BRIEF COMMUNICATION

Sago: an alternative cheap gelling agent for potato *in vitro* culture

P.S. NAIK and D. SARKAR

Division of Crop Improvement, Central Potato Research Institute, Shimla-171 001, Himachal Pradesh, India

Abstract

Sago, a processed (gelatinized) edible starch, was successfully used as a gelling agent in culture medium. The efficacy of sago-gelled (80 g dm^{-3}) medium was studied in ten potato (*Solanum tuberosum* L.) genotypes during micropropagation and minimal growth conservation. Sago starch provided a firm gelling surface throughout the entire culture period, and fostered optimum plantlet growth in terms of shoot height, number of nodes per plant, number of leaves and fresh mass. No softening of the sago-gelled medium occurred over prolonged (six months) storage. The study showed that sago starch could be used as a substitute to agar in culture medium to substantially reduce the medium cost.

Additional key words: cassava starch, *in vitro* conservation, *Mannihot esculenta*, micropropagation, *Solanum tuberosum*, tissue culture.

Agar is most frequently used solidifying agent in plant tissue culture media. It is commercially extracted from species of red algae genera *Gelidium*, *Gracillaria* and *Pterocladia* (McLachlan 1985). The main reasons for its wide use are its stability, high clarity, non-toxicity and metabolic inactiveness (McLachlan 1985). However, the cost of agar is high. In addition, some investigators have raised doubts about the non-toxic nature of agar (Kohlenbach and Wernicke 1983, Arnold and Ericksson 1984). In view of these, since the late 1980s, attempts have been made to find cheap alternative gelling agents. Starches from various sources such as barley, maize, potato, rice and wheat have been used with varying degrees of success (Sorvari 1986a,b,c, Henderson and Kinnersley 1988, Tiwari and Rahimbaev 1992, Zimmerman *et al.* 1995). Recently, *Isubgol*, the husk derived from the seeds of *Plantago ovata* has been used (Babbar and Jain 1998).

Sago, a processed (gelatinized) edible starch available in the form of small globules, pearls or flakes is valued as food for invalids and infants. The sago was originally manufactured from sago palms (*Metroxylon* spp.) in Malaysia and Indonesia. However, cassava (*Manihot esculenta*) and potato (*Solanum tuberosum*) starches are now the major sources of sago. It is produced from cassava in Malaysia, India, Indonesia and Thailand, and from potato and sweet potato in Germany and Japan. The sago produced from grains like jowar, maize, rice and wheat yields an opaque product, whereas sago made from roots and tubers yields a translucent mass. Besides starch, sago contains small amounts of sugars, fibre, protein, calcium and other minerals (Anonymous 1971).

In potato *in vitro* culture (using commercial grade saccharose), agar makes up approximately 80 % of the total medium cost. Identification of a cheap alternative gelling agent will greatly reduce the cost of production, especially in large-scale commercial potato micropropagation. One main reason of the limited use of starch as a gelling agent is perhaps due to its tendency to be metabolized, which results in a gradual decrease in the consistency of the medium during the course of culture period. Therefore, in the present investigation, the efficacy of sago as a gelling agent was also studied during prolonged maintenance of potato plantlets *in vitro*.

Received 10 May 2000, *accepted* 12 September 2000.
Acknowledgements: We thank Dr. G.S. Shekhawat, Director, Central Potato Research Institute and Dr. S.K. Pandey, Head, Division of Crop Improvement for providing facilities. Photographic assistance from S.K. Dey is much appreciated. Comments and suggestions on the manuscript from an anonymous reviewer are gratefully acknowledged.
Fax: (+91) 177 224460, e-mail: naikps@excite.com

Ten potato (*Solanum tuberosum* L.) genotypes were used in the present study. CP-2270, I-1035, Kufri Badshah, Kufri Jyoti, Kufri Neela belonged to *S. tuberosum* ssp. *tuberosum*, CUP-199, Huachapa and Imilla Blanca to *S. tuberosum* ssp. *andigena*, and Kufri Lalima and Kufri Sindhuri were hybrids of *S. tuberosum* ssp. *andigena* and *S. tuberosum* ssp. *tuberosum*. These genotypes were selected because of their contrasting response to both micropropagation and *in vitro* minimal growth conservation. Disease-free plantlets of these genotypes were maintained and multiplied through shoot cuttings following the method previously described (Sarkar *et al.* 1997).

