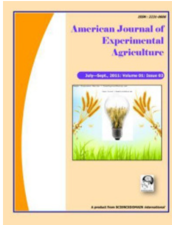
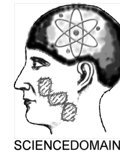


American Journal of Experimental Agriculture
2(1): 81-89, 2012

SCIENCEDOMAIN *international*
www.sciencedomain.org



Comparing Liquid and Solid Media on the Growth of Plantlets from Three Kenyan Potato Cultivars

Miriam Mbiyu[1], Jane Muthoni[1*], Jackson Kabira[1], Christine Muchira[1], Patrick Pwaipwai[1], Joseph Ngaruiya[1], John Onditi[1] and Susan Otieno[1]

[1]*Kenya Agricultural Research Institute (KARI), National Potato Research Centre, Tigoni, Kenya.*

Research Article

Received 2nd August 2011
Accepted 2nd October 2011
Online Ready 21st December 2011

ABSTRACT

Tissue culture has been used to produce high quality and clean planting material. In addition to viral elimination, tissue culture offers other advantages such as rapid multiplication of seed stock for basic seed production within a short period of time. The method is also useful for germplasm conservation. Although, *in-vitro* multiplication of potato was started 40 years ago and is promising, extensive use in developing countries has been limited by the high costs of media. There is thus need to explore cheaper alternatives without compromising on quality of *in -vitro* plantlets. A study was carried out to compare the effects of the liquid medium and solid medium on performance of three popular Kenyan potato cultivars i.e. Dutch, Kenya Sifa and Tigoni. Liquid medium consisted of Murashige and Skoog medium supplemented with normal vitamins and sucrose while solid medium consisted of the same chemicals into which phytagel were added to solidify the medium. Data taken were number of roots, nodes and leaves per plantlet over time. From the results, the liquid medium gave more roots, more nodes and more leaves per plantlet than the solid medium. The results therefore indicated that liquid media have a significant effect on the plant growth. In addition, liquid medium was found to be cheaper than solid media by USD 1.65. It appears more economical to use liquid media than solid media for *in vitro* micro-propagation of potato. However, the experiment needs to be repeated especially with different potato cultivars so as to come up with useful recommendations.

Keywords: Liquid medium; potato; in-vitro; plantlets.

**Corresponding author: Email: jayney480@yahoo.com;*

1. INTRODUCTION

Potato (*Solanum tuberosum* L.) is the fourth most important food crop in the world after rice, wheat and maize (FAO, 2008; Nyende *et al.,* 2005). In Kenya, the crop is an important food and cash crop, second after maize in production (MoA, 2008). Potato is grown by about 500 000 farmers, on 120 000 hectares per season, with an annual production of about 1 million tonnes in two growing seasons (MoA, 2008). Annual production of the crop is estimated to be about Ksh. 5 billion at farm gate level and 10 billion at the customer level, potato industry employs thousands as market agents, transporters, processors and vendors (MoA, 2008). Although potato yields of 40 t ha^{-1} have been recorded under research conditions, yields on farmers' fields range from 4.4 to 10 t ha^{-1} with an average of 6.7 t ha^{-1} (Lung'aho *et al.,* 1997; MoA, 2008). Low yields in farmer fields is due to many production constraints such as low soil fertility, lack of good quality seeds and diseases such late blight, bacterial wilt and viruses (Jane and Nyamongo, 2009). Lack of clean certified seeds is the most important production constraint with less than 1% of potato farmers in Kenya having access to good quality seeds (Jane and Nyamongo, 2009). This forces the farmers to get seeds from informal sources (farm-saved, local markets or neighbors) leading to a buildup of diseases and viruses (Jane *et al.,* 2010; Nyende *et al*., 2005).

