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Cost-effective tissue culture media for large-scale propagation of three commercial banana (*Musa* spp.) varieties

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ABSTRACT

Cost-effective tissue culture protocols have been established for the commercial multiplication of three banana varieties, 'Rasthali' (AAB – Silk), 'Grand Naine' (AAA – Cavendish), and 'Udhayam' (ABB – Pisang Awak). Reverse osmosis water and 3% (w/v) table sugar were used as the low-cost water and carbon source, respectively. Six different gelling agent treatments were tested: sago alone (T1), Isabgol alone (T2), sago + agar (T3), Isabgol + agar (T4), sago + Isabgol (T5), and agar alone as a control (T6). Full-strength Murashige and Skoog (MS) medium supplemented with 3 mg l^{-1} 6-benzylaminopurine (BAP) and 1 mg l^{-1} indole-3-acetic acid (IAA) were used for culture initiation and subculturing. Rooting was accomplished on low-cost MS medium containing 1.0 mg l^{-1} *α*-napthaleneacetic acid (NAA), 1.0 mg l^{-1} indole-3-butyric acid (IBA), and 250 mg l^{-1} activated charcoal. Statistical analysis indicated that sago + Isabgol (T5) produced the maximum number of shoots (10 per explant) in 'Udhayam' and 'Rasthali', while sago alone (T1) produced the maximum number of shoots (6 per explant) in 'Grand Naine'. The genetic stability of tissue-cultured banana plantlets produced using these low-cost substitutes was assessed using inter-simple sequence repeat (ISSR) markers. The results indicated that the ISSR profiles of the five treatments and the control (T6) were similar, indicating genetic stability using these cost-effective tissue culture protocols. Reductions in cost over the control (l^{-1} of MS medium) ranged from 65% to 86%, while the per plant production cost was reduced by 12.5%–20.0%. Adoption of these treatments (T1–T5) as low-cost tissue culture protocols for *in vitro* propagation would reduce production costs significantly, leading to an expansion of the area planted with tissue-cultured banana, thereby increasing productivity.

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Banana; tissue culture; propagation; ISSR markers

Micropropagation was the first biotechnological method to be exploited commercially for the production of high-quality, uniform planting material in economically important crops such as banana, papaya, and medicinal plants (Agnihotri, Prem, & Gupta, 2004; Costa et al., 2004). However, *in vitro* techniques are hampered by high unit production costs, and/or poor multiplication, and/or low survival rates during acclimatisation (Kozai, Kobuta, & Watanabe, 1988). The high cost of production has also prevented small- or medium-scale laboratories with limited resources from accessing the potential benefits of plant tissue culture technology, which has led to the closure of >30 of the 90 commercial micropropagation units established in India. The concept of low-cost production protocols has therefore stimulated researchers.

Prakash, Hoque, and Brinks (2004) reported that double-distilled water, the main component of all tissue culture media, is normally considered to be free from ions and contamination. However, the production of double-distilled water is expensive; hence, alternative, cheaper sources of water which could lower the costs of the media used without compromising on quality were investigated. Subsequently, Raghu, Martin, Priya, Geetha, and Balachandran (2007) reported that the use of tap water for tissue culture did not adversely affect *in vitro* plant growth.

Agar, which is an expensive gelling agent, has been widely used for solid media due to its clarity, non-toxic nature, and resistance to metabolism during tissue culture (McLachlan, 1985). However, starches from various sources such as potato, rice, barley, wheat, tapioca, or corn have also been used as gelling agents, either singly or in combination, with varied degrees of success (Nene & Sheila, 1996).

Sucrose is the preferred carbon source for most plant tissue culture protocols as it is an easily assimilated macronutrient that rapidly provides energy. However, commercially available table sugar has been used to replace tissue culture-grade sucrose. This reduced medium costs without compromising on the micropropagation rate or on the quality of the plants produced (Raghu et al., 2007).