The experiment was conducted with single node cuttings (SNCs) dissected from aseptically grown plants. Three SNCs were cultured per tube (25 × 150 mm) containing 13 cm^3 of Murashige and Skoog (1962) medium supplemented with 8.39 μM D-calcium pantothenate, 0.29 μM gibberellic acid (GA_3), 0.054 μM 1-naphthaleneacetic acid (NAA) and 30 g dm^{-3} saccharose. The medium was solidified with 80 g dm^{-3} sago (cassava sago globules finely ground in a grinder). Agar medium (8 g dm^{-3}) was used as the control. Culture tubes were closed with polypropylene closures. The cultures were maintained under a 16-h photoperiod (irradiance of 40 μmol m^{-2} s^{-1}) at temperature of 24 ± 1 °C.

For minimal growth conservation of microplants, three SNCs were cultured per tube (25 × 150 mm) containing 15 cm^3 of MS medium supplemented with 20 g dm^{-3} mannitol and 40 g dm^{-3} saccharose. The medium was solidified with 80 g dm^{-3} sago, and agar (8 g dm^{-3}) medium was used as the control. Culture tubes were closed with polypropylene caps, and sealed with *Parafilm M*™ (*American National Can*, Neenah, USA). The cultures were preconditioned for 14 d (Sarkar and Naik 1998) before transfer to a walk-in-chamber, and incubated under a 16-h photoperiod (irradiance of 20 μmol m^{-2} s^{-1}) at temperature of 6 ± 1 °C.

Shoot height, number of nodes per plant, number of leaves per plant and plant fresh mass were recorded after 30 d and six months of micropropagation and *in vitro* storage, respectively. The experiment was laid out in a factorial (10 × 2) completely randomized design with six replicate culture tubes per treatment, and replicated twice. The homogeneity of error variance was tested using the *F*-test, and the *ANOVAs* were pooled over time using the standard procedure (Gomez and Gomez 1984).

In a preliminary experiment, different concentrations of sago (50, 60, 70, 80, 90 and 100 g dm^{-3}) were supplemented in the media to test their gelling strength. Sago starch provided a firm gelling surface throughout when used at a concentration of 80 g dm^{-3}. No softening of sago-gelled media occurred during the entire course of short-term micropropagation and also long-term storage of potato plants.

The analyses of variance showed that except for number of nodes per plant, gelling agent did not significantly ($P \leq 0.05$) affect potato microplant growth during micropropagation. Variations due to genotypic differences were significant ($P \leq 0.05$) for all the growth parameters studied. Genotype × gelling agent interaction was significant ($P \leq 0.05$) only for microshoot height, suggesting that the effect of gelling agent on microshoot height was not consistent over the genotypes tested. In cvs CP-2270, CUP-199, Kufri Badshah, Kufri Lalima, Kufri Neela and Kufri Sindhuri, no significant differences in growth parameters were observed between the microplants grown in sago and agar media (Table 1). The microplants of cv. I-1035 gave significantly ($P \leq 0.05$) higher shoot height, number of nodes per plant and fresh mass in sago medium. Similarly, sago medium fostered higher number of nodes and leaves per plant in cv. Kufri Jyoti, and higher plant fresh mass in cv. Huachapa. On the contrary, in cv. Imilla Blanca, higher shoot height and fresh mass were observed when the microplants were cultured on agar medium. Microplants cultured on sago medium did not show any growth abnormalities, and there was no adverse effect on rooting (Fig. 1*A*).

Table 1. Effect of gelling agent on potato micropropagation.

Genotype	Shoot height [mm]		Number of nodes [plant^{-1}]		Number of leaves [plant^{-1}]		Fresh mass [mg]	
	sago	agar	sago	agar	sago	agar	sago	agar
CP-2270	68.4	82.1	8.0	9.5	8.9	9.5	81.38	80.73
CUP-199	45.6	56.9	7.0	6.6	7.6	7.5	94.02	108.72
Huachapa	82.2	75.1	8.4	7.9	9.3	8.7	177.83	122.05
I-1035	69.0	31.1	8.9	6.9	10.6	8.6	162.17	102.07
Imilla Blanca	94.8	119.4	9.7	10.9	10.4	12.0	144.40	193.40
Kufri Badshah	99.3	83.2	10.0	8.5	10.8	9.0	118.93	93.50
Kufri Jyoti	69.9	66.1	10.2	8.0	11.2	9.0	87.78	93.57
Kufri Lalima	66.4	62.4	7.3	7.1	7.6	7.6	88.93	83.35
Kufri Neela	75.5	75.7	8.3	7.0	8.6	8.2	86.17	95.17
Kufri Sindhuri	74.7	70.9	6.8	6.3	7.7	7.0	92.62	91.28
$LSD_{0.05}$	18.3		1.9		2.1		42.51	