Kenya Agricultural Research Institute (KARI), Tigoni has the mandate of producing basic seed. Production of disease-free in vitro plants is initiated through tissue culture technique using meristem tip culture. These high quality starting materials are produced year round in *in-vitro* conditions (micro-tubers, plantlets) at a high density. Depending on the potato cultivar 5 to 10-fold multiplications are achieved in four weeks time. In addition to viral elimination, tissue culture offers other advantages such as rapid multiplication of seed stock for basic seed production within a short period of time. The method is also useful in germplasm conservation (Jane *et al.,* 2010).

Although, *in-vitro* multiplication of potato was started 40 years ago and is promising, extensive use has been limited by the high costs of media especially in developing countries (Coleman *et al.,* 2001). Therefore, tissue culture laboratory must be cost effective and production of low cost high value plantlets should be an ultimate objective which should be achieved by appropriate choice of media components. Prakash (1993) classified *in-vitro* culture media into two groups; liquid and solid media. Solid media is prepared from adding a solidifying agent into the liquid media. There are many brands and grades of agar such as agarose, phytagel and gerlite, which are used as solidifying agents (Deberg, 1983; Prakash *et al,* 2000). These are usually added into liquid media to increase viscosity and ensure that explants remain in an upright position; these agents contribute 70% of the total production costs (Mohamed, 2009; Prakash 1993).

The *in-vitro* development is dependent on the explant and the medium interaction. Agar, the conventional gelling agent has been reported to have a number of drawbacks that negatively affect culture growth and differentiation. This is because the slow uptake of nutrients in the solid medium may be leading in lower nutrient availability to the plants and hence a reduction in growth rate (Scholten and Pierik, 1998). Debergh (1983) and George (1993) reported that an agar-solidified medium has lower water availability and uptake by the plants than liquid medium. This lower uptake of nutrients could explain the lower rate of development of plantlets in solid media compared to liquid media (Kuria *et al*., 2008). However, use of phytagel has not been widely reported.

The objective of this study was to evaluate the effect of liquid and solid medium on the growth of three different potato cultivars in Kenya.

2. MATERIALS AND METHODS

The study was carried out in the plant tissue culture laboratory at the National Potato Research Centre, Tigoni between February and March 2010 (first trial) and May to July 2010 (second trial). The experiment was set up as a factorial arrangement in a randomized complete block design (RCBD) with three replications. Each block consisted of six treatment combinations. Factor one was the media i.e. liquid medium (without phytagel) and solid medium (with phytagel) while factor two was the potato cultivars i.e. Dutch Robyjn, Kenya Sifa and Tigoni. Phytagel is the gelling agent that thickens the liquid medium into solid form and hence helps hold the plantlets into an upright position. Within each block, each treatment combination consisted of five test tubes.

2.1 Preparation of the Plant Materials

Ten tubers of each of the three popular Kenyan potato cultivars i.e. Tigoni, Dutch Robyjin, and Kenya Sifa were potted, three weeks after potting; the plants were transferred to thermotherapy chamber for heat treatment for one month as described by Espinoza (1984). The thermotherapy room was maintained at a constant temperature of $36^{0}C \pm 2$ and photoperiod of 16\8 during the entire period; the high temperature increased the rate of production of virus-free materials. Thereafter, 3-4 nodes from the apical end of the sprouted plants were excised and placed for 10 minutes in 5% hypochlorite solution and then rinsed 3-4 times with distilled water. The meristems were then dissected from each node under a microscope and each meristem tip was carefully transferred into the culture media in the test tube prepared as described by Murashige and Skoog (1962). Twenty five meristem tips of each variety were cultured, they were then incubated in a growth chamber under 16 hrs of light and 8 hrs of dark at 22 ± 2 ℃. After 8 weeks the meristems developed into plantlets, which were then further multiplied through the *in-vitro* nodal cutting system. In this system, the individual plantlets were cut into single nodes measuring 4 millimeters which were placed on culture media. The nodes sprouted and developed into new plantlets over a period of 4 weeks; these plantlets were used for setting up the experiment.

