Potential of Alternative Cheap Gelling Agents for *in vitro* Micro-Propagation of *Rice* (*Oryza sativa L*)

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Abstract

Plant tissue culture techniques often require optimizing cost reduction by substituting the culture medium with alternative gelling agents. This study was conducted to investigate various commercial products; agar, stabilizer, corn flour, Kithul flour, gelatine and potato as gelling agents in culture media preparation for *in vitro* propagation of rice. MS basal medium was prepared without plant growth regulators supplemented with different alternative gelling agents and agar. Results showed that agar and alternative gelling agents successfully produced shoots from the seed explants of *At 401 and AT 402* after 4 weeks in culture. The highest length of shoots were obtained in MS basal medium containing 8 g L^{-1} agar (11.89 cm, 11.95 cm) for both varieties. However, 50 g L^{-1} stabilizer (9.85 cm, 10.13 cm), 60 g L^{-1} corn flour (7.17 cm, 7.25 cm), 80 g L^{-1} Kithul flour (11.03 cm, 10.79 cm), 60 g L^{-1} gelatine (10.08 cm, 10.05 cm) and 80 g L^{-1} potato starch (4.64 cm, 4.63 cm) were also supported shoot growth in both rice varieties AT 401 and AT 402, respectively. Kithul flour has shown a greater potential as a cheaper alternative gelling agent for agar based medium according to the cost analysis.

Key words: *At401, At402,* Alternative Gelling agents, Agar, Corn flour, Gelatin, Kithul flour, Micro-propagation, Potato and Stabilizer

Introduction

The commercial use of plant tissue culture involves the production of large number of plants with minimum expenses. Due to high cost involved in governing plant tissue culture techniques, many researchers tried to find 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 when preparing tissue culture media, thus reducing the cost while successfully 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 micro-propagation. The low cost methods include washing and sterilization operations types of plantlets containers and culture media. Any low cost material selected to prepare a tissue culture medium should be able to induce shoot generation One of the factors that contribute to the efficiency of micro-propagation 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 the most expensive components as well. Various commercial products of agar, agarose, phytagel and gelrite have been tested for in vitro micro-propagation (Debergh 1983). For over 100 years, agar has being 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 conducted to find out alternative substances that have same functions as the agar and also to reduce the cost using agar substitutes in preparing tissue culture media. Naik and Sarkar (2001) used a cheaper gelling agent sago while Gebre and Sathyanarayana (2001) used commercial cassava and sago for micro propagation of potato (*Solanum tuberosum* L). Other researches have tried preparing the media by mix agar and other gelling agents at different quantity. Combination of agar with commercial corn starch and potato starch reported efficient for potato micro-propagation by nodal explants (Mohamed et al. 2009). Maliro and Lamerck (2004) had worked with cassava flour as a gelling agent and reported that the gel was improved by mixing with some agar. Zapata (2001) successfully reduced the cost of banana tissue culture by mixing corn and potato starch with gelrite as alternative gelling agent.

The aim of this study was to evaluate potential of various commercial products; agar, stabilizer, corn flour, Kithul flour, gelatine and potato as gelling agents in culture media preparation. An attempt has also been made to minimize the cost of the medium.

Materials and methods

Plant materials: All experiments were conducted at the research laboratory in the Faculty of Agriculture, University of Ruhuna. The explants used were, seeds of *rice (AT 401 and AT 402).* Firstly, seeds rinsed with distilled water and then washed using 70% (v/v) alcohol for 1 min. Then the seeds were soaked in 20% (v/v) Chlorox solution (Sodium hypochlorite) and added with 2 drops of Tween-20 for 20 minutes. Those seeds rinsed in sterile distilled water for 4-5 times. The seeds were then cultured in MS basal medium which was solidified by different gelling agents. In these experiments, seed were used as the explant.

Preparation of culture medium: The culture medium used for all experiments was based on MS medium with 30.0 g L^{-1} sucrose, and addition of different gelling agents (stabilizer, corn flour, Kithul flour, gelatine and potato and agar). No hormones were added to the medium. The medium was autoclaved for 20 min at 121°C after adjusting the pH to 5.8.

Culture condition: Seeds were inoculated in prepared culture medium. Cultures were incubated for 4 weeks in the culture room with light intensity of 1000 µmol/m^2/sec and at 25±1°C and 70-80% relative humidity with a 16/8 hrs light/dark photoperiod.

