Proceeding of
2nd International
Conference on
Horticulture
Godavari, Lalitpur
3-4 April 2023

TECHNICAL PAPER

A Low-cost Medium for Potato Micro Propagation

Anuja Rijal*, Neela Paudel, Bihani Thapa, Kabita Kisi, Prakash Bhattarai and Giridhari Subedi
National Potato Research Program
*Corresponding author's email: anu.rij013@gmail.com
*ORCID iD: 0000-0002-6073-3770

Abstract

Plant tissue culture is a technology for the production of disease free planting materials but it is constricted by the high cost of production. A laboratory experiment was carried out to evaluate the use of Ispaghol *(Plantago ovata)* as a substitute for agar which is used as a gelling agent for the solidification of plant growth media for the in-vitro mass propagation. This study was aimed at identifying the potential use of a cheaper substitute for agar in the tissue culture media. The experiment was performed using different amount of ispaghol (10g/l, 15 g/l, 20 g/l) and agar @8 g/l as a control for the two successive seasons. One explant per tube was used in the experiment. Plant growth parameters like shoot length, number of nodes per plant and number of leaves per plant was recorded. The results showed that the production of healthy plantlets in the ispaghol gelled media @ 15g/l and @20 g/l was statistically at par with the agar solidified media. Cost of production was compared and ispaghol was found to be 11.4 times cheaper. The resembling performance of ispaghol and agar as a gelling agent in the tissue culture media for the growth of potato plantlets fully suggests that ispaghol husk has solidifying qualities like agar that ultimately could reduce the cost of production of in-vitro plantlets.

Keywords: Agar, gelling agent, ispaghol, media

Introduction

Potato (*Solanum tuberosum* L.) is an important food crop and consumed by people all over the world. Seed is the most expensive input in potato production which consists of 40-50% of production cost. In-vitro micro-propagation technique has enabled maintaining the nucleus stocks free viruses and other diseases for potato seed production. *In vitro* propagation by nodal cutting has become an established method of rapid multiplication in potatoes (Ranalli et al.,1994).Tissue culture technique is gaining popularity for the production, multiplication and maintenance of disease free potato clones. Tissue culture technology has opened a new frontier in agricultural science by addressing food security and agricultural production issues (Oggema et al.,2007) However, the high cost of production has limited the use of technology to few institutions, private sector and resourceful farmers. The expensive chemicals required for nutrient medium preparation is one factor contributing to high cost of production (Savangikar, 2004). Low cost options should lower the cost of production without compromising the quality of the micro propagules and plants (Savangikar, 2004). Agar is most frequently used as a solidifying agent in tissue culture media (Sreet, 1977). The most expensive and extensively used component of semisolid nutrient media for in-vitro propagation is agar (Pierik, 1989). The 'Ispaghol husk' derived from the seeds of (*Plantago ovata*)a stem less herb of the plantaginaceae family, is used as emollient, demulcent and laxative and is used in the treatment of dysentery and diarrhea (Khan et al., 2012). It is essential to develop the strategies to minimize the cost of in-vitro plantlets production, so that smallholder farmers can adopt the technology. In the present experiment “Ispaghol” is evaluated as a gelling agent for micro-propagation of potatoes to substitute agar, thereby to develop a cost effective technology for potato micro propagation.

Materials and Methods

The experiment was conducted at tissue culture laboratory of National Potato Research Program, Khumaltar Lalitpur during 2021 in two seasons autumn and spring. The study was carried out on potato variety "MS 42-3", in-vitro propagated plantlets produced in the laboratory of NPRP.

Basal Murashige and Skoog (1962) medium containing 0.50mg/l Gibberellic acid(GA3), 100 mg/l mio-inositol, 30g/l sucrose solidified with agar and ispaghol at PH 5.7 were used in this study. Single nodal cuttings were taken as explant from in-vitro propagated virus free plantlets and were aseptically cultured in 15X150 mm glass tubes containing 15 ml media. The cultures were kept in the incubation room at25±2°C under 16/8 (day/night) photoperiod. Ispaghol at different concentration was compared with standard agar concentration 8g/l as a solidifying agent. Ispaghol was used in three concentration of 10 g/l, 15g/l and 20g/l.

