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PLANT TISSUE CULTURE

Evaluating suitable low-cost agar substitutes, clarity, stability, and toxicity for resource-poor countries' tissue culture media

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Abstract

Over 20% of plant species assessed are threatened with extinction. Most of these plants have food security implications, especially in developing countries. Traditional seeds and cutting propagation techniques cannot counter the loss rate, and tissue culture provides a fast alternative to conventional propagation techniques. However, tissue culture has been considered too expensive for developing countries negatively impacted by food insecurity. A gelling agent is the costliest media component in plant tissue culture. This study aims to assess different gelling agents to find suitable ones with low cost and acceptable gelling properties for developing countries, especially in rural areas. Plantain explants were propagated on 16 starch-based substrates to evaluate their suitability as tissue culture gelling agents. This study compared the cost of various substrates and their gelling properties, such as clarity, toxicity, and texture, with agar as a reference gelling agent. Some substrates, such as xanthan, had good gelling properties, but their cost was too high (5.98 Euro L^{-1}) to be considered low-cost. Other substrates, such as cassava starch, did not have suitable gelling properties; however, the cost was low (0.99 Euro L^{-1}). Two of the substrates, mung bean and Isabgol, had suitable gelling properties and cost less than one euro. Therefore, smallholder banana and plantain farmers in resource-poor countries can undertake tissue culture operations with mung bean and Isabgol as gelling agents with minimum cost.

Keywords Tissue culture · Food security · Gelling agent · Mung bean · Isabgol

Introduction

Anthropogenic activities, diseases, and climate change manifestations, such as drought, have endangered isolated populations of crops, and it is estimated that nearly three species of seed-bearing plants disappear each year (Woodruff 2001; Petrescu-Mag and Păpuc 2019). Furthermore, Brummitt *et al*. (2015) have shown that more than 20% of plant species assessed are threatened with extinction. A decline in plant population affects crop production in resource-poor countries, which further negatively impacts the food and nutrition security of the indigenes (Steege *et al*. 2015). Therefore, the conservation of plant species, especially food crops and their regionally well-adapted cultivars, is vital to ensure a sustainable food system. Agricultural conservation practices might be associated with land treatment techniques designed to preserve, enhance, or protect soil, water, vegetation, and other natural resources (Krutilla 2016). Specifically, propagating crops are essential for conservation (Murashige 1974; Delgado *et al*. 2011). However, the traditional methods for plant propagation are often not efficient to produce plantlets for the trading of well-adapted resistant cultivars or, in some cases, not efficient to mitigate the loss rate of crops with food security implications (Debnath and Goyali 2020). Tissue culture is one of the rapid methods to propagate plants (Hussain *et al*. 2012; Alikina *et al*. 2016) and will help with the selection of viable plants and also help prevent the extinction of some plants (Pegg 2002).

Although suitable to select plants with superior traits, high-tech tissue culture techniques are expensive, which limits their application in resource-poor countries (Agrawal *et al*. 2010; Basit *et al*. 2020). The gelling agent is one of the

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costliest media components. The cost of gelling agents *per* unit of media is about three-fourths of the overall expense of the media, which might slightly differ based on the details of the recipe and local costs of the ingredients (Gour and Kant 2011; Teixeira Da Silva 2014). Typically, agar is used as a gelling agent in tissue culture because of its convenient gelling properties. However, agar is expensive, which limits its usage, especially in developing countries (Henderson and Kinnersley, 1988; Singh and Kaur 2011; Sanchez-Cardozo *et al*. 2019). Consequently, attempts have been made to find cheaper alternative gelling agents to agar (Daud *et al*. 2011; Raina and Babbar 2011). These include starches derived from corn (Henderson and Kinnersley 1988; Zimmerman *et al*. 1995), Isobgol (Jain *et al.* 1997), sago (Naik PS 2001) potato, cassava and yam (Kodym and Zapata-Arias 2001), guar gum (Babbar *et al*. 2005), locust bean gum (Gonçalves and Romano 2005), corn flour, cassava flour, rice flour and potato starch (Kuria *et al*. 2008), and xanthan gum (R. Jain and Babbar 2006). All the above alternative gelling agents have been used either singly or combined with others with varying degrees of success. However, it is essential to further search for a gelling agent with suitable properties and affordability, which would increase the application of tissue culture for plant propagation in developing countries and thereby increase also food security.