Tissue-cultured banana plants have become an integral part of commercial banana production

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(Singh, Uma, Selvarajan, & Karihaloo, 2011). Therefore, it is essential to search for reliable, low-cost substitutes for use in banana tissue culture to overcome cost constraints and to harness the full potential of banana tissue culture. The objectives of the present study were: (i) to develop a reliable, yet simple and cost-effective micropropagation protocol for three commercial varieties of banana using low-cost sources of water, carbon, and gelling agent; and (ii) to assess the effect of using low-cost substitutes on the genetic stability of tissue-cultured plantlets using inter-simple sequence repeat (ISSR) markers. The responses of banana shoot tip explants on media containing different low-cost substitutes, and the reductions in media and production costs are discussed in this paper.

Materials and methods

Water sources

Based on previous reports (Gitonga, Ombori, Murithi, & Ngugi, 2010), three sources of water [reverse osmosis water (RO), bore-well water, or single-distilled water] were tested and compared with double-distilled water (control) for media preparation in preliminary trials on the ABB banana cultivar 'Udhayam'. The different sources of water were tested for microbial contaminants by the plate spreading method (Sanders, 2012) on nutrient agar medium. Precipitation of salts was observed when water sources other than double-distilled water were used due to their high concentrations of bicarbonates. This was overcome by boiling and filtration, as reported by Das and Gupta (2009). The pHs of all water sources were measured before and after boiling. After boiling, pH values ranged from 5.20 to 6.52 and were adjusted to 5.80 with 1.0 M NaOH or HCl, which was the final pH of the Murashige and Skoog medium used in these studies.

Carbon sources

Three different carbon sources [table sugar, rock candy, and small candy (EID Parry India Ltd, Chennai, India)] were tested, each at three different concentrations [3.0%, 4.0%, or 5.0% (w/v)] in preliminary trials, with 3.0% (w/v) tissue culture-grade sucrose (Hi-Media, Mumbai, India) as the control.

Gelling agents

Three different gelling agents [sago (Sagoserve, Salem, India), Isabgol (Agro-Organic Exports, Ahmadabad, India), and corn flour (Vitarich Agrofoods Pvt. Ltd, West Bengal, India)] along with conventional agar (Hi-Media) were tested in different combinations in preliminary trials.

Based on the results of preliminary trials, RO water, table sugar, and six gelling agent combinations: T1, 10.0% (w/v) sago; T2, 3.0% (w/v) Isabgol; T3, 9.0% (w/v) sago plus 0.04% (w/v) agar; T4, 3.0% (w/v) Isabgol plus 0.08% (w/v) agar; T5, 5.0% (w/v) sago plus 1.5% (w/v) Isabgol; and T6, 0.70% (w/v) agar alone, control, were tested on three commercial banana (*Musa* spp.) varieties 'Udhayam' (Pisang Awak, ABB), 'Rasthali' (Silk, AAB), and 'Grand Naine' (Cavendish, AAA).

Culture initiation

Shoot meristems approximately 5.0 cm^3 in size were isolated from 2–3-month-old "sword suckers" collected from disease-free mother plants at the ICAR-NRCB Farm, Podavur, Tiruchirappalli and brought to the laboratory. They were initially washed in 200 $\mu l\ l^{-1}$ Tween-20 (Hi-Media) followed by running tap water for 20–30 min to remove all traces of the detergent. They were then surface-sterilised for 10 min each in 4.0% (v/v) sodium hypochlorite followed by 0.10% (w/v) mercuric chloride, with three rinses in sterile water in between each treatment. The surface-sterilised shoot tips (n = 10 per cultivar) were trimmed to a final size of 1.5 cm^3 and initiated on full-strength MS medium supplemented with 3 mg l^{-1} 6-benzylaminopurine (BAP; Sigma-Aldrich Co., St. Louis, MO, USA) and 1 mg l^{-1} indole-3-acetic acid (IAA; Sigma-Aldrich Co.). All media were prepared with RO water, and contained 3% (w/v) table sugar with different combinations of the gelling agents (as above). The pH of each medium was adjusted to 5.8 with 1.0 M NaOH or HCl prior to autoclaving at 121°C for 20 min at 100 kPa. All cultures were maintained at 26±2°C under a 16-h photoperiod at a light intensity of 30–40 µmol photons $m^{-2}\ s^{-1}$. The percentage of greening and the number of days taken for the greening of shoot tips were recorded during the culture initiation phase.