The experiment was carried out in completely randomized design (CRD) with four replications over a period of 28 days. After 7, 14, 21 and 28 days of culture, observations were recorded on plant morphological characteristics like micro-plant height (cm), number of nodes/plant, number of green leaves/plant. The recorded data was analyzed statistically using the software STAR for obtaining analysis of variance and means were separated according to the Duncan's multiple range test at 0.05 level of significance.

Result

Plant height

Potato variety MS 42-3 showed significant differences in plant height in different days after culture in both set of experiment conducted in autumn and spring season (Table 1). Plantlets cultured in media containing ispaghol @15g/l and 20g/l were found to be statistically at par with the media containing agar as a gelling agent in the first season (Table 1). Media containing ispaghol @10 g/l was not fully solidified and plant growth was not optimum like in higher concentration. In the first season, at 28 days after culture plant height was observed highest (6.55 cm) in the media containing agar as a gelling agent and use of ispaghol @15g/l and 20 g/l were found to be statistically at par with media containing agar (Table 1). In the second season, at 28 days after culture plant height was observed highest (7.76 cm) in the media containing ispaghol as a gelling agent @20g/l and use of ispaghol @15g/l and agar @ 8g/l were found to be statistically at par with media containing ispaghol @ 20g/l.

Table 1. Effect of different concentration of gelling agent on the plant height of in-vitro potato plantlets in two seasons of experiment, 2021 at NPRP

	Plant height (cm) @ days of culture							
	1st season				2nd season			
Treatments	**7**	**14**	**21**	**28**	**7**	**14**	**21**	**28**
Ispaghol (10g/l)	1.91	2.69	3.86	5.67	3.24	4.08	4.7	5.12
Ispaghol (15g/l)	2.77	3.85	4.72	6.64	4.12	5.28	5.92	7.16
Ispaghol (20g/l)	2.56	3.61	4.6	6.3	4.44	5.6	6.58	7.76
Agar(8g/l)	2.94	3.75	4.93	6.55	4.38	5.44	6.58	7.64
Grand mean	2.54	3.47	4.53	6.29	4.040	5.100	5.950	6.920
CV (%)	14.58	17.34	10.93	12.48	6.300	4.920	5.71	5.190
P-value	0.002	0.028	0.018	0.024	0.000	0.000	0.000	0.000

Number of leaf

With the increasing days of culture, leaf number per plant was also increased in all treatments (Table 2). Data related to leaf number per plant was found to be non-significant in 7, 14 and 21 days after culture in the first season (Table 2). At 28 days of culture, treatments were significantly different and the highest leaf number per plant (10.60) was recorded in the treatment of Agar @ (8g/l) followed by the treatment isabgol @ (15g/l) (10.20). Media containing ispaghol@ 10g/l showed the lowest (8.2) number of leaf/plant at 28 days after culture in the first season. Likewise, in the second season treatments were found to be significantly different at 21 and 28 days after culture. Culture media containing agar @8/l, ispaghol @ 15g/l and 20g/l were found to be statistically similar containing the higher number of leaf per plant in the second season.

Table 2. Effect of different concentration of gelling agent on the leaf number of in-vitro potato plantlets in two seasons of experiment, 2021 at NPRP

Number of leaves per plant@ days of culture								
	1st season 2nd season							
Treatments	**7**	**14**	**21**	**28**	**7**	**14**	**21**	**28**
Ispaghol (10g/l)	2.60	4.80	7.00	8.20	3.60	5.80	7.60	9.60
Ispaghol (15g/l)	4.00	6.40	8.20	10.20	4.60	7.00	10.60	14.20
Ispaghol (20g/l)	3.60	5.60	7.20	9.40	5.00	7.60	11.00	14.00
Agar(8g/l)	3.40	5.40	7.80	10.60	5.00	8.40	11.20	14.80
Grand mean	3.40	5.55	7.55	9.60	4.5500	7.2000	10.100	13.150
CV (%)	25.04	17.09	14.2	10.42	26.0000	15.9900	15.500	12.440
P-value	0.106	0.103	0.302	0.008	0.2300	0.0170	0.007	0.0004