Gelling properties, such as clarity, texture, and toxicity, must be considered before choosing a gelling agent (Teixeira da Silva and Retired 2015). Clarity refers to the gelling agent's transparency, and clarity is vital to monitoring the development of the roots during tissue culture (Lin and Casida 1984). The media's texture is suitable when the gelling agent can support the explant to stand upright even under changing temperatures (Das *et al*. 2015). A suitable gelling agent should also be resistant to digestion by explant enzymes produced during their propagation (Puchooa 1999; Hussain *et al*. 2012). During tissue culture, plants get cut and therefore injured, which leads to the formation of a protective layer around that injury, which is formed from polyphenolic oxidation for pathogen protection (Chikezie 2012). This is called the phenolic compound problem (browning) in tissue culture (Vincenzo *et al*. 2015) because this light-toxic brown coat reduced the growth of the explants (Wetzstein *et al*. 1994). Therefore, a suitable gelling agent should be affordable and match the mentioned requirements.

Many studies have been conducted to assess different gelling agents as low-cost alternatives to agar. However, as far as literature is concerned, no study has assessed low-cost agents to get the cheapest and in combination with their most suitable tissue culture properties. This present study uses two local plantain cultivars, "Apantu and Apem," which are locally essential plantain varieties of Sub-Sahara Africa (Dzomeku *et al*. 2020). Generally, tissue culture plantain propagation results in optimal yield, uniformity, and disease-free planting material (Ngomuo *et al*. 2014). All parts of the plantain containing a meristem are potentially suitable as an initial explant for tissue culture, making the plant an ideal model crop to assess potential gelling agents (Agbadje *et al*. 2021). This study aims to use plantain as a model plant to assess its responses in terms of shoot proliferation to some starch-based substrates as an alternative low-cost gelling agent in developing countries. Specifically, the study will evaluate and compare the cost of sixteen starch-based substrates and their suitable tissue culture gelling properties, such as clarity, toxicity, and texture. It is hypothesized that low-cost gelling agents will reduce the cost of media preparation while still allowing for highly effective plant propagation through tissue culture.

Materials and methods

Plant material "Apantu and Apem" were selected to represent the AAB genotype bananas cultivated and consumed in West Africa. Shoot cultures of "Apantu and Apem" were established *in vitro* using suckers obtained from the Crops Research Institute in Kumasi, Ghana. The following steps were performed under a flow bench (ESCO Airstream Class II BSC; Tanah Merah, Singapore). The shoot tips of the explants (2 to 3 cm diameter) were cleaned by cutting off the outer leaf sheaths and then soaked in a commercial bleach solution (20% *w/v* sodium hypochlorite) with a few drops of Tween 20 ® for 20 min. The outer leaf sheaths of the same shoot tip explant were further removed and soaked in sodium hypochlorite (10% *w/v*) for 10 min. The explants were then rinsed three times with sterile deionized water with each rinsing taking about 1 min. The explant was subjected to final leaf sheath removal leaving a shoot tip of about 1 cm × 1 cm containing the central block of the meristem (Fig. 1).