Shoot proliferation, rooting and hardening

Shoot proliferation was achieved on the same shoot induction medium by subculturing five or six times at 4–5-week intervals. The number of days taken for multiple shoot initiation after the second subculture, shoot lengths (cm), and numbers of shoots produced per explant were recorded during shoot proliferation.

Single shoots obtained after repeated subculturing were rooted in low-cost MS medium supplemented with 1.0 mg l^{-1} α-napthaleneacetic acid (NAA; Sigma-Aldrich Co.), 1.0 mg l^{-1} indole-3-butyric acid (IBA; Sigma-Aldrich Co.), and 250 mg l^{-1} activated charcoal. The number of days taken for root initiation, root numbers, and the longest root lengths were recorded during

rooting. Plantlet hardening involved the transfer of well-developed, rooted plantlets from semi-solid, soft-textured MS media to hard-textured coco peat, and then to soil-based media. Coco peat-filled trays were used as the support matrix for primary hardening. Secondary hardening was done in hard medium containing a 1:1:1 (v/v/v) mix of farmyard manure, sand and native soil or red earth.

Assessment of genetic stability using ISSR markers

The protocol of Gawel and Jarret (1991) was adopted, with minor modifications, for the isolation of genomic DNA from the tissue-cultured plantlets. DNA quantification was done by UV spectrophotometry and DNA quality was confirmed by electrophoresis in 0.8% (w/v) agarose gels.

Each 25-μl polymerase chain reaction (PCR) was performed in a Multigene Opt Max gradient master cycler (Labnet International Inc., Edison, NJ, USA) and contained 10 ng of genomic DNA, 100 μM of each dNTP, 0.4 μM of each primer, 1× PCR buffer [100 mM Tris–HCl, pH 9.0, 500 mM KCl, 10 mM $MgCl_2$, and 0.1% (w/v) gelatin] and 2 Units of *Taq* DNA polymerase (Sigma-Aldrich Co.). Each PCR programme consisted of an initial denaturation step at 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, annealing at the primer-specific temperature for 45 s, and extension at 72°C for 2 min, with a final extension step at 72°C for 8 min, followed by incubation at 4°C.

Five ISSR markers from the University of British Columbia (UBC Series), namely 807, 812, 834, 841, and 868, were used to assess genetic stability. The PCR-amplified products were separated by electrophoresis in 1.5% (w/v) agarose gels (BIO-RAD Laboratories Inc., Hercules, CA, USA) at 100 V for 3–4 h. The PCR-amplified ISSR marker products were scored across the different treatments and compared with the controls for all three banana varieties.

Economics

The production costs per plant were calculated for sago-, Isabgol-, and agar-based media considering both variable and fixed costs. Variable costs included the cost of the medium, plantlet hardening, electricity, and labour, while fixed costs included the cost of buildings and infra-structure required for the laboratory with a production capacity of 10^5 plants per annum. Medium costs were calculated based on the costs of MS macro- and micro-salts, vitamins, hormones, water, carbon source, and gelling agent. Similarly, plantlet hardening costs included the costs of containers, substrates, fertilisers, and pesticides. Electricity and labour costs were calculated separately for all individual activities.

Experimental design and data analysis

The experiment was laid out in a completely randomised design with 10 replicates of each treatment and each banana variety. All experiments were repeated at least three times. Data were subjected to one-way analysis of variance (ANOVA; $P \leq 0.05$) using AGRES software (Tamil Nadu Agricultural University, Coimbatore, India). Differences within and between treatments were estimated by means separation using Duncan's multiple range test.