Number of node

With the increasing days of culture, node number per plant was also increased in all treatments (Table 3). Data related to node number per plant was found to be non-significant in 7, 14 and 21 days after culture. At 28 days of culture, treatments were significantly different and the highest node number per plant (10.60) was recorded in the treatment of Agar @ (8g/l) followed by the treatment isabgol @ (15g/l) (10.20). In the second season, treatments were found to be significantly different for the number of node per plant at 14, 21 and 28 days after culture (Table 3). Highest number of node per plant (8.20) was observed in the culture media containing agar @ 8g/l followed by the media containing ispaghol @ 20g/l and 15 g/l.

Table 3. Effect of different concentration of gelling agent on the node number of in-vitro potato plantlets in two seasons of experiment, 2021 at NPRP

Number of node per plant@ days of culture								
Treatments	**1st season**				**2nd season**			
	7	**14**	**21**	**28**	**7**	**14**	**21**	**28**
Ispaghol (10g/l)	2.60	4.80	7.00	8.20	2.00	2.40	4.20	5.80
Ispaghol (15g/l)	4.00	6.20	8.00	10.20	2.60	3.00	5.00	6.80
Ispaghol (20g/l)	3.40	5.40	7.20	9.40	2.80	3.40	5.40	7.40
Agar(8g/l)	3.40	5.40	8.00	10.60	2.40	3.40	5.60	8.20
Grand mean	3.35	5.45	7.55	9.60	2.450	3.050	5.050	7.050
CV (%)	27.52	19.46	15.81	10.42	18.250	15.550	8.860	13.080
P-value	0.163	0.261	0.43	0.008	0.060	0.013	0.001	0.006

Table 4. Cost of agar and ispaghol gelled MS medium

Gelling agent	Price per 500g	Amount used per liter MS medium	Cost for preparing 1 liter medium	Cost reduction
Agar	7500	8 g	120	
Ispaghol	350	15 g	10.5	11.4 times

Discussion

Ispaghol was compared with agar as a solidifying agent in the culture media for micro propagation of potato through the method of single nodal culture. Data related to different micro plant characteristics like plant height, number of node per plant and number of green leaf per plant was recorded at 7,14,21 and 28 days after culture in two seasons of experiment. Plant growth was observed healthy in all treatments during the experiment time and full root growth in the plant was observed. The data (Table 1,2,3) showed that plantlets growth in the test-tube (plant height, number of node per plant, number of green leaf per plant) was observed lower in the media containing ispaghol @ 10g/l. Plantlets growth was observed superior in the culture media containing isbaghol@15 g/l and 20 g/l which was statistically at par with the culture media containing agar @8g/l (Table 1,2,3). The performance of

ispaghol as a solidifying agent was similar with that of agar and the plant appeared to be healthy in appearance as well. In some of the culture vessel, plantlets on ispaghol used media were observed to be more vigorous and healthier than on agar used media. Thus, the plant development on Ispaghol husk medium was as good as in the agar solidified medium and did not showed any adverse effect on shoot proliferation. From this experiment, it was observed that there was no softening of the Ispaghol gelled medium during the entire course of culture, proving that ispaghol is not metabolized during culture. The in vitro growth of plants is the result of interaction between explant and medium, which is dependent upon the gel quality (Shah et al., 2003). Scholten and Pierik (1998) reported that the gel quality of the plant growth medium showed positive correlation with gel strength. As a result, the change in the type of gelling agent and concentration of gelling agent affects the overall nutrient concentration in the medium, which influence the plant growth. Babbar and jain (1998) also reported that the response of plant growth on media gelled with `Ispaghol' was similar to that on media solidified with agar. The efficacy of 'Ispaghol husk' is entirely due to the large quantity of mucilage present in the husk. The properties of 'Isubgol husk', including its polysaccharide like and colloidal nature, reported resistance to enzymatic activity, good gelling ability even in cold water and reasonable clarity in gelled form, are indicative of its potential to become an universal gelling agent in tissue culture media (Babbar and Jain 1998).