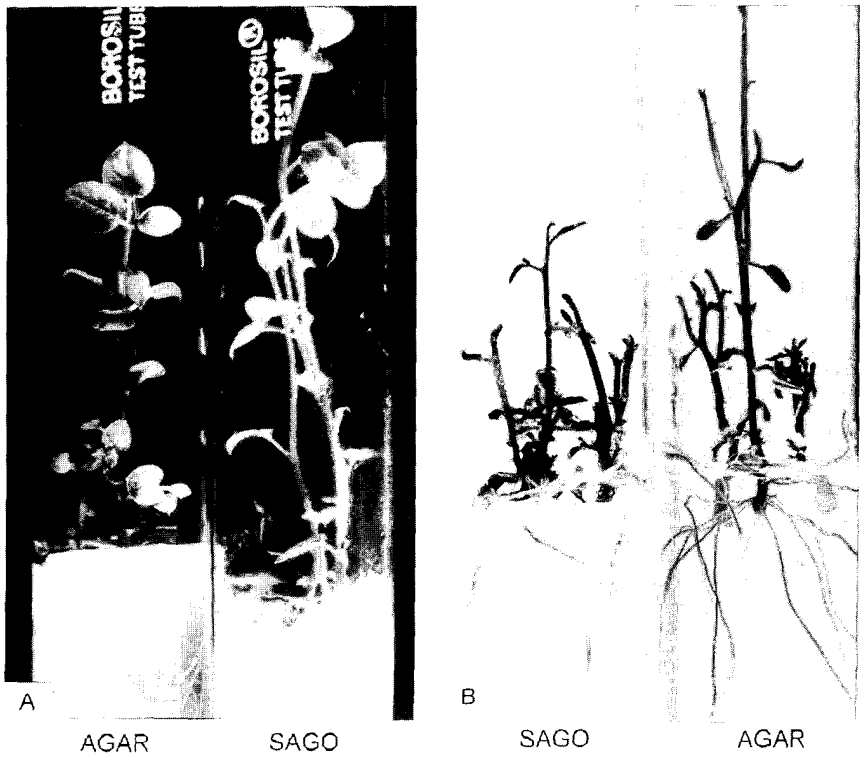

Fig. 1. Effect of gelling agent on potato micropropagation and minimal growth conservation of microplants in cv. I-1035. *A* -microplants after 30-d incubation on agar- (*left*) and sago- (*right*) gelled MS media supplemented with 8.39 μM D-calcium pantothenate, 0.29 μM GA_3, 0.054 μM NAA and 30 g dm^{-3} saccharose. *B* - microplants after 6-month conservation in sago- (*left*) and agar- (*right*) gelled minimal growth media supplemented with 20 g dm^{-3} mannitol and 40 g dm^{-3} sucrose.

Table 2. Effect of gelling agent on minimal growth conservation of potato microplants.

Genotype	Shoot height [mm]		Number of nodes [plant^{-1}]		Number of leaves [plant^{-1}]		Fresh mass [mg]	
	sago	agar	sago	agar	sago	agar	sago	agar
CP-2270	36.3	33.9	18.0	17.3	19.5	18.7	96.32	93.65
CUP-199	50.7	47.2	12.4	9.9	7.8	8.3	99.05	111.12
Huachapa	24.7	31.2	8.5	9.6	6.7	8.5	115.43	98.68
I-1035	32.7	30.5	21.0	18.5	22.9	20.6	122.05	109.02
Imilla Blanca	61.2	54.1	15.5	17.5	17.0	17.2	195.05	140.00
Kufri Badshah	52.9	48.3	14.1	12.5	15.9	14.7	103.52	235.17
Kufri Jyoti	35.9	34.4	15.2	13.4	16.5	14.8	85.33	75.55
Kufri Lalima	45.7	46.1	13.1	11.2	14.0	12.1	157.95	136.27
Kufri Neela	33.3	35.0	6.9	10.6	8.8	11.6	149.27	153.90
Kufri Sindhuri	30.3	36.3	10.5	13.6	11.9	15.0	115.27	137.22
$LSD_{0.05}$	8.3		2.8		2.9		49.50	

For storage *in vitro*, the analyses of variance showed no significant ($P \leq 0.05$) effect of gelling agent on potato plantlet growth. Variations due to genotypic differences were significant ($P \leq 0.05$) for all the growth parameters, except for fresh mass. Genotype × gelling agent interaction was significant ($P \leq 0.05$) only for number of nodes per microplant, suggesting that the effect of gelling agent on number of nodes per plant was not consistent over the genotypes tested. In cultivars Kufri Neela and Kufri Sindhuri, number of nodes and leaves per plant increased significantly ($P \leq 0.05$) in the control agar medium. However, in other cultivars, the mean values of

these two growth parameters were at par in both the media. In cv. Imilla Blanca, a significant ($P \leq 0.05$) increase in fresh mass occurred when the plants were conserved in sago medium. Microplant fresh mass decreased in sago medium in cv. Kufri Badshah. The microplants conserved in sago medium were morphologically identical to those conserved in agar medium (Fig. 1*B*).