Medium composition and preparation Preliminary experiments with different types of gelling substitutes to obtain suitable concentrations that would support the growing explants were carried out. This experiment used different kinds of substitutes from Africa, Europe, and the Asian markets, for example, agar powder (food additive), pearl sago, guar gum, xanthan, corn starch, Isabgol, mung bean starch, yam starch, rice flour, glutinous rice flour, white maize meal, sago, tapioca, potato starch, and cassava starch. These were all tested in different amounts to find which ones to use as a suitable alternative gelling agent in tissue culture with the prescribed model plant, as shown in Table 1. These tissue culture experiments used a Murashige and Skoog (MS; Murashige and Skoog 1962) medium. The composition of the MS-medium used was modified for banana and plantain following the recommendation of the Bioversity

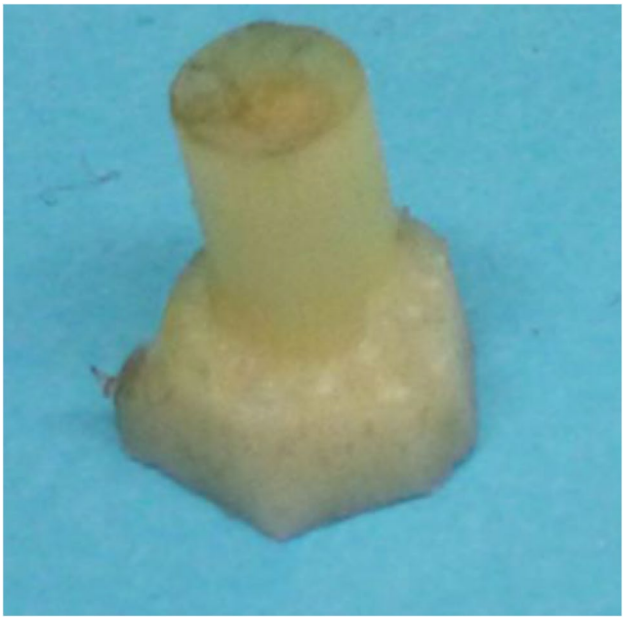

Figure 1. Explant of Apantu (*Musa* AAB) with central block meristem ready for transfer into media.

International *Musa* Germplasm Transit Center. The following components were added to modify the MS-medium.

1. The macronutrients used were NH_4NO_3 1650 mg L^{-1}, KNO_3 1900 mg L^{-1}, $CaCl_2{\cdot}2H_2O$ 440 mg L^{-1}, $MgSO_4{\cdot}7H_2O$ 370 mg L^{-1}, and KH_2HPO_4 400 mg L^{-1}.
2. Micronutrients used were H_3BO_3 6.18 mg L^{-1}, $MnSO_4{\cdot}H_2O$ 16.9 mg L^{-1}, $ZnSO_4{\cdot}7H_2O$ 8.6 mg L^{-1}, KI 0.83 mg L^{-1}, $Na_2MoO_4{\cdot}2H_2O$ 0.24 mg L^{-1}, $CoCl_2{\cdot}6H_2O$ 0.024 mg L^{-1}, and $CuSO_4{\cdot}5H_2O$ 0.025 mg L^{-1}.
3. Iron: $FeSO_4{\cdot}7H_2O$ 27.80 mg L^{-1} and $NA_2{\cdot}EDTA{\cdot}2H_2O$ 37.22 mg L^{-1}.
4. Vitamins: glycine 2.0 g L^{-1}, thiamine hydrochloride 0.1 mg L^{-1}, nicotine acid 0.5 mg L^{-1}, and pyridoxine hydrochloride 0.5 mg L^{-1}.
5. Antioxidant: ascorbic acid 10.0 mg L^{-1}.
6. Sucrose 30 g L^{-1}.
7. Gelrite 2.0 g L^{-1}.
8. Plant growth regulator N6-benzylaminopurine (BAP) 2.25 mg L^{-1} and (indole-3-acetic acid; IAA) 0.175 mg L^{-1}. Stock solutions of 10 × macronutrients and 100 × micronutrients, vitamins, iron salts, and growth regulators were prepared, diluted, and mixed with deionized highly purified water (Sartorius, Germany).

