have tried household sugar and tap water to replace laboratory sucrose and double distilled water used in plant tissue culture. As the result, it responsive in reduce the cost needed and successful promoting the plantlet induction. While, according to Ezekiel (2010) that have been studied about vegetation of tropical trees, due to external factor like climate change influences them to develop low cost technique in micropropagation and also macropropagation. The low cost option include in washing and sterilizing operation which is most important aspect, types of plantlets containers and culture media. In talking culture media composition, selection of low cost materials must be able to induce the shoots regeneration. One of the factors that contribute to the efficiency of micropropagation is composition of culture medium (Rashid *et al*., 2000).

Agar is the most frequently used solidifier in plant tissue culture media (Afrasiab and Jafar, 2011) and it is a most expensive components used in plant tissue culture media. Various brands and grades of agar, agarose, phytagel and gelrite were used for *in vitro* micro-propagation (Debergh, 1983). More than 100 years ago, agar has been widely used as a gelling agent in plant tissue culture technique. This is because its stability, high clarity, non toxic nature and resistance to its metabolism Henderson and Kinnersley (1988). Some studies have been made to find alternative substances that have same ability as agar and also the effect of using agar substitutes in order to reduce cost in preparing tissue culture media. Bhattacharya *et al*. (1994) and Naik and Sarkar (2001) used a cheaper gelling agent sago for while

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Gebre and Sathyanarayana (2001) used commercial cassava and sago for potato (*Solanum tuberosum* L.). Other researches tried prepare the media by mix agar and other gelling agent at different quantity. Combination of agar with commercial corn starch and potato starch reported are efficient for potato micro-propagation by nodal explants (Mohamed *et al*., 2009). Maliro and Lamerck (2004) worked with cassava flour as a gelling agent and found that the gel was improved by mixing with some agar. While, Zapata (2001) successfully reduced the cost of banana tissue culture by mixing corn and potato starch with gelrite as alternative gelling agent.

Barley starch used as gelling agent was experimented by Tiwari and Rahimbaev (1992) cited in Jain-Raina and Babbar (2011). As the result, combination of barley starch and agarose in anther culture of barley responsive better gelling effect. The effect includes firm surface formation and prevention of explant sinking during degradation of starch. Besides that, other researchers tried on guar gum (Babbar *et al*., 2005), isubgol (Jain-Raina and Babbar, 2011; Saglam and Cifict, 2010), ispaghol (Hussain Shah *et al*., 2003; Jain *et al*., 1997) gum katira (Jain and Babbar, 2002), locust bean gum (Goncalves and Romano, 2005) and glass bead (Goel *et al*., 2007). The performance of these low-cost gelling agents was found satisfactory and could compare well with agar.

The aim of this study was to evaluate potential of various commercial starches namely; cassava flour, rice flour, corn flour as alternative gelling agent in culture media. Besides that, comparison done by comparing the number of shoots regeneration on stem segments of *Celosia* sp. in the presence and absence of oxoid technical agar. A successful attempt has been made to minimize the cost of media culture preparation.

MATERIALS AND METHODS

Plant materials: All experiments were conducted at Laboratory B2.5, Institute of Biological Sciences, Faculty of Sciences, University of Malaya, Kuala Lumpur, Malaysia in year 2010. In these experiments, explants used were seed of the *Celosia* sp. Firstly, the seeds were soaked with Clorox solution (sodium hypochlorite) 100% (v/v) and added with 2 drops of Tween-20 for 5 min and then 70% (v/v) Clorox solution and shaken for 3 min. Seeds of further rinsed with distilled water and then washed using 70% (v/v) alcohol for 1 min. After the last washing, the seeds rinsed with sterile distilled water at 4-5 times and ready to be cultured. The seeds were cultured in MS basal media. In these experiments, explants used were stem segment of *Celosia* sp. excised from 2 months-old *in vitro* seedling.

Preparation of culture medium: The culture medium used for all experiments was based on Murashige and Skoog (1962) medium with 30.0 g L^{-1} sucrose, addition of alternative gelling agents and Oxoid technical agar. No hormones were added in the medium. The medium was autoclaved for 21 min at 121°C after adjusted the pH to 5.8.

Culture condition: Stem segment of explants (2 cm) were inoculated into the vial containing culture medium. Cultures were incubated 8 weeks under the culture room condition of light intensity (1000 μmol/m^2/sec), temperature at 25±1°C and 70-80% relative humidity with a 16/8 h light/dark photoperiod.

Experimental design: The numbers of shoot per explants were recorded weekly for 8 weeks in culture and the mean were calculated. The data were subjected to analysis of variance (ANOVA) using SPSS version 16 statistical package. Least significant differences between means were analyzed to identify treatment means which different significantly at the 5% level of significance.