Results and discussion

Identification of low-cost substitutes for double-distilled water, laboratory-grade sucrose, and agar

In a preliminary trial to identify the optimum low-cost substitute for double-distilled water in the banana cultivar 'Udhayam', earlier greening was observed with RO water at 5.4 days, followed by double-distilled water (control) at 5.6 days. Delayed greening was recorded with bore-well water (7.8 days), followed by single-distilled water (7.0 days). The percentage of greening was maximum in the controls (80%), followed by 75% in both RO and single-distilled water. The results obtained with RO water were similar to the controls with respect to the percentage of greening and days taken for greening of shoot tips (data not shown). Similarly, Sunandakumari, Martin, Chitra, Sini, and Madhusoodanan (2004) reported that the media prepared with tap water or with double-distilled water did not differ significantly with respect to *in vitro* shoot induction in *Mentha piperita*.

Bananas cultured on different carbon sources exhibited differences in their growth. This might be due to the differential sensitivity of tissues to the furfural or hydroxyl furfural formed during autoclaving of the carbon source (Hsiao & Bornman, 1989). Table sugar at 3% (w/v) resulted in early greening and produced the maximum number of shoots per explant (5.75), followed by 3% (w/v) rock candy (3.75), and 4% (w/v) small candy (1.75) in 'Udhayam' (data not shown). This might be attributed to the presence of 96%–97% sucrose in table sugar, compared to 99.98% in laboratory-grade sucrose (Tyagi, Agrawal, Mahalakshmi, Hussain, & Tyagi, 2007). A 95% reduction in cost was achieved by replacing laboratory-grade sucrose with commercially-available table sugar during media preparation. Similarly, Kodym and Arias (2001) achieved a 90% resource cost reduction by replacing

sucrose and Gelrite™ with locally available commercial sugar and a starch–Gelrite™ mixture, respectively.

Among the three gelling agents tested, minimum greening (10%–20%) was observed in medium with corn-flour. This might be due to the greater osmotic stress and ionic contamination which would have impaired the optimum growth of banana under *in vitro* conditions (Gebre & Sathyanarayana, 2001). Furthermore, drying of shoot meristems was observed at higher concentrations, making corn-flour unfit as a gelling agent for use in banana tissue culture media.

Culture establishment

In the present study, shoot tips cultured on various gelling agents showed differences in their growth and proliferation (Figure 1). This might be due to their different nutritional supplements, as reported earlier by Gebre and Sathyanarayana (2001). Earlier greening was observed in all three commercial banana cultivars in the controls (T6), followed by the different treatments (T1–T5). Among all three cultivars, the number of days taken for the greening of shoot tips was non-significant in 'Udhayam' and 'Rasthali', and was significantly different only in 'Grand Naine'. Earlier greening was observed in 'Rasthali' (4.6–5.2 days), followed by 'Grand Naine' (7.0–9.0 days), and 'Udhayam' (8.9–10.4 days), which might be due to genetic differences. In 'Udhayam', earlier greening was recorded in treatment T2 followed by treatment T4. In 'Grand Naine', earlier greening was observed in treatments T1, T2, and T3, which were similar to the control (T6).

Isabgol-based medium enhanced the initial establishment of shoot tips in 'Udhayam' and 'Rasthali'. This might have been due to its high mucilage content (>30%), or its polysaccharide and colloidal nature, as reported by Tyagi et al. (2007). Several workers have used Isabgol as a potential gelling agent for tissue-culture multiplication of crops such as chrysanthemum (Bhattacharya, Satyahari, & Bhattacharya, 1994), turmeric (Tyagi et al., 2007), tobacco (Ozel, Khawar, & Arslan, 2008), and banana (Agrawal, Sanayaima, Tandon, & Tyagi, 2010). In 'Grand Naine', sago-based medium accelerated the greening of shoot tips. The firmness of the medium also provided adequate support for the growth of banana explants throughout the culture period (Naik & Sarkar, 2001). The low amounts of sugars, fibre, protein, calcium and starch might have caused earlier greening, equivalent to the controls (Anon., 2010).

