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Cost Effective Micropropagation Technology for Potatoes

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Abstract: Ispaghol (*Plantago ovata*) husk was evaluated as a gelling agent for their performance in solidification of media for *in vitro* micropropagation/production of virus free potatoes (*Solanum tuberosum* L.). The objective of this study was to find a cheaper substitute for agar. After pilot study 12 gl^{-1} ispaghol was selected as suitable concentration for micropropagation. The data were recorded for growth parameters i.e. number of roots and number of nodes per plantlet, shoot length and relative growth rates. The results revealed that the performance of "Ispaghol gelled media" was excellently at par with agar solidified media. Cost of "Ispaghol" per plantlet was found to be 54 times lower than agar grown plantlets. It was concluded that ispaghol husk might emerge as a cheaper alternative for agar that could lead to substantial reduction in cost of production per potato plantlet.

Key words: Agar, gelling agent, *Plantago ovata,* semisolid media, *Solanum tuberosum*

Introduction

Potato (*Solanum tuberosum* L.) is an important food crop. It is good and cheap source of carbohydrates, vitamins, minerals, and proteins. It also provides most of the trace elements which can meet the energy requirements of peoples (Malik, 1995). Potato has a wide distribution over the globe and grown successfully in Pakistan. In the year 1999-2000, it was grown on an area of 108.8 thousand ha with total production of 1616 thousand tones For the year 1999-2000 annual seed requirement for Pakistan was 248572 tones. On the other hand, the total seed provided both by public and private sector was 4320 tones, that is 1.738% of the national demand, while 2.07% of the total seed requirement (5153 tones of seed potato) was met through import. Overall, only 4% Pakistan's seed requirement is fulfilled through certified seed and rest of 96% seed requirement relay on non-certified, low quality, impure and contaminated seed (Anonymous, 1999-2000). This results the far lower national yield of potatoes than the production in developed countries. To narrow the gap between seed demand and supply and to minimize the foreign exchange spending on seed import, the sustainable strategy would be to accelerate and strengthen.

Potato is normally vegetatively propagated, contamination of seed material by pathogens (bacteria, virus and fungi) cause severe reduction in yield. That is why, despite tremendous efforts little success has been achieved in conventional seed potato production scheme. In this event plant biotechnology offers a great potential to complement conventional breeding methodology for potato improvement and production via plant tissue culture techniques (mass production of pathogen free potato material through micropropagation). But lack of budget, limited resource allocation and relatively high recurrent cost (chemical expenses) of this technology has been envisaged as a major obstacle in benefiting from this technology in developing countries particularly, in Pakistan.

The only alternative is to develop cost-effective technology for *in vitro* clonal propagation of disease free potatoes. *In vitro* propagation by nodal cutting has become an established method of rapid multiplication in potatoes (Roca *et al.*, 1978; Dodds *et al.*, 1991; Ranalli *et al.*, 1994). The most expensive and extensively used component of semisolid nutrient media for in vitro micropropagation is agar (Pierik, 1989) which is most frequently used as a solidifying agent in tissue culture media (Street, 1977). In the present study an attempt has been made to evaluate "Ispaghol" (*Plantago ovata*) as a gelling agent for micropropagation of potatoes to select a cheaper substitute for agar, thereby to develop a cost effective micropropagation technology for potatoes.

Materials and Methods

This experiment was carried out at the Institute of Biotechnology and Genetic Engineering NWFP, Agriculture University Peshawar during 2001-2002. The study was conducted on potato cultivar "cardinal", *in vitro* propagated virus free plantlets were kindly provided by Potato Research Center, Abbottabad.

Basal Murashige and Skoog (1962) medium containing 1.0 mgl^{-1} Ca-pentothenate, 0.50 mg l^{-1} Gibberellic acid (GA_3), 100 mgl^{-1} Meso-inositol, 30 g l^{-1} sucrose solidified with 6 g l^{-1} agar at pH 5.7 were used in this study. Single nodal cuttings from in vitro propagated virus free plantlets were

aseptically cultured in 15X150 mm glass tubes containing 15 ml medium. After inoculation cultures were maintained in the growth room at 21±2^{0}C under 16/8 (day/night) photoperiod with 30μ mol/m^2/s photosynthetic photon flux density provided by white cool fluorescent illumination.

In tissue culture technology explant's physiological condition is crucial for morphogenesis. To maintain the physiological uniformity of explants three epical nodes were used through the study. The shoot of aseptically grown plantlets was selected for explant material for single node culture.