2.2 Preparation of Nutrient Medium

Two nutrient medium were prepared; liquid medium [without gelling agent, phytagel)] and solid medium (with phytagel). The two medium consisted of the Murashige and Skoog (1962) (MS) basal salts supplemented with glycine 0. 2 g /L, nicotinic acid 0.05 g/ L, pyridoxine 0.05 g/L, inositol 10g/l thiamine 0.01 g /L, gibberellic acid 0.001 g/L, and sucrose 30 g /L . The pH of the medium was adjusted to 5.8 using 1 N NaOH or 0.1 M HCL. Two and a half grams of phytagel was added to one litre of media as a solidifying agent. Ten millilitres of culture medium was dispensed into 50 millilitres test tubes (Pyrex Co.) and covered using aluminium foil and then autoclaved at $121^{0}C$ for 15 min.

2.3 Inoculation of Potato Nodal Cuttings

Four-millimetre single nodal cuttings were dissected from twelve week-old in-vitro plantlets of the potato cultivars Tigoni, Dutch Robyjin, and Kenya Sifa using sterile blades and forceps under a sterilised laminar hood chamber. The single nodal cuttings (4 millimetres long) were

put into 50 millilitres test tubes (Pyrex Co.) containing 10 ml MS medium supplemented with Phytagel (solid) and without phytagel (liquid). The process was continued until complete plantlets were obtained in sufficient numbers (10 test tubes for the three varieties in each treatment in three replications). The nodal cuttings were floating on the media where they were sufficiently aerated. The test-tube cultures were then randomly placed in the growth chamber having temperatures of 22 ± 2°C and a 16-hr photoperiod having light intensity of 3,000 lux.

2.4 Data Collection and Analysis

Fourteen days after innoculation, data collection on in-vitro plantlet performance commenced; subsequent data were taken at 7 days interval for 35 days. The following parameters were recorded: Number of roots, number of nodes, and number of leaves per plantlet. In each block data were taken on all the thirty test tubes.

Data were subjected to analysis of variance (ANOVA) to test for differences between treatments using Genstat statistical package (Gensat, 2009). Where ANOVA test showed significant differences, treatment means were separated using the Least Significant Difference test at P=0.05.

3. RESULTS

In the first trial, the medium had no significant effect (P=0.05) on the number of roots per plantlets (Table 1). The interactions between the potato cultivar and the medium were significant at 14, 21 and 35 days after inoculation.

Table 1. ANOVA: Number of roots per plantlet during the first trial

Source of variation	Degrees of freedom	F Probability			
		14 DAI	21 DAI	28 DAI	35 DAI
Rep	2				
Cultivar	2	0.098ns	0.003**	0.005**	0.003**
Medium	1	0.731ns	0.126ns	0.172ns	0.196ns
Cultivar x medium	2	0.025*	0.030*	0.121ns	0.042*

**= significant at P=5%, **= significant at P=1%, ns= not significant, DAI= Days after inoculation*

The liquid medium had more roots than the solid medium. Generally, Kenya Sifa had the shortest roots throughout while Dutch Robyjin had the longest (Table 2).

Table 2. Mean number of roots of different potato cultivars over time during the first trial

Cultivar	Days after inoculation (DAI)			
	14	21	28	35
Dutch Robyjin	1.567a	4.33a	7.03a	8.60a
Tigoni	1.067a	3.60a	6.00a	7.80a
Kenya Sifa	1.200a	2.60b	4.50b	5.87b
LSD (5%)	0.4713	0.982	1.494	1.579

Within the same column, figures followed by the same letter are not significantly different at LSD=0.05

The medium as well as interaction between it and cultivar did not significantly affect the number of nodes per plantlet in the first trial. However, there were significant differences (P=0.05) in the number of nodes among cultivars at 21 and 28 days after inoculation (Table 3).

Table 3. Means of the nodes per plantlet in the first trial

Nodes per plantlet	Days after inoculation			
	14	21	28	35
Dutch Robyjin	2.67	4.67	6.27	7.67
Tigoni	2.30	3.87	5.30	7.23
Kenya Sifa	2.17	4.20	5.60	7.73
Solid medium	2.24	4.31	5.51	7.29
Liquid medium	2.51	4.18	5.93	7.80
LSD (5%) Cultivar	0.47	0.58	0.66	0.70
LSD (5%) Medium	0.38	0.48	0.54	0.57
CV (%)	38.6	26.7	22.5	18.1

The medium had no significant effects on the number of leaves per plantlet in the first trial (Table 4). The cultivars were significantly different at 21 and 28 days after inoculation (Table 4).