Shoot proliferation

During shoot proliferation, the number of shoots per explant was higher in all treatments (T1–T5) compared to the control (T6; Figure 1). The number of shoots produced per explant was highly significant among treatments in 'Rasthali', significant in 'Udhayam', and non-significant in 'Grand Naine', which indicated a genome-specific response (Table I). The non-significant effect of low-cost gelling agents in 'Grand Naine' might be due to the high regeneration potential of 'Grand Naine' compared to other banana varieties. Treatment T5 produced the maximum numbers of shoots per explant (10.0) compared to the controls in 'Udhayam' and 'Rasthali' (8.0 and 7.0 shoots per explant, respectively).

Sago and Isabgol performed better as gelling agents for many crops including banana (Babbar & Jain, 1998; Naik & Sarkar, 2001; Bhattacharya et al., 1994). However, this is the first report in which a combination of sago and Isabgol (T5) performed significantly better, possibly due to a positive synergistic effect on the *in vitro* growth of banana.

In 'Grand Naine', the maximum number of shoots per plant (6.0) was produced in treatment T1 followed by the control (T6; 5.0), which agreed with the findings of Nene and Sheila (1996), who showed that sago performed better, except for the opaqueness of medium which reduced visibility to check for fungal growth under *in vitro* conditions. Lower concentrations of Isabgol (2.5 g l^{-1}) produced fewer shoots per explant in all three varieties tested. This might be due to the reverse osmosis of carbohydrates from the explants to the gel rather than diffusion of nutrients and growth regulators from the gel to the

Figure 1. Effect of single use or combinations of low-cost gelling agent(s) on the number of shoots produced per explant in the banana cultivar 'Rasthali'. Sample C, control [0.70% (w/v) agar alone]; T1 [10.0% (w/v) sago]; T2 [3.0% (w/v) Isabgol]; T3 [9.0% (w/v) sago plus 0.04% (w/v) agar]; T4 [3.0% (w/v) Isabgol plus 0.08% (w/v) agar]; and T5 [5.0% (w/v) sago plus 1.5% (w/v) Isabgol].

Table 1. Effect of gelling agent and combinations on shoot proliferation in the commercial banana (*Musa* spp.) cultivars 'Udhayam', 'Grand Naine', and 'Rasthali'.

Gelling agent [% (w/v)]			'Udhayam' (ABB)		'Grand Naine' (AAA)		'Rasthali' (AAB)	
Sago	Isabgol	Agar	Days taken for greening	No. of shoots/ explant	Days taken for greening	No. of shoots/ explant	Days taken for greening	No. of shoots/ explant
10.00	–	–	10.37	8.0b*	7.30a	6.0	5.10	9.0ab
–	3.00	–	9.62	9.0ab	7.50a	4.0	5.20	8.0bc
9.00	–	0.04	10.25	9.0ab	7.50a	5.0	4.70	8.0bc
–	2.50	0.08	9.75	9.0ab	8.10ab	3.0	5.10	8.0c
5.00	1.50	–	10.00	10.0a	9.00b	4.0	5.70	10.0a
–	–	0.75	8.87	8.0b	7.00a	5.0	4.60	7.0c
Significance†			NS	1.38	1.19	NS	NS	1.28

*Mean values (n = 10) followed by the same lower-case letters in each column for each cultivar are not significantly different at $P \leq 0.05$ using Duncan's multiple range test.
†NS, not-significantly different at $P \leq 0.05$.

explants (Ozel et al., 2008). The lower relative matric potential might also lead to slow transport of water and nutrients resulting in slow multiplication (Tyagi et al., 2007). The number of shoots produced per explant varied with cultivar and gelling agent. The variable hardness of the gelling agents, due to their biochemical composition and structural differences, might have affected the diffusion of plant growth regulators and other nutrients into the medium, ultimately resulting in variation in the numbers of shoots produced per explant (George, 1993).