Ispaghol concentration: A pilot experiment was set up to select suitable concentration of "Ispaghol" husk as gelling agent in comparison with standard agar concentration (6 g l^{-1}) for potato micropropagation. For this purpose an experiment with nine treatments of Ispaghol, 0, 6, 8, 10, 12, 14, 16, 18, 20 mg l^{-1}, each treatment had 20 replicates was conducted. 0.0 mg Ispaghol denotes standard concentration of agar i.e. 6.0 g l^{-1} agar (Martinez *et al*., 1996). The Ispaghol husk in ground form was added in medium at room temperature, vigorously shaken and dispensed quickly in tubes.

Measurement of Growth

Shoot length was measured after 3, 7, 10, 14, 17 days by the method of Martinez *et al*. (1996). After 17 days root number, number of nodes per plantlets and shoot relative growth rates (RGR) weeks^{-1} were calculated as described by Shah *et al*. (1997).

$$RGR = \ln(\text{Final length}) \ln(\text{initial length})/2 \text{ Week}^{-1}$$

Results

Selection of Ispaghol concentration: Results of pilot experiment showed that 6 and 8 g l^{-1} ispaghol had very low gelling capacity and was very difficult to culture on, after culturing all explants found either floated on the surface of the media or sunk in the media. While media supplemented with 16, 18 and 20 g l^{-1} ispaghol showed very high gelling capacity, the medium was too thick and hard to culture on. These concentrations caused repulsion to explant and almost all explants came to surface of the media after inoculation.

Media containing 10, 12 and 14 g ispaghol/l were successfully cultured and plantlets obtained were quite health in appearance. The relative growth rate of plantlets on these levels were comparable to agar supplemented medium (Fig. 1a). The graph showed that the mean relative growth rate on 12 g per liter ispaghol was closer to the growth obtained on standard medium. The culturing on

Table 1: The effect of gelling agent on root and node number per plantlet of potato cv. Cardinal (Mean values of pooled data from both experiments ± standard error in parenthesis)

		No. of roots per plantlet	No. of nodes per plantlet
Gelling Agent	n	Mean	Mean
Agar	100	3.67 (0.809)	4.72 (1.396)
Ispaghol husk	100	3.77 (0.724)	4.78 (1.223)

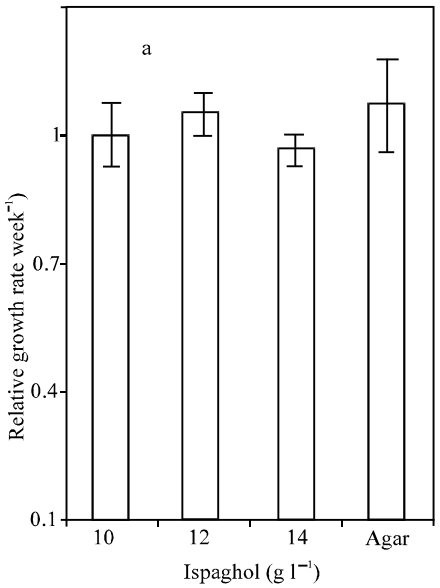

Fig. 1a: Relative growth rates of potatoes' plantlets on media solidified with 10, 12 and 14 gl^{-1} Ispaghol's husk respectively. The data are presented as mean ± SE

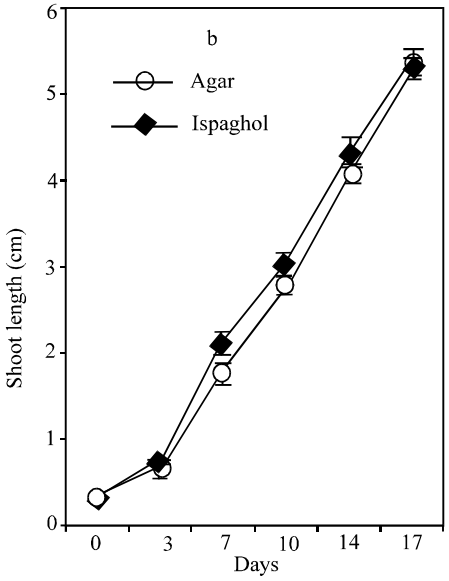

Fig. 1b: Growth curves (expressed as cm increase in shoot length) of Potato plantlets on agar and Ispaghol solidified media. The data presented are mean values of 100 replicate ± standard errors

