Low Cost Gelling Agents for *In Vitro* Seed Germination of Radish (*Raphanus sativus* L.) Var. Beeralu Rabu

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Abstract

***In vitro* cultivation of plant tissues is generally carried out in a solid or semi-solid nutrient medium, using gelling agents. Agar is a polysaccharide extracted from sea weeds is used commonly in plant tissue culture to solidify the medium. Unreasonable price of agar and fear of over-exploitation of its resources necessitated the search for low costs materials as alternative to agar. Radish (*Raphanus sativus* L., Brassicaceae) is an important vegetable plant with varied medicinal properties. Therefore, the present study was designed to examine the potential of alternative cheap gelling agents (*corn* flour, *kithul* flour, *barley*, *saw*, *wheat* flour and *undu* flour) for seed germination of radish (*Raphanus sativus* L.) var. Beeralu Rabu. Seed germination was observed on MS basal medium supplemented with different alternative gelling agents (10%) and agar (0.8%). Plantlets weights, heights and number of germinated seeds were recorded after 4 weeks of culture. Results showed that agar and alternative gelling agents successfully produced plantlets from the seed explants of radish after 4 weeks. The highest mean height (16.89cm) of plantlets was obtained in MS basal medium with agar and highest mean weight (1.65g) of plantlets were obtained from MS medium supplemented with corn flour. Furthermore highest number of seeds (3.4) out of 5 seeds was germinated in the medium with corn flour. Mean height, weight and number of seeds germinated in MS media with corn flour were not significantly different from agar. Cost of gelling agent was reduced in 95% by using corn flour as solidification agent instead of agar. Corn flour (10% w/v) has shown a greater potential as a cheaper alternative gelling agent for agar based medium as per the cost analysis**

***Keywords**: Raphanus sativus L., Alternative gelling agents, Agar, Corn flour, Seed germination*

INTRODUCTION

Plant tissue culture provides reliable method of producing and propagating pathogen free plants. It may be also useful in providing plant material for in vitro conservation and genetic transformation studies. However, this method is more expensive than the conventional methods of plant propagation. It requires highly responsive regeneration protocol, expensive culture medium, sophisticated instruments and skilled workers. For successful establishment of tissue culture laboratories cost effective culture medium is required (Singh *et al.*, 2013). The in vitro cultivation of plant tissues is generally carried out in a solid or semi-solid nutrient medium, using gelling agents. Traditionally, agar is used, which is a polysaccharide extracted from seaweeds (Lima *et al.*, 2012). During last two decades, there has been increase in the efforts to look for suitable substitutes for agar (Singh *et al.*, 2013). Mohomad *et al.* (2009) stated that gelling agent such as agar which is usually added to increase media viscosity contributes 70% of the media costs. Various brands and grades of agar, agarose, phytagel and gelrite were used for in vitro propagation. Agar, the conventional gelling agent, has a number of drawbacks that negatively affect culture growth and differentiation in many cases (Mohomad *et al.*, 2009). Cheaper agar alternatives include various types of starch and gums, which have been investigated in commercial micro-propagation. For example gelrite can be replaced with starch gelrite mixture. Other options include white flour, laundry starch, semolina, potato starch, rice powder and sago (Mohomad *et al.*, 2009) and corn

starch, tapioca, isubgol, guar gum, xanthan gum and karaya gum (Singh *et al.*, 2013). Radish (*Raphanus sativus*) is an edible root vegetable. It is grown and consumed throughout the world. Radish has numerous varieties, sizes, color and duration of cultivation time. Radishes are an alternative treatment for a variety of ailments including whooping cough, cancer, gastric discomfort, liver problems, constipation, dyspepsia, gallbladder problems, arthritis, gallstones, kidney stones *etc.* (Singh and Singh, 2013). A low cost micro-propagation method for Radish is required to its improvement especially for local Radish varieties to used medicinal properties as well. Wheat flour (*Triticum aestivium*), corn flour (*Zea mays*), kithul flour (*Cariyota urenus*), undu flour (*Vigna mungo*), saw (*Cladium californicum*) and barley flour (*Hordeum vulgure*) is easily available in the market of Sri Lanka and have a possibility to be used as potential solidifying agents. Therefore, present study was carried out to evaluate the feasibility of various commercial products; agar, *wheat* flour, *corn* flour, *kithul* flour, *undu* flour, saw and barley flour as solidification agents to minimize the cost of gelling agents.