Experimental design: The lengths of the shoots were recorded after a 4 weeks period. All experiments reported here were repeated at least three times with a minimum of ten replicates. Statistical analysis was performed with the Student Newman-Kuells Means Separation Test using SAS software (Version 9.1) (SAS Institute, Cary, NC 1995).

Results and discussion

Seeds were cultured in the MS basal medium supplemented with alternative gelling agents and agar. The length of the shoot of both rice varieties (AT 401 and AT 402) when using different gelling agents is presented in the Table 1 and Figure 1. The general characteristics of the best medium stability were semi solid media which adhere to the surface of the test tube and showed individually different transparency from each alternative gelling agent.

Based on alternative gelling agents in the medium, the highest shoot length was recorded in MS basal medium containing 8g/L agar (11.8 cm, 11.9 cm) while minimum shoot length was observed in the medium with 80 g L^{-1} potato starch (4.6 cm, 4.6 cm) in both rice varieties AT 401 and AT 402, respectively. The shoot length of the rice plants in the other tested gelling agents; 50g/L stabilizer (9.8 cm, 10.1 cm), 60g/L corn flour (7.1 cm, 7.2 cm), 80g/L Kithul flour

Table 1: Cost of preparing media and shoot length of seed explants after 4 weeks, cultured in MS basal medium supplemented with different gelling agents

Treatment	Price of 1 kg gelling agent Rs.	Cost deduction compared with agar %	Concentration of gelling agent g/L	At 401 (V1)	At 402 (V2)
Agar	12,000	-	8	11.89[a]	11.95a
Stabilizer	1,200	37.5	50	9.85[c]	10.13[c]
Corn flour	600	62.5	60	7.17[d]	7.25[d]
Kithul flour	600	50	80	11.03[b]	10.79[b]
Potato	70	94.1	80	4.64[e]	4.63[e]
Gelatine	2,000		60	10.08[c]	10.05[c]

Means fallowed by the same lower case letters in each column are not significantly different at 5% level

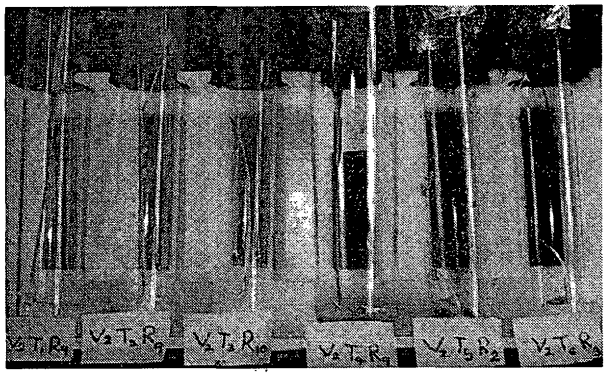

Fig 1: Shoot length of seed explants after 4 weeks cultured in MS basal medium supplemented with different gelling agents

(11.03cm, 10.7 cm) and 60g/L gelatin (10.0 cm, 10.0 cm) was also differed significantly in both rice varieties AT 401 and AT 402, respectively. The gelatin and stabilizer both supported the growth of rice shoot similarly and these two can also be considered as the third best gelling agents among the other selected gelling agents. The response on starches gelled media could be due to the absence of inhibitors which have been reported to be present in agar (Debergh 1983). Mohamed et al. (2009) reported, using 40, 50 and 60g/L starches (corn starch and potato starch) with low levels of agar (0, 1 and 2g/L of agar) for Solanum tuberosum micro-propagation, produced higher shoot regeneration than using 7g/L agar alone. Our study also showed corn flour could use as a gelling agent at 60g/L concentration. Kithul (Caryota urens) which is a native tree in Sri Lanka and the Kithul flour is available in the local market at a low price. This indicates its potential to use as a solidifying agent in the tissue culture medium while reducing the cost.

Cost analysis of gelling agents

Cost benefit ratio of various gelling agents was also calculated by comparing their price with the standard price of agar. The cost of 1kg of agar is about Rs. 12,000/ and it the most expensive gelling agent comparing to the other gelling agents tested in the study. Kithul flour alone at 80g/L in the MS basal medium resulted in a 50% cost reduction. Further, the medium solidified by Kithul flour showed the second highest shoot length of rice (table 1) seed culture and it was next to the agar.

Conclusion

The best alternative gelling agent for agar is Kithul flour (80 g/L) based on highest shoot length and largest cost reduction. 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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