The pH 5.7 was adjusted with KOH/HCL 1 M. Then, agar 8 g L^{-1} was used as control, and all the other alternative gelling agents with concentrations as shown in Table 1 were added to their respective treatments. In choosing the concentrations of the different alternative gelling agents, we carried out a preliminary experiment where we assessed different concentrations of these gelling agents to get a suitable concentration that we used in our main experiment. The concentration of gelling agents has been shown to influence the solidification of the media (Mohamed *et al*. 2021). The media components were autoclaved at 121 °C for 15 min. In the case of media which required IAA, it was added through

Table 1 Different kinds of alternative gelling agents that were used in the tissue culture media and their assigned codes

Treatment no	Alternative gelling agents (g L^{-1})	Agar (g L^{-1})	Code of the media
1	Mung bean starch 30 g	4.0	MbA
2	Mung bean starch 80 g	0.0	Mb
3	Sago starch 30 g	4.0	SA
4	Sago starch 220 g	0.0	S
5	Xanthan 30 g	4.0	XA
6	Xanthan 60 g	0.0	X
7	Isabgol 20 g	4.0	IA
8	Isabgol 30 g	0.0	I
9	Guar gum 30 g	4.0	GKA
10	Guar gum 60 g	0.0	GK
11	Pear sago 60 g	4.0	PSA
12	Pear sago 250 g	0.0	PS
13	Cassava starch 40 g	4.0	CSA
14	Cassava starch 250 g	0.0	CS
15	Tapioca starch 80 g	4.0	TPA
16	Tapioca starch 130 g	0.0	TP
17	Agar	8.0	Control (A)

*__Note:__ When the gelling agent has agar in the mixture, they carry the letter "A" in the media code

a sterile 0.2-µm filter tip after autoclaving when the media had cooled down to about 50 °C. IAA was added at 50 °C because, as a hormone of its kind, it will be destroyed at a higher temperature. All chemicals were purchased at either VWR Chemicals located in Hessen Darmstadt, Germany; Sigma-Aldrich located in Steinheim am Albuch, Baden-Württemberg, Germany; or Duchefa in the city of Haarlem, Netherlands.

Treatments and growth conditions The study tested 17 different gelling agents, including the control (agar), as shown in Table 1. Ten 250-mL jars each were filled with 30 mL of MS-modified medium containing the respective gelling agent for each treatment. The explants were then placed in the MS-modified medium containing the respective gelling agent for each treatment. The jars were placed in a growth chamber (Rumed, Teyp 1200, Rubarth Apparate GmbH, Germany) under 12 h d/night and illuminated by fluorescent lamps (Osram Fluora L36W/77) with a light intensity of 40 ± 4 µmol·m^{-2} s^{-1} at 25 ± 1 °C. Browning is a known problem in banana and plantain tissue culture (Fig. 2) (Onuoha *et al*. 2011). For browning prevention, the brown parts were cut out from all explants that survived after 1 wk after the start of the experiment. Then, plantlets were placed on a new MS-modified medium.

Data collection Data collection started 4 wk after the explants had been sub-cultured (explants are put into the new media every 4 wk). The clarity of the gelling agents was evaluated visually by observing the transparency (Kumar *et al*. 2019). As outlined by Kumar *et al*. (2019), the clarity of the media was assessed under a black and white background by visual inspection. We observed the appearance for the presence of suspended particulate matter. The more the particulate matter in the media, the cloudier was the media and therefore less transparent. The stability was assessed by observing how easily an explant could be submerged into the medium without falling over during growth (Puchooa 1999). Toxicity as a result of browning was assessed indirectly by assessing the number of explants that survived. Plant growth characteristics, such as the number of shoots per explants, height, leaf, and root number, were recorded. The percentage of explants that survived were statistically calculated, and the amount of phenolic compounds problems (browning) was graded as follows: 0 to 4 of phenolic problems, where 0 = non-presence of phenolic compounds problem, 1 = a slight presence of phenolic compounds problems, 2 = high phenolic problems, 3 = higher phenolic compounds problems (but still survived), and 4 = higher presence of phenolic compounds problems (almost dead). The cost of the different acceptable gelling agents was compared to that of agar in Euros. This means that if the cost was above one Euro L^{-1}, the study did not consider the alternative gelling agent as suitable. The second criteria was the gelling agent properties, and the study examined three of these properties (clarity, texture, and toxicity). It was considered suitable if a gelling agent fulfilled at least two of the requirements. However, for a gelling agent to be considered low-cost suitable for developing countries, it should have from two to three suitable properties and be less than 1 Euro L^{-1}. The study used "Suitable" and "Unsuitable" to validate the suitability of the gelling agents' properties and cost, as shown in Table 4. The physical properties of the alternative gelling agents were confirmed using "Suitable" if it was suitable and "Unsuitable" if it was not suitable. Three fundamental properties were assessed; and if the alternative gelling agent met two of the three properties, it was considered a suitable alternative gelling agent.