MATERIALS AND METHODS

Plant materials: Seeds of radish (variety: Beeralu) were purchased from the Seed and Planting Material Division, Department of Agriculture, Sri Lanka. First, seeds rinsed with distilled water and then washed using 70% (v/v) alcohol for 1 min. Thereafter, the seeds were soaked in 20% (v/v) Chlorox solution (Sodium hypochlorite) for 20 min. Those seeds were rinsed in sterile distilled water for 4-5 times. The seeds were then cultured on Murashige and Skoog (MS) basal medium, which was solidified by different gelling agents as stated earlier. The culture medium used for all the experiments was based on MS medium with 30.0 gl^{-1} sucrose (Dahanayake *et al*, 2012), and addition of 10% different gelling agents (agar - control, wheat flour, corn flour, kithul flour, undu flour, saw and barley flour) without plant growth regulators. Medium was autoclaved for 21 minutes 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, weights of the aseptically raised plants and number of seeds germinated were recorded after a 4 weeks period. All experiments reported here were repeated at least five times with five replicates. Statistical analysis was performed with the Student Newman-Kuells Means Separation Test using SAS software (version 9.1.3). The cost analysis was performed using the following formulae;

$$\text{Cost reduction compared with agar \%} = 100 - \frac{\text{Cost of Agar (1kg)}}{\text{Cost of alternative gelling agent (1kg)}} \times 100$$

RESULTS AND DISCUSSION

The lengths, weights and number of seeds germinated out of 5 seeds per bottle are presented in the Table 1 and seed germination in different gelling agents is presented in Figure 1.

Plantlets weight as affected by different gelling agents: Highest seedling mean weight (1.65g) was obtained by MS medium solidified with corn flour and lowest mean seedling weight (0.00g) was obtained by MS medium solidified with undu flour. However, it was not significantly different from seedling mean weight (1.43g), which was in agar (control). Plantlets weights of treatment 1 (agar), treatment 2 (corn flour; 1.65g), treatment 4 (barley; 0.5g), treatment 5 (wheat flour; 1.04g), and treatment 6 (kithul flour; 0.47g) were not significantly different from each other, while treatment 3 (saw; 0.03g) and treatment 7 (undu; 0.01g) significantly differed from agar and

corn flour. Furthermore, treatment 3, treatment4, treatment 5, treatment 6 and treatment 7 were not significantly different.

Seedling height as influenced by different gelling agents: MS medium solidified with agar registered highest seedling height (16.89cm), while lowest seedling height was observed in medium solidified with undu (0.00cm). Treatment 1(agar) and 2 (corn flour; 15.64cm) were at par with the control. In addition, treatment 4 (barley; 5.46cm), treatment 5 (wheat; 7.66cm) and treatment 6 (kithul; 7.16cm) were not significantly different from each other, while treatment 3 (saw; 1.56cm) and treatment 4 (barley) were also at par with each other, statistically. Similarly, treatment 3 (saw) and treatment 7 (undu; 0.00cm) were not significantly different.

Seed germination percentage: Highest numbers of seed germination out of 5 seeds (3.4/5) were observed in medium solidified with corn flour followed by agar (3.2/5). Number of germinated seeds in treatment 3 (0.4/5), treatment 4 (1.8/5), treatment5 (2.0/5) and treatment 6 (1.2/5) were not significantly different from each other; besides, treatment 3, 6 and 7 also not differing significantly with each others. No seed germination was noted in treatment 4 (undu; 0/5) (Table 1 and Figure 1).