RESULTS AND DISCUSSION

Explants of stem segment were cultured in the MS media supplemented with alternative gelling agents and in combination of agar have shown responsiveness to the shoots regeneration. All 40 formulas tested in the experiment were promising as solidifying agent on *Celosia* sp. stem segments culture with low cost materials. The number of shoots regeneration on *Celosia* sp. using different gelling agents in the presence or absence of agar were presented in Table 1-4 while the performance of *in vitro* regeneration of *Celosia* sp. are show in Fig. 1 and 2. General characteristics of the best medium stability were semi solid media which adhered to the surface of the jam jar and showed individually different transparency from each alternative gelling agent.

All alternative gelling agents sustained cultures in all used concentrations. Based on combination of alternative gelling agent in medium, the highest of shoot regeneration were obtained on cultured in MS medium containing 40 g L^{-1} corn flour (20.10±9.17 shoots per explants) in Table 1 as shown in Fig. 1a, 60 g L^{-1} cassava flour (40.00±6.73) in Table 2 as shown in Fig. 1b, 40 g L^{-1} rice flour (18.70±9.56 shoots per explants) in Table 3 as shown in Fig. 1c and the combination of 40 g L^{-1} potato starch plus 2 g L^{-1} agar (27.20±8.59 per explants) in Table 4 as shown in Fig. 1d. The number of shoot regenerations on MS medium supplemented with 60 g L^{-1} cassava flour had higher compared to the other media treatment used. MS

Table 1: Effects on *in vitro* in term of number shoot regeneration of stem segments explants after 8 weeks cultured in MS medium supplemented with corn flour and agar

Concentration of corn flour (g L^{-1})	Concentration of agar (g L^{-1})		
	0	1	2
40	20.10±9.17^{ab}	18.30±7.66^{ab}	19.60±5.08^{ab}
50	17.80±3.01^{ab}	11.50±5.03^{a}	11.90±3.92^{a}
60	18.30±8.00^{ab}	12.50±4.85^{a}	15.80±8.89^{ab}
100	10.50±3.59^{a}	nd	nd

nd = not determined. Values are Mean±SD with 14 replicates followed by the same alphabet letter are not significantly different at 5% level by Tukey test

Table 2: Effects on *in vitro* in term of number shoot regeneration of stem segments explants after 8 weeks cultured in MS medium supplemented with cassava flour and agar

Concentration of cassava flour (g L^{-1})	Concentration of agar (g L^{-1})		
	0	1	2
40	6.00±2.98^{a}	22.00±13.39^{bc}	22.6±10.78^{bc}
50	11.30±6.25^{a}	26.90±7.86^{bc}	24.50±9.59^{bc}
60	40.00±6.73^{d}	35.1±8.51^{cd}	27.90±9.30^{bc}
100	14.80±11.82^{ab}	nd	nd

nd = not determined. Values are Mean±SD with 14 replicates followed by the same alphabet letter are not significantly different at 5% level by Tukey test

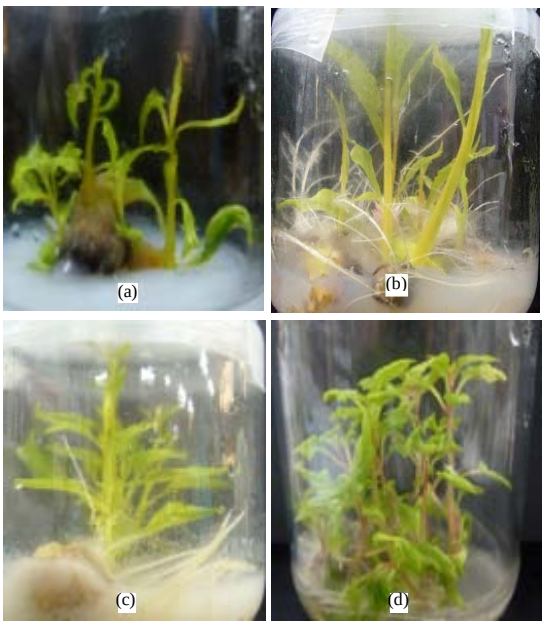

Fig. 1(a-d): *In vitro* regenerated shoots from stem segment explants of *Celosia* sp. cultured in MS medium containing different concentrations of gelling agent: (a) 40 g L^{-1} corn starch, (b) 40 g L^{-1} cassava flour+2 g L^{-1} agar, (c) 40 g L^{-1} rice flour (d) 60 g L^{-1} potato flour

Table 3: Effects on *in vitro* in term of number shoot regeneration of stem segments explants after 8 weeks cultured in MS medium supplemented with rice flour and agar