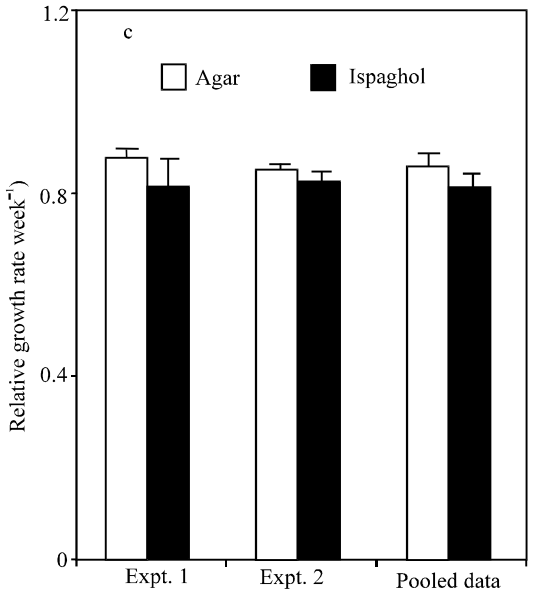

Fig. 1c: Relative growth rates of potato plantlets on agar and Ispaghol solidified media. Experiment 1 and 2 each had 50 replicates for each treatment

Fig. 2 a: Plantlet of potato cultivar cardinal on ispaghol solidified medium
b: Plantlet of potato cultivar cardinal on agar solidified medium

this concentration was easier and better than on rest of the concentrations. Based on these results 12 g ispaghol was selected for further study.

After selection of ispaghol concentrations, two set of large-scale experiments were conducted to evaluate Ispaghol-supplemented medium in comparison with standard medium. Each set had two treatments (i.e. agar and Ispaghol supplemented medium) with 50 replicates each.

Number of roots/plantlet: The data were recorded on 17th day of culturing. The average number of roots/plantlet from pooled data of both experiment were 3.67 for agar gelled medium and 3.77 for ispaghol supplemented medium, respectively (Table 1). Statistical analysis showed that gelling agent had non-significant effect on number of root per plantlet.

Number of nodes/plant: The average number of nodes/plant in agar solidified medium were 4.72 and 4.78 for ispaghol added medium (Table 1). Statistical analysis of the data revealed non-significance difference between gelling agent in production of nodes.

Shoot length: The data pertaining to shoot growth from both experiments as measured by shoot length in centimeters is presented in Fig. 1b and Fig. 2a,b, respectively. It is evident from the figures that shoot growth was similar in both gelling agents as both growth curves run parallel to each other exhibiting a typical growth curve. When the pooled data from both experiments were expressed on relative growth rate basis (Fig. 1c) again the growth response of plantlets on both media were found very similar showing a non-significant difference.

Discussion

The comparative efficacy of gelling agents like starches from various sources such as barley, corn, potato, rice and wheat, synthetic polymers (polyacrylamide pellets), gelrite in comparison with agar on medium solidification for *in vitro* culture of plants/calluses have been widely studied but agar proved to be the best (Sorvari, 1986a,b,c; Pierik, 1989).

The evaluation of 'ispaghol's husk' as gelling agent was studied for in vitro micropropagation of potato from single node cuttings for which semisolid media are commonly used. The data (Table 1, Fig. 1) showed that plantlets growth (number of roots per plant, number of nodes per plantlet and shoot growth) and plant health in both media was at par. The similarity in performance of gelling

property of ispaghol with that of agar was not only for growth but plantlet morphology and their appearance was also similar in both media (Fig. 2a,b). Even in some replicates plantlets on 'Ispaghol' solidified media were more vigorous and healthier in appearance than on agar solidified media. The in vitro growth of plants is the result of interaction between explant and medium, which is dependent upon the gel quality. The gel quality interns showed positive correlation with gel strength (Scholten and Pierik, 1998). Consequently, a change in the gelling agent or concentration of gelling agent affects the nutrients present in it as well as overall nutrient concentration in the medium, ultimately growth is affected. Lobban and Wynne (1981) have reported that the gel quality of agar is due to its colloidal and polysaccharidic nature and greatly influenced by the inorganic components of agar and the interaction of agar-medium-tissue. Low sulphate and Cl contents have been proved a good parameter for the purity of agar. Inorganic components of agar and their interaction with explant are reported to be responsible for growth disorders (Scholten and Pierik, 1998). 'Ispaghol' has large quantity of mucilage and is used as laxative, and in the treatment of dysentery and diarrhea (normally recommended by local Hakeems/Homeo Doctors) and remain practically unaffected by the digestive enzymes and bacteria (Chopra *et al*., 1958). Laidlaw and Percival (1950) reported that 'ispaghol's ' mucilage is colloidal and polysaccharidic in nature like agar.