Table 4. Means of the leaves per plantlet in the first trial

Leaves per plantlet	Days after inoculation			
	14	21	28	35
Dutch Robyjin	2.70	4.93	7.37	8.67
Tigoni	2.30	3.80	5.97	8.00
Kenya Sifa	2.50	4.33	6.50	8.57
Solid medium	2.71	4.31	6.38	8.27
Liquid medium	2.29	4.40	6.84	8.56
LSD (5%) Cultivar	0.53	0.54	0.73	0.71
LSD (5%) Medium	0.43	0.94	0.59	0.58
CV (%)	41.0	29.6	21.4	16.4

In the second trial, the cultivars and medium were significant at 21, 28 and 35 days after inoculation (Table 5). Generally, the liquid medium had more roots than the solid medium.

In the second trial, the medium had a significant (P=0.05) effect on number of nodes per plantlet (Table 6). The liquid medium had significantly (P=0.05) more nodes than the solid medium throughout and the interaction between medium and cultivars was not significant (Table 6).

Table 5. Means of the root per plantlet in the second trial

Roots per plantlet	Days after inoculation			
	14	21	28	35
Dutch Robyjin	1.73	4.20	6.73	8.53
Tigoni	1.87	4.33	6.30	8.60
Kenya Sifa	1.43	3.07	4.80	6.60
Solid medium	1.47	3.49	5.27	7.09
Liquid medium	1.89	4.24	6.62	8.73
LSD (5%) Cultivar	0.64	0.87	1.40	1.58
LSD (5%) Medium	0.52	0.71	1.15	1.29
CV (%)	73.7	44.0	46.0	38.8

Table 6. Means of nodes per plantlet in the second trial

Nodes per plantlet	Days after inoculation			
	14	21	28	35
Dutch Robyjin	2.87	4.93	7.03	8.43
Tigoni	2.40	4.33	5.93	8.03
Kenya Sifa	2.17	4.47	6.30	8.63
Solid medium	1.98	3.84	5.42	7.24
Liquid medium	2.98	5.31	7.42	9.49
LSD (5%) Cultivar	0.66	0.77	0.83	0.81
LSD (5%) Medium	0.53	0.63	0.67	0.66
CV (%)	51.4	32.9	25.0	18.80

The cultivars were not significantly different in the number of leaves per plantlet in the second trial. Liquid medium gave more leaves than the solid medium throughout. The interaction between medium and cultivars were significant at 28 and 35 days after inoculation (Figure 1).

4. DISCUSSION

From the results, the liquid medium gave more roots, more nodes and more leaves per plantlet than the solid medium. Root proliferation in the liquid medium could have been due to the ease at which roots penetrated the liquid medium compared to the solid medium. This could have had led to faster plantlet growth and hence more nodes (axilliary buds) and leaves. Due to faster growth, it means that sub-culturing can be done more frequently with liquid medium than with solid medium. In addition, no physiologic differences were noted in the plantlets in the two media. The above results are in agreement with those of Kuria *et al.* (2008) who reported higher biomass accumulation in liquid media than in solid media.

Use of liquid culture media has been reported by a number of people to show better shoot and root growth in many plant species (Sandal *et al.*, 2001; Smith and Spomer, 1994; Ziv, 1989). Pierik (1997) pointed out that high shoot proliferation in liquid media could have been due to availability, ease of uptake of water and nutrients and closer contact between explants and the medium. However, plants in liquid media or in a media having low concentration of the gelling agent suffer from hyperhydricity (Pierik, 1997).