The cost of production for one liter of MS medium using two different component as a solidifying agent was compared. The solidifying agents used for the media preparation were agar and ispaghol. The results showed that the cost of production of plantlets in micro propagation medium could be reduced with the use of ispaghol as a substitute for agar. From the present study, it was inferred that the agar can be successfully replaced by an ordinary ispaghol, which is 21 times times cheaper than the agar. The low cost alternative for agar presented in this experiment allow a low cost strategy for successful micro propagation of potato without compromising on quality of plants. 'Ispaghol' reduced price of gelling agent approximately by 11.4 times in preparation of plant tissue culture media (Table 4). There are emerging commercial tissue culture laboratories in our country. Agar has remained to be the most expensive constituent of tissue culture media, making the tissue culture technology expensive. The use of Ispaghol can reduce the cost of solidifying agent in the culture medium. With the concern of making the tissue culture technology less expensive, this study shows an economic feasibility of using ispaghol as a gelling agent in the plant tissue culture media.

Conclusion

From this experiment, it was observed that ispaghol can be used as an alternative as a gelling agent in MS media for micro propagation of potato. The successful use of alternative solidifying agent for potato tissue culture is an indication that it is possible to produce disease free plantlets at lower cost and maintain the quality as well. This will reduce the cost of plantlets which will significantly increase productivity of the crop. Ispaghol @ 15g/l and 20 g/l were found to be statistically at par with the use of agar as a solidifying agent in the culture media for plant establishment and healthy growth of leaves and root. There is huge possibility of using ispaghol in the commercial scale as a cheaper substitute for agar for plant tissue culture.

Acknowledgement

We would like to express our sincere thanks to Nepal Agricultural conflict of interest. Research Council for providing financial support and National Potato Research Program for other research facilities.

Declaration of the conflict of interest

We have no conflict of interest to disclose.

References

Babbar, S. B., & Jain, N. (1998). Isubgol'as an alternative gelling agent in plant tissue culture media. *Plant Cell Reports*, *17*, 318-322.

Chopra, R. N., & Chopra, I. C. (1994). *Indigenous drugs of India*. Academic publishers.

Khan, S., Al Maruf, A., Aktera, S., Habiba, A., Banua, T. A., & Islam, S. (2012). Admixture of Isubgol husk together with conventionally used agar as gelling agent for Potato and Stevia regeneration. *Bangladesh Journal of Scientific and Industrial Research*, *47*(2), 161-166.

Lobban, C. S., & Wynne, M. J. (Eds.). (1981). *The biology of seaweeds* (Vol. 17). Univ of California Press.

Oggema, J. N., Kinyua, M. G., Ouma, J. P., & Owuoche, J. O. (2007). Agronomic performance of locally adapted sweet potato (Ipomoea batatas (L) Lam.) cultivars derived from tissue culture regenerated plants. *African Journal of Biotechnology*, *6*(12).

Pierik, R. L. M. (1997). *In vitro culture of higher plants*. Springer science & business media.

Ranalli, P., Bassi, F., Ruaro, G., Del Re, P., Di Candilo, M., & Mandolino, G. (1994). Microtuber and minituber production and field performance compared with normal tubers. *Potato Research*, *37*, 383-391.

Savangikar, V. A. (2004, February). Role of low cost options in tissue culture. In *Low cost options for tissue culture technology in developing countries. Proceedings of a Technical Meeting organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture* (pp. 11-15).

Scholten, H. J., & Pierik, R. L. M. (1998). Agar as a gelling agent: chemical and physical analysis. *Plant Cell Reports*, *17*, 230-235.

Shah, A. H., Shah, S. H., Swati, Z. A., & Hussain, Z. (2003). Cost effective micropropagation technology for potatoes. *Pakistan Journal of Biological Sciences*, *6*(4), 336-340.

Street, H. E. (1977). Plant Tissue and Cell Culture Black Well Scientific Publication.