The wide use of agar as a gelling agent has been attributed to its stability, high clarity, resistant to metabolism (not digested by plant enzymes and remain stable at all feasible incubation temperatures), limited diffusion of medium components (do not react with media constituents) and water (McLachlan, 1985; Henderson and Kinnersly, 1988 ; Romberger and Tabor, 1971) and gel strength (Debergh, 1983). During this study ispaghol gelled media remained stable and did not show any softening through out culture period, which indicated that ispaghol did not metabolize during culture.

The almost exclusive use of agar has resulted exorbitant price of tissue culture grade agar. In terms of cost 'Ispaghol' is 54 times cheaper than agar in Pakistani currency, we can easily save Rs.1.5 per plantlet for potatoes micropropagation if ispaghol was used as solidifying agent for semisolid media. For commercial purpose it might be a tremendous reduction in cost of production per plantlet.

The clearly parallel performance of ispaghol with that of agar for medium solidification for micropropagation of potatoes with out any adverse effect on plantlet development and health strongly suggests that 'Ispaghol's husk' possesses gelling qualities (strength) like agar. There is every possibility of ispaghol becoming a universal gelling agent and a cheaper substitute for agar for micropropagation.

References

Anonymous, 1999-00. Government of Pakistan Ministry of Food, Agriculture and Live Stock, Agriculture and Live Stock Division (Economic Wing), Islamabad, Pakistan.

Chopra, R.N., L.C. Chopra, K. L.Handa and L. D. Kapur, 1958. Chopra's indiginous drugs of India. UN Dhurand Sons Pvt, Calcutta.

Debergh, P.C., 1983. Effects of agar brand and concentration on the tissue culture medium. Physiol. Plant, 59: 270-276.

Dodds, J.H., Z. Huaman and R. Lizarraga, 1991. Potato germplasm conservation. In: J.H. Doods (ed). Methods for conservation of genetic resources. Chapman and Hall, Laondon, pp: 93-109.

Henderson, W.E. and A.M. Kinnersly, 1988. Corn starch as an alternative gelling agent for plant tissue culture. Plant Cell Tissue Organ Culture, pp: 15: 17-22.

Laidlaw, R.A. and E.G.V. Percival, 1950. Studies of seed mucilage. Part V. examination of polysaccharide extracted from the seeds of *Plantago ovata* Fork. By hot water. J. Chem. Soc., pp: 528-537.

Lobban, C.S. and M.J. Wynne, 1981. The biology of sea weeds. Bot. Monograh Blackwell.

Malik, N.J., 1995. Potatoes in Pakistan. Word Mate, Islamabad, Pakistan.

Martinez, C.A., M. Maestri and E.G. Lani, 1996. *In vitro* salt tolerance and proline accumulation in Andean potato (*Solanum* sp.) differing in frost resistance. Plant Sci., 116: 177-184.

McLachlan, J., 1985. Macroalgea (sea weed): Industrial sucrose and their utilization. Plant Soil, 89: 137-157.

Murashige,T. and F. Skoog, 1962. A revised medium for rapid growth and bio assay with tobacco tissue culture. Physiol. Plant, 15: 473-497.

Pierik, R.L.M., 1989. *In vitro* culture of higher plant. Martinus Nijhoff Publishers, Bostan, Lancaster, Dordrecht.

Ranalli, P., B.G. Ruaro, P. Delre, M. Dicandilo and G. Mandolino, 1994. Microtuber and mini tuber production and field performance compared with normal tubers. Potato Res., 37: 383-391.

Roca, W., M.R. Roca and J.E. Bryan, 1978. A tissue culture method for the rapid propagation of potatoes. Am. Potato J., 55: 691-701.

Romberger, J.A. and C.A. Tabor, 1971. The *Picea abies* shoot meristem in culture. I Agar and autoclaving effects. Am. J. Bot., 58: 131-140.

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

Shah, S.H., S. Tobita and S. Yashima, 1997. NaCl stress in rice seedlings: The interaction of sodium and calcium chlorides on relative growth rates, ion relation and proline accumulation. Jpn. J. Crop Sci., 66: 90-91.

Sorvari, S., 1986a. The effect of starch gelatinized nutrient media in barley anther culture. Ann. Agric. Fenn, 25: 127-133.

Sorvari, S., 1986b. Differentiation of potato tuber discs in barley starch gelatinized nutrient media. Ann. Agric. Fenn, 25: 135-138.

Sorvari, S., 1986c. Comparison of anther culture of barley cultivars in barley starch and agar gelatinized media. Ann. Agric. Fenn, 25: 249-254.

Street, H. E., 1977. Plant Tissue and Cell Culture. Blackwell Sci. Publ. water. J. Chem. Soc., pp: 528-537.