Rooting and hardening

Among the three varieties, significant differences were observed in plant height, numbers of roots, and longest root length, which controls acclimatisation, except in 'Udhayam' (data not shown). In the case of gelling agent, combinations of sago and agar, sago and Isabgol, and Isabgol and agar were found to be optimal for 'Rasthali', 'Grand Naine', and 'Udhayam', respectively. Rooting was >95%, irrespective of gelling agent and variety, which indicated that the gelling agents did not inhibit root initiation or penetration into the medium. Although plantlets grown on various gelling agents showed differences in their growth parameters, their performance during the hardening stage and percentage field survival were similar, as reported by Bhattacharya et al. (1994).

Assessment of genetic stability using ISSR markers

ISSR markers, which are simple and cost-effective (Gupta & Varshney, 1999), were to assess the genetic stability of tissue-cultured banana plantlets derived from the various low-cost media. The polymorphism was <15%, which was substantiated by the uniformity of ISSR profiles (Figure 2). This might be due to direct regeneration of plantlets from shoot tip explants (Tyagi et al., 2007). Our results further suggested that low-cost substitutes could be used in tissue culture media for the multiplication of commercial banana varieties as they caused no significant variation at the genetic level.

Economics

The costs of standard and low-cost media were compared (Table II). Substantial cost reductions were observed by using low-cost substitutes for double-distilled water, sucrose, and/or agar. The maximum cost reduction (86.4%) was observed when sago alone was used as the gelling agent, followed by sago plus agar

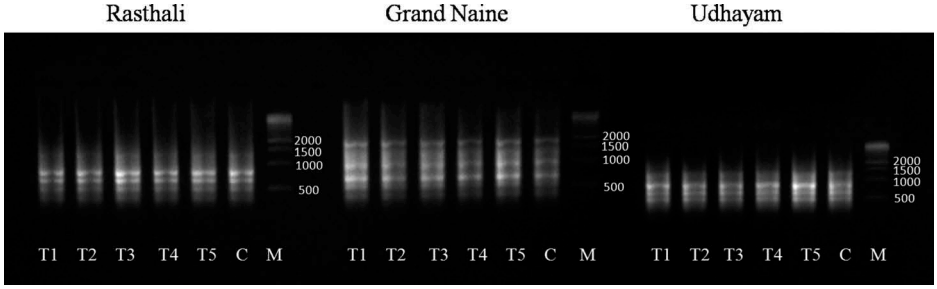

Figure 2. Genetic stability of low-cost tissue-cultured banana plantlets assessed using the ISSR primer UBC 807 in three commercial banana (*Musa* spp.) cultivars: 'Rasthali', 'Grand Naine', and 'Udhayam'. T1 [10.0% (w/v) sago]; T2 [3.0% (w/v) Isabgol]; T3 [9.0% (w/v) sago plus 0.04% (w/v) agar]; T4 [3.0% (w/v) Isabgol plus 0.08% (w/v) agar]; T5 [5.0% (w/v) sago plus 1.5% (w/v) Isabgol]; and C, [control, 0.70% (w/v) agar alone]. Marker lanes (M) show the sizes in bp.

Table 2. Economics (in US$ l^{-1}) of low-cost and standard media used for the micropropagation of three commercial cultivars of banana (*Musa* spp.).