The present study showed that sago-gelled medium could safely be used for potato microplant culture. Sago starch did not show any detrimental effect on potato growth or rooting during short-term micropropagation as well as long-term storage. Even in some genotypes, during micropropagation, the beneficial effect of sago on microplant growth was in terms of number of nodes per plant. Higher rate of node formation in sago medium might increase the sub-culturing efficiency. Although starches from various other sources like barley, corn, potato, rice and wheat were used as alternative gelling agents in plant tissue culture media, a major limitation of using starch as a gelling agent was that the starch-gelled medium was weakly solidified. Therefore, starches were used either in combination with other solidifying agents like agar, agarose, gelrite, *etc.* (Henderson and Kinnerseley 1988, Tiwari and Rahimbaev 1992, Zimmerman *et al.* 1995) or in the presence of some physical support like polyester nets to prevent the submersion of tissues into the medium (Sorvari 1986a,b,c). However, in the present study, sago starch provided a firm gelling surface throughout, and gave adequate support to growing potato microplants during the entire course of culture period. In contrast to micropropagation medium, storage medium is osmotically enriched with high concentrations of saccharose (40 g dm^{-3}) plus mannitol (20 g dm^{-3}). Starches, upon autoclaving, yield sugars that may further increase the osmotic potential of the storage medium. This may perhaps be responsible for growth reduction in sago medium over prolonged storage of potato plantlets *in vitro*, because the water availability to the growing cultures is reduced (Thorpe 1979). This is beneficial where the primary objective is to retard the plant growth to prolong the subcultural intervals.

The cost of tissue culture grade agar is approximately USD 130 per kg whereas that of the sago starch is USD 0.36 per kg. Thus agar-gelled medium at 8 g dm^{-3} concentration is 36 times more costly than sago-gelled medium at 80 g dm^{-3}. The use of sago starch as a gelling agent in semisolid culture medium will, therefore, substantially reduce the cost of production of potato microplants *vis-a-vis* microtubers.

References

Anonymous: The Wealth of India - A Dictionary of Indian Raw Materials and Industrial Products. Vol. 8. - Council of Scientific & Industrial Research, New Delhi 1971.

Arnold, S.V., Ericksson, T.: Effect of agar concentration on growth and anatomy of adventitious shoots of *Picea abies* (L.) Karst. - Plant Cell Tissue Organ Cult. **3**: 257-264, 1984.

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

Gomez, K.A., Gomez, A.A.: Statistical Procedures for Agricultural Research. - John Wiley & Sons, New York 1984.

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

Kohlenbach, H.W., Wernicke, W.: Investigations on the inhibitory effect of agar and the function of active carbon in anther culture. - Z. Pflanzenphysiol. **86**: 463-472, 1978.

McLachlan, J.: Macroalgae (sea weeds): industrial sources and their utilization. - Plant Soil **89**: 137-157, 1985.

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

Sarkar, D., Naik, P.S.: Factors affecting minimal growth conservation of potato microplants *in vitro*. - Euphytica **102**: 275-280, 1998.

Sarkar, D., Chandra, R., Naik, P.S.: Effect of inoculation density on potato micropropagation. - Plant Cell Tissue Organ Cult. **48**: 63-66, 1997.

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

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

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

Thorpe, T.A.: Regulation of organogenesis *in vitro*. - In: Hughes, K.W., Henke, R., Constantin, M. (ed.): Propagation of Higher Plants Through Tissue Culture: A Bridge Between Research and Application. Pp. 87-101. United States Technical and Information Service, Virginia 1979.

Tiwari, S., Rahimbaev, I.: Effect of barley starch in comparison and in combination with agar and agarose on anther culture of *Hordeum vulgare* L. - Curr. Sci. **62**: 430-432, 1992.

Zimmerman, R.H., Bhardwaj, S.V., Fordham, I.: Use of starch gelled medium for tissue culture of some fruit crops. - Plant Cell Tissue Organ Cult. **43**: 207-213, 1995.