Concentration of rice flour (g L^{-1})	Concentration of agar (g L^{-1})		
	0	1	2
40	18.70±9.56^{a}	13.40±8.24^{a}	14.40±9.08^{a}
50	13.20±8.80^{a}	12.80±3.74^{a}	10.80±7.88^{a}
60	12.70±7.90^{a}	11.10±4.77^{a}	12.30±6.84^{a}
100	13.70±3.74^{a}	nd	nd

nd = not determined. Values are Mean±SD with 14 replicates followed by the same alphabet letter are not significantly different at 5% level by Tukey test

Table 4: Effects on *in vitro* in term of number shoot regeneration of stem segments explants after 8 weeks cultured in MS medium supplemented with potato starch and agar

Concentration of potato starch (g L^{-1})	Concentration of agar (g L^{-1})		
	0	1	2
40	18.90±9.79^{ab}	25.30±3.97^{ab}	27.20±8.59^{bc}
50	21.50±8.73^{ab}	21.00±8.43^{ab}	14.60±7.69^{a}
60	26.50±5.06^{bc}	17.40±6.73^{a}	18.30±9.41^{ab}
100	26.20±9.64^{ab}	nd	nd

nd = not determined. Values are Mean±SD with 14 replicates followed by the same alphabet letter are not significantly different at 5% level by Tukey test

medium supplemented with cassava flour plus agar also gave better performance shoot regeneration compared to corn, rice and potato starch as substitutes agar.

The effect of starches (especially cassava flour) can act as an additional carbon source to the medium and improve morphogenesis. Powell and Uhrig (1987) reported that some solidifying agents have inhibitory substances that affect morphogenesis of *Solanum* genotypes. The better response on starches gelled media could also be due to the absence of inhibitors which have been reported to be present in agar (Debergh, 1983; Puchooa *et al*., 1999). Mohamed *et al*. (2009) supported, using 40, 50 and 60 g L^{-1} starches (corn starch and potato starch) with low 0, 1 and 2 g L^{-1} of agar for *Solanum tuberosum* micro-propagation produced higher shoot regeneration than using 7 g L^{-1} agar alone. Besides that, as the concentration of agar increased the number of shoots regeneration not necessary increased too. For example in this present study used 60 g L^{-1} cassava starch without agar showed the high shoot regeneration than addition with 1 or 2 g L^{-1} of agar as shown in Table 2. Such results show the same pattern with a previous study by Karim *et al*. (2003) that used *Chrysanthemum morifolium* as experimental species.

According to Debergh (1983) gel strength is often regarded as an important criterion for agar quality. Media gelled with 80 g L^{-1} cassava starch mixed with 2.5 g L^{-1} agar provided the same firmness as media gelled with 80 g L^{-1} agar. Cassava starch of 100 g L^{-1} gave adequate support and orientation of potato nodes explants. Gebre and Sathyanarayana (2001) reported the response of

Table 5: Comparison of gelling agent costs

Gelling agents	Cost/kilogram (RM kg^{-1})	Amounts in medium	Cost/liter (RM L^{-1})*
Technical oxoid agar	560.00	8 g L^{-1}	4.48
Corn flour	3.00	100 g	0.30[a]
Rice flour	4.40	100 g	0.44[b]
Cassava flour	3.00	100 g	0.30[c]
Potato starch	15.00	100 g	1.50[d]

*RM: Ringgit Malaysia. Means cost of alternative gelling agent, [a,b,c,d] = RM 0.64

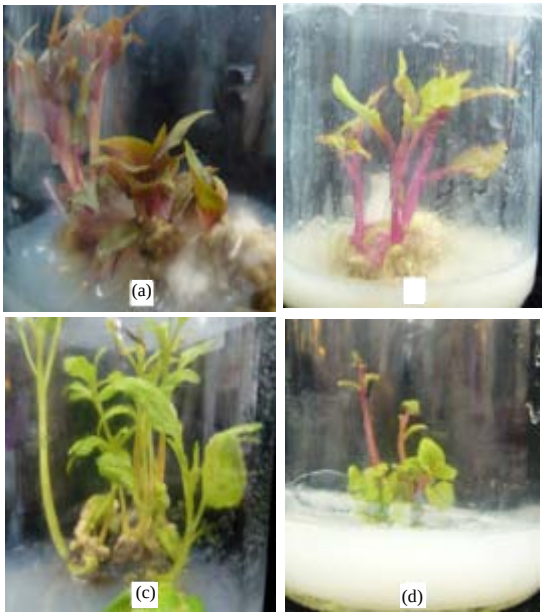

Fig. 2(a-d): *In vitro* regenerated shoots from the stem segment explants of *Celosia* sp. that affected by MS medium with combination 100 g L^{-1} of different gelling agent: (a) cassava flour, (b) rice flour, (c) of potato starch and (d) corn flour

shoot regeneration on gelled media with cassava starch concentration is lower than 140 g L^{-1} and higher than the 80 g L^{-1} reported by Nene and Sheila (1994). Based to Gebre and Sathyanarayana (2001) they have confirmed that cassava flour have gelling ability and suggested it is a potential cheap substitute for agar.