Since agar was introduced as gelling agent more than 100 years ago, it has been extensively used for microbial and plant tissue culture media. Agar is useful for the purposes due to its stability, high clarity, nontoxic nature and resistance to its metabolism. In the recent past, several attempts have been made to look for suitable substrata that could possibly replace agar in culture medium because of doubts about its inertness and nontoxic nature, fear of over-exploration of its sources above all (Deb and Pongener, 2013).

Agar was used as a control treatment in the present study. Performance of corn flour was not significantly different from those of agar. Corn flour has been found to be the best alternative to agar among gelling agents, which we used in the present study. Similar results were observed by Henderson and Kinnersley (1988) and Daud *et al.* (2011). Growth and differentiation of plant cell cultures was increased when media were gelled with corn starch instead of agar. Dry weight of tobacco and wild carrot cell cultures on media gelled with starch was more than three times that of cultures on media gelled with agar (Kinnersley, 1988). Higher yield of anthocyanin and dry weight of embryos were found in wild carrot cultures grown on media gelled with corn starch. The starch-mediated increase in growth and differentiation of wild carrot cells was accompanied by an increase in density of the cultures shown by higher dry weight/fresh weight ratios (Henderson and Kinnersley, 1988). Furthermore the present study revealed that *corn* flour was better than agar when considering mean weight of plantlets and number of seeds germinated. Alternative gelling agents such as *wheat* flour and rice powder were better than *corn* starch (Daud *et al.*, 2011). However, *corn* starch was the best gelling agent than rice and *wheat* flour in the present study. Kithul (*Caryota urens*), which is a native tree of Sri Lanka and is easily available in the local market at a low price, was the best gelling agent for rice seed germination than corn flour (Dahanayake *et al.*, 2012). Nevertheless the present study indicated that *kithul* flour was not a suitable gelling agent as compare to corn flour. There are no reports, which show that barley, saw and *undu* were used as gelling agent for micro propagation studies. However *barley* saw and *undu* were used in the present study and those solidifying agents were not better than *corn* flour and agar.

Cost analysis of agar and corn flour: Economics of using alternative gelling agents was calculated by comparing the standard price of agar (Table 2). The cost of 1 kg of agar was about Rs. 12,000.00 Sri Lankan currency and it the most expensive gelling agent compared to the other gelling agents tested in this study. Use of corn flour, as an alternative solidifying agent, reduced 95% of expenditure made towards purchase of gelling agent *i.e.* agar.