Experimental design and data analysis The experiment was set up in a completely randomized design with seventeen treatments, each treatment had five replications. SPSS software (version 22, IBM, Armonk North Castle, New York) was used to analyze quantitative data, and results were expressed as means of the independent replications ± standard error (SE). Data were subjected to a one-way analysis of variance, and the mean values were compared using Tukey's

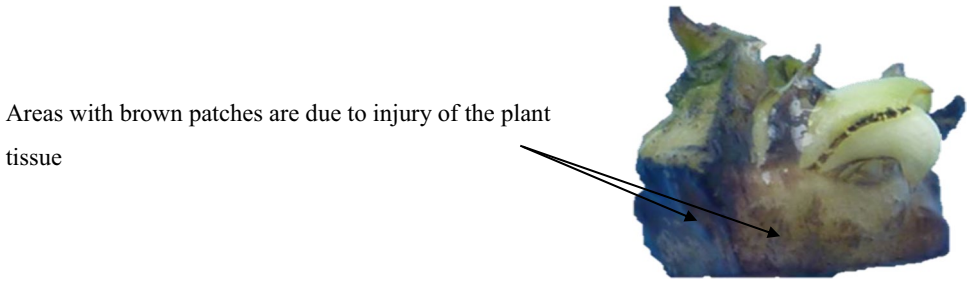

Figure 2. Apantu (*Musa* AAB) develop dark brown areas (browning problems) after 1 wk in the media due to the cutting off of the outer leaf sheaths of the explants. Browning is due to polyphenolic oxidation for pathogen protection of the wounded areas. The explant has to be taken out, the dark brown areas cut out, and the explant is then placed in a new media.

post hoc test ($P \leq 0.05$); alternatively, Kruskal–Wallis and Mann–Whitney's *post hoc* test ($P \leq 0.05$) was used.

Results

Cost comparison of the alternative gelling agents The lowest costs of the alternative gelling agents were found with treatments that used Mb, I, CS, and TP. On average, these treatments had about 82% cost reduction compared to the cost of agar (Fig. 1). Again, treatments such as MbA, SA, S, IA, GKA, PSA, PS, CSA, and TPA also had between 32 and 53% cost reduction compared to agar. Interestingly, it was observed that treatments derived from XA and X as a low-cost alternative gelling agent were more expensive than the agar (Fig. 3).

Comparison of physical properties of the different alternative gelling agents Clarity of the media

Four treatments that used IA, I, SA, and GKA as alternative gelling agents were comparable to the agar (control) in terms of clarity of the medium. The results demonstrated (Fig. 4) that most of the gelling agents with good clarity had agar as one of the components (IA, SA, and GKA), except I which had no agar in the mixture. Gelling agents with good clarity are those with the least amount of suspended particulate matter when visually inspected under a black and white background. Despite demonstrating good quality in terms of clarity, they were more expensive because of their respective combinations with agar.