Statistical results showed that there were significant differences interactions effects ($p<0.05$) in terms of number of shoot regenerated between the concentration gelling agents used (40, 50 and 100 g L^{-1}). However, there were no significant different interaction effects ($p>0.05$) between MS medium with combination of 60 g L^{-1} gelling agent and agar in terms of number of shoot regenerated. Production of shoots on media supplemented with 60 g L^{-1} of alternative agar alone or in combination with

Table 6: Comparison of culture medium costs

Medium	1	2	3 (control)
Combination	Alternative gelling agent (RM 0.64)+ MS powder (RM 33.00)+ Source (RM 2.70)	Alternative gelling agent (RM 0.64)+ MS powder (RM 33.00)+ Sucrose (RM 2.70) +Agar (RM 4.48)	MS powder (RM 33.00)+ 2.0 mg L^{-1} BAP+1.0 mg L^{-1} NAA+Vitamins (RM 0.52)+ Source (RM 2.70) +Agar (RM 4.48)
Cost (RM)*	36.34	40.82	40.70
Number of shoots regenerated	26.20±9.64	27.90±9.30	15.50±1.67

*RM: Ringgit Malaysia. The price given are cost preparing 1 L of culture media that consist of 4.4 g L^{-1} of MS powder, 30 g L^{-1} of sucrose, 8 g L^{-1} of oxoid technical agar and 2.0 mg L^{-1} BAP+1.0 mg L^{-1} NAA

agar (1 or 2 g L^{-1}) had no significant difference among the other gelling agent. Shoots regeneration of them are strongly influenced by the physical consistency of the medium. Apparently, the viscosity of medium and its component play an important role in shoot regeneration. Media culture that fully saturated with alternative gelling agent without addition of Oxoid technical agar also promising ability on shoot regeneration. Figure 2c showed, the best number of shoot regeneration were supported by potato starch media culture with 26.20 number of shoots income. The least shoot produced as shown at Fig. 2b, cultured in rice flour. While, in Fig. 2a and d showed the intermediate number of shoot production cultured in cassava flour and corn flour, respectively.

Cost required in preparing three different media culture shown at Table 5. Although, the composition and cost required of each medium are different but it still managed to produce regenerated shoots of *Celosia* sp. Researchers have used an alternative gelling agent material costly RM 0.64. This price derived from the mean of each type of powder as the price of each is roughly the same as shown in Table 5. Preparation of media 3 costly about RM 40.70 which more expensive than cost of media 1 preparation that about RM 36.34 which differ about 10.71% calculated from the cost shown as in Table 6. While comparison cost between media 2 show additions about 0.29% cost needed compare to RM 40.70 in media 3 combinations. Media 1 and 2 produced high production of shoots regeneration (26.20±9.64 and 27.90±9.30) rather than media 3 (15.50±1.67) as also shown in Table 6. In percentage, the different cost between media 2 and 3 as shown in Table 6 was about 0.29%. Even extra 0.29% needed in media 2 preparation, it can be said as worthwhile due to actively promising number of shoots regeneration income in media 2. Big differences in the number of shoot that about 12.4 pieces are preferred over the addition of 0.29% or approximately ten cent of cost

provision in order to choose the optimum media that providing high shoots regeneration as shown in Table 6. The control of shoot regeneration media with 8 g L^{-1} of oxoid agar for in combination with plant regulators (NAA and BAP) limit of less number of shoot regeneration as represented in media 3. Optional of media 1 and 2 in Table 6 allowed lower the cost of production without compromising the quality of the micropropagules and plants.

CONCLUSION

Four commercial starches (not pure starch) namely; corn starch, rice flour, cassava flour and potato starch tested in the experiment can be an alternative for agar which responsive in shoots regeneration stem segment on *Celosia* sp. Although, the agar alternatives may differ in their composition than agar which may affect culture growth, this phenomenon was not observed in *Celosia* sp. cultures. Besides sustaining the cultures, this agar alternative was found cheap and available. The results of the present study offer new possibilities of using low cost raw materials as agar alternatives which will reduce materials costs considerably and will help in popularizing plant tissue culture techniques.

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