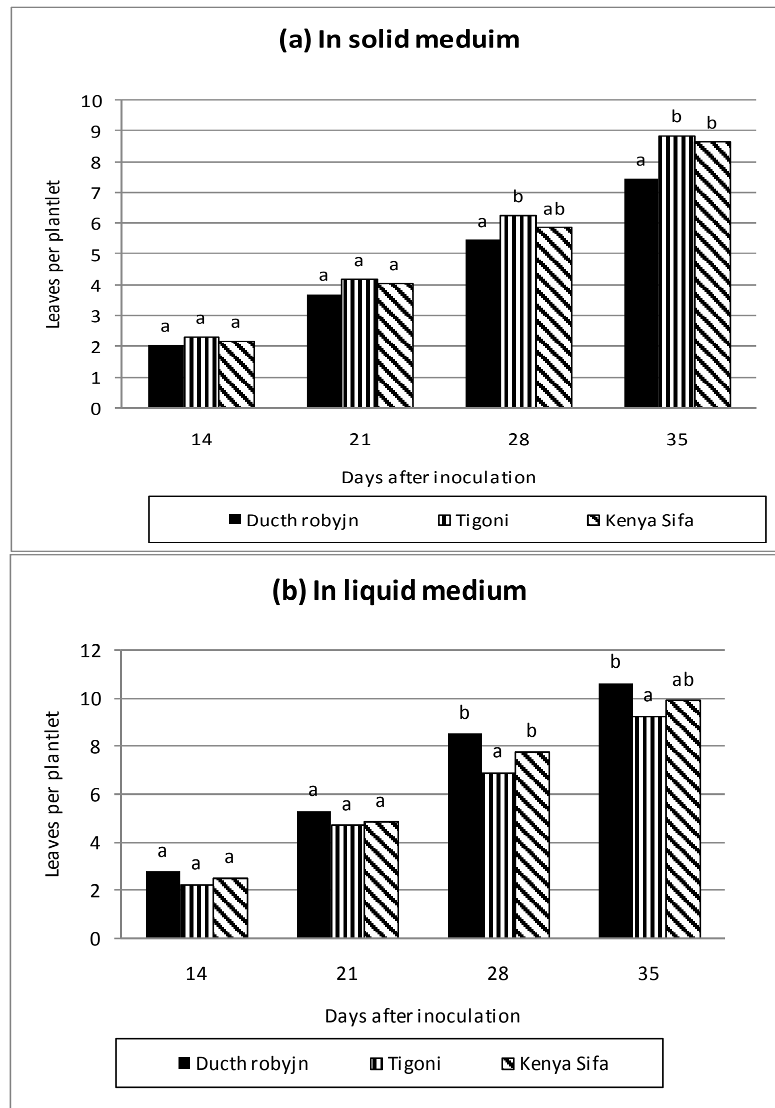


Figure 1. Leaves per plantlet in the second trial in (a) solid medium and (b) liquid medium

Preece (2011) reported that many plant species have reduced growth as agar levels increases; he concluded that eliminating agar or other gelling agent can improve micro-shoot proliferation and growth.

Phytagel is normally used in tissue culture to solidify the medium and hence support the plantlets into an upright position; however this means extra cost. The cost of phytagel is USD 660 per kg in the local Kenyan market currently. The cost of one litre of MS medium using phytagel at 0.25% was found to be USD 1.65 more than the medium without phytagel (Table 7).

Table 7. Cost of phytagel-supplemented medium compared to liquid medium

Type of gelling agent	Cost/kg (USD)	Conc./ litre (w/v)	Cost of 1 Litre of MS medium supplemented with GA3 and sucrose (USD)	Cost of .25% of phytagel (USD)	Total cost of 1litre of media (USD)
Phytagel	660	0.25%	2.97	1.65	4.62
Without gelling agent	0	0	2.97	-	2.97

5. CONCLUSION AND RECOMMENDATIONS

Liquid medium was found to induce more roots, more nodes and more leaves in the plantlets than the solid medium; in addition, liquid medium was cheaper than the solid medium. It was therefore concluded that it is more economical to use liquid media than solid media for potato *in vitro* micro-propagation. The experiment needs to be repeated especially with different potato cultivars so as to come up with useful recommendations.