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Figure

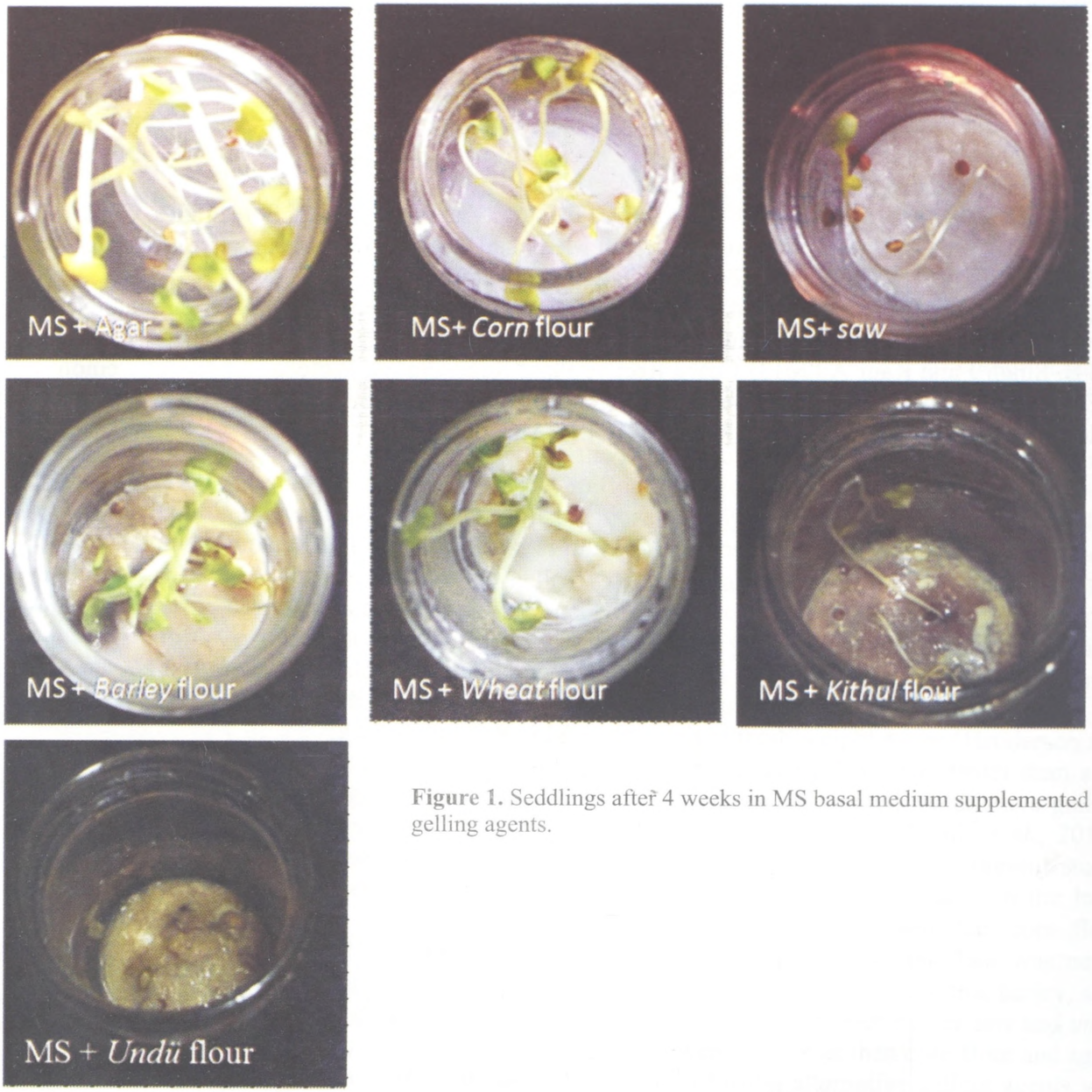

Figure 1. Seddlings after 4 weeks in MS basal medium supplemented gelling agents.

Tables

Table 1: Effect of different gelling agents on seed germination and seedling growth of radish

Treatments(Gelling agents)	Mean weight of the seedlings (g)	Mean height of the seedlings (cm)	Seed germination (%)
T1 (Agar) / Control	1.43^{a}	16.89^{a}	3.2^{a}
T2 (Cone flour)	1.65^{a}	15.64^{a}	3.4^{a}
T3 (Saw)	0.03^{b}	1.56^{cd}	0.4^{bc}
T4 (Barley flour)	0.50^{ab}	5.46^{bc}	1.8^{ab}
T5 (Wheat flour)	1.04^{ab}	7.66^{b}	2.0^{ab}
T6 (Kithul flour)	0.47^{ab}	7.16^{b}	1.2^{bc}
T7 (Undu flour)	0.01^{b}	0.00^{d}	0.0^{c}

Means fallowed by the same lower case letters in each column are not significantly different at 5% level with Duncan's Multiple Range Test

Table 2: Comparison of cost different gelling agents

Treatment	Price of 1 kg gelling agent Rs.	Concentration of gelling agent g/L	Cost deduction compared with agar %
T1 (Agar)	12,000.00	8	-
T2 (*Corn* flour)	600.00	100	95
T3 (*Saw*)	180.00	100	98
T4 (*Barley* flour)	522.00	100	95
T5 (*Wheat* flour)	105.00	100	99
T6 (*Kithul* flour)	600.00	100	95
T7 (*Undu* flour)	550.00	100	95