Treatment [gelling agent; % (w/v)]	MS salts with vitamins and hormones	Carbon source	Gelling agent	Water source	Total cost (US$)	Cost reduction (%)
Control*	0.06	0.29	0.56	0.15	1.07	0.00
Sago (10.0)	0.06	0.02	0.05	0.02	0.15	86.40
Isabgol (3.0)	0.06	0.02	0.22	0.02	0.31	70.87
Sago + agar (9.00 + 0.04)	0.06	0.02	0.08	0.02	0.17	83.89
Isabgol + agar (3.00 + 0.08)	0.06	0.02	0.28	0.02	0.38	64.76
Sago + Isabgol (5.00 + 1.50)	0.06	0.02	0.13	0.02	0.23	78.64

*Hi-Media (Mumbai, India) agar, sucrose, and double-distilled water were used for standard (control) medium preparation compared to low-cost gelling agents, table sugar, and RO water in the low-cost media.

Table 3. Costs of single banana plant production in low-cost and standard media.

	Variable cost (US$)							
Treatment [gelling agent; % (w/v)]	MS medium	Hardening	Labour	Electricity	Total	Fixed cost (US$)	Total production cost/plant (US$)	Reduction in cost (%)
Control*	0.020	0.028	0.008	0.009	0.063	0.023	0.086	0.0
Sago (10.0)	0.001	0.028	0.008	0.009	0.046	0.023	0.068	20.0
Isabgol (3.0)	0.002	0.028	0.008	0.009	0.046	0.023	0.070	18.5
Sago + agar (9.00 + 0.04)	0.003	0.028	0.008	0.009	0.048	0.023	0.071	17.1
Isabgol + agar (3.00 + 0.08)	0.007	0.028	0.008	0.009	0.052	0.023	0.075	12.5
Sago + Isabgol (5.00 + 1.50)	0.002	0.028	0.008	0.009	0.047	0.023	0.069	18.9

*Hi-Media (Mumbai, India) agar, sucrose, and double-distilled water were used for standard (control) media preparation compared to low-cost gelling agents, table sugar, and RO water in the low-cost media.

(83.9%), sago plus Isabgol (78.7%), Isabgol alone (70.9%), or Isabgol plus agar (64%). Similarly, the costs of water and carbon sources were reduced by 86% and 95%, respectively, by using low-cost substitutes. Cost reductions due to low-cost substitutes have previously been reported by several authors (Bhattacharya et al., 1994; Das & Gupta, 2009; Buah, Tachie-Menson, Addae, Chambel, & Pereira, 2011).

The production cost per plant was calculated by adding all fixed and variable costs (Table III). Unit variable costs remained constant, regardless of changes in production volume, but the total variable cost and unit fixed cost varied in proportion to changes in production volume. Accordingly, the fixed unit cost for a laboratory with a production capacity of 10^5 plants per annum was US$0.02. Variable costs remained constant for all parameters except the medium, due to the use of different low-cost gelling agents, water source, and carbon source. The variable cost for the standard medium was US $0.06, with a unit production cost of US$0.08, while the variable cost for a low-cost medium with RO water and table sugar ranged from US$0.04 to US $0.05 with a total unit cost ranging from US$0.06 to US$0.07 for production of a single plant. Single plant production costs were therefore reduced by 12.5%–20.0% by using low-cost media. However, the percentage reduction in costs could improve further based on the capacity of the tissue culture unit.

Conclusions

Although several studies exist on the use of low-cost substitutes for agar, this is the first report where a combination of sago and Isabgol (T5) performed significantly better in two varieties of banana, 'Udhayam' and 'Rasthali', producing a maximum of 10 shoots per explant in a single cycle. Genetic stability tests using ISSR markers suggested that including low-cost substitutes in the tissue culture media did not cause any significant variation at the genetic level. The percentage reduction in medium costs compared to the controls ranged from 65% to 86%, while the production cost per plant was reduced by 12.5%–20.0%. This cost-effective mass multiplication protocol will enable plant tissue culture companies to produce varieties other than 'Grand Naine' in a more economical way. Commercial adoption of these low-cost tissue culture protocol would minimise production costs, leading to an expansion in the area grown with tissue-cultured bananas.

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