ACKNOWLEDGEMENTS

The authors are grateful to the centre director National Potato Research Centre (NPRC)-Tigoni, Kenya for provision of potato genotypes and funds. The technical team is acknowledged for their assistance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

Coleman, W.K., Donnelly, D, J., Coleman, S.E. (2001). Potato micro-tubers as research tools: A review. American Potato Research Journal, 78, 47-55.

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

Food and Agriculture Organisation of the United Nations. (2008). International year of the potato [Online]. Available at http://www.potato2008.org. (Accessed 10 March 2010). FAO, Rome.

GenStat for Windows (2009). Lawes Agricultural Trust, Rothamsted Experimental Station, Harpenden, UK.

George, E.F. (1993). Plant propagation by tissue culture. Part 1- The technology. Edington: Exegetics limited, pp. 337-356.

Jane, Muthoni, Miriam, W.M., Nyamongo, D.O. (2010). A review of potato seed systems and germplasm conservation in Kenya. Journal of Agriculture and Food Information, 11, 157–167.

Jane, Muthoni, Nyamongo, D.O. (2009). A review of constraints to ware Irish potatoes production in Kenya. Journal of Horticulture and Forestry, 1(7), 98-102.

Kuria, P., Demo, P., Nyende, A.B., Kahangi, E.M. (2008). Cassava starch as an alternative cheap gelling agent for the in vitro micro-propagation of potato (*Solanum tuberosum* L.). African Journal of Biotechnology, 7(3), 301-307.

Lung'aho, C., M'makwa, C., Kidanemariam, H.M. (1997). Effect of source of mother plant, variety and growing conditions on the production of stem cuttings and subsequent yields of minitubers in the Kenyan potato program. In *Proceedings of the 4th Triennial Congress of the African Potato Association* Pretoria, South Africa: National Agricultural Research Organization, 275–283.

MoA (Ministry of Agriculture). (2008). National policy on potato industry. Policy reforms to revitalize the potato industry.

Mohamed, M.A.H., Alsadon, A.A., Al Mohaidib, M.S. (2009). Corn and potato starch as an agar alternative for *Solanum tuberosum* micropropagation. African Journal of Biotechnology, 8(19).

Murashige, T., Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant, 15, 473-97.

Nyende, A.B., Schittenhelm, S., Mix-Wagner, G., Greef, J.M. (2005). Yield and Canopy growth characteristics of field grown synthetic seeds of potato. European Journal of Agronomy, 22, 175-184.

Pierik, R.L.M. (1997). *In vitro* culture of higher plants. Kluwer academic publishers, Dordrecht, Netherlands, pp. 21-146.

Prakash, S. (1993). Production of ginger and turmeric through tissue culture methods and investigations into making tissue culture propagation less expensive. Ph.D Thesis. Bangalore University, Bangalore, India.

Prakash, S., Hoque, M.I., Brinks, T. (2000). Culture media and containers. Biotechnology and Eco-development Research Foundation, Bangalore, India. pp. 29-30.

Preece, J.E. (2011). Micropropagation in stationary liquid media. Propagation of Ornamental Plants. 10(4), 183-187.

Sandal, I., Bhattacharya, A., Ahuja, P.S. (2001). An efficient liquid culture system for tea shoot proliferation. Plant Cell Tissue Organ Cult., 65, 75-80.

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

Smith, M.A.L., Spomer, L.A. (1994). Vessels, gels, liquid media and support systems. In: Aiktken-Christie, J., Kosai, T., Smith, M.A.L. (eds.). (1994). Automation and Environmental control in plant tissue culture. Kluwer Academic Publishers, Dordrecht., 371-404.

Ziv, M. (1989)**.** Enhanced shoot and cornlet proliferation in liquid cultured gladiolus buds by growth retardants. Plant Cell Tissue Organ Cult., 17, 101-110.

© 2012 Mbiyu et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.