The texture of the media Based on the preliminary studies of the texture of the various media, the study separated all treatments into four groups. The first group was classified as "soft," although these were not as viscous as agar (control), and they included Mb, MbA, SA, IA, PSA, CSA, and TPA. The second group was classified as "too soft" (medium could not support the proper growth or development of explants); this included GKA and GK. In the third group, the gelling agents were gluey and consisted of XA, X, I, and S. The fourth group was classified as "hard and gluey," including PS, CS, and TP. The gelling agents in this group had a sticky consistency.

Phenolic compound problem (toxicity) according to the response of Apantu The first group was the treatment that had no or minor phenolic compound problems (< 1.0 times), such as MbA, Mb, SA, I, IA, PSA, and CSA. There was no treatment effect ($P > 0.05$) in terms of phenolic compound production. Agar showed the highest mean number of shoots, 4.6 shoots per explant, significantly different from all treatments (Table 2). This was followed by Mb and MbA, which had 13% and 17% fewer shoots, respectively. The least shoots were produced by PSA and CSA, which had 78% fewer shoots compared to the agar (Table 2). Explants from MbA and SA had the highest average shoot height followed by I, A, Mb, and PSA, significantly different from other treatments. Interestingly, the shoot height derived from the agar treatment was about 13% shorter than the shoot height derived from MbA and SA treatments. Explants from all treatments in the first group had an average number of leaves between 3 and 4. Only explants from Mb had 2.4 leaves *per* explant, which was significantly different from the other treatments in this group. The explant from "I" produced the highest number of roots 4.8 roots *per* explant followed by Mb (3.8), and both treatments were significantly different

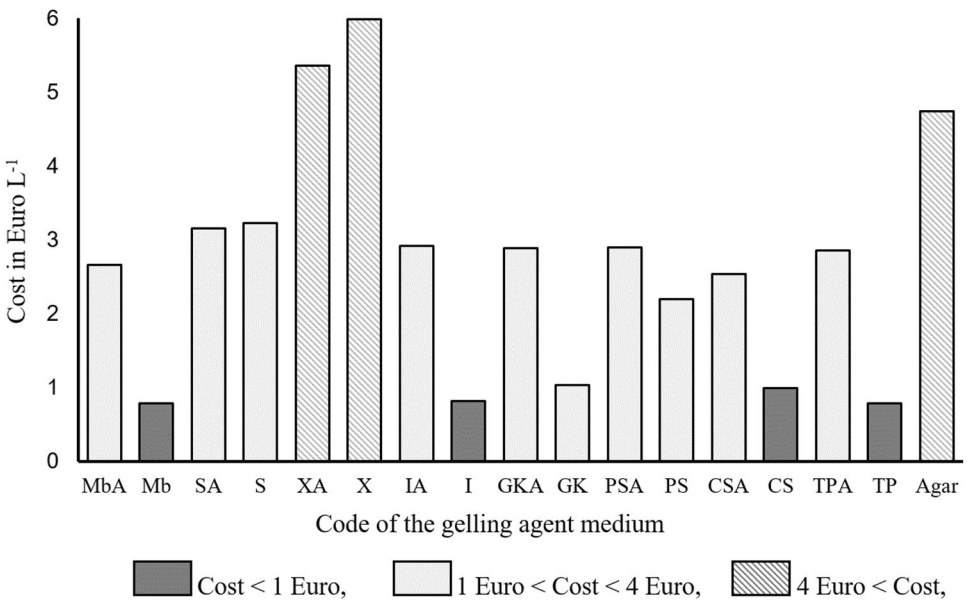

Figure 3. The cost of all different alternative gelling agents in Euro $L.^{-1}$. Agar (A), mung bean (Mb), mung bean plus agar (MbA), sago (S), sago plus agar (SA), xanthan (X), xanthan plus agar (XA), Isabgol (I), Isabgol plus agar (IA), guar gum (GK), guar gum plus agar (GKA), pear sago (PS), pear sago plus agar (PSA), cassava starch (CS), cassava starch plus agar (CSA), tapioca starch (TP), tapioca starch plus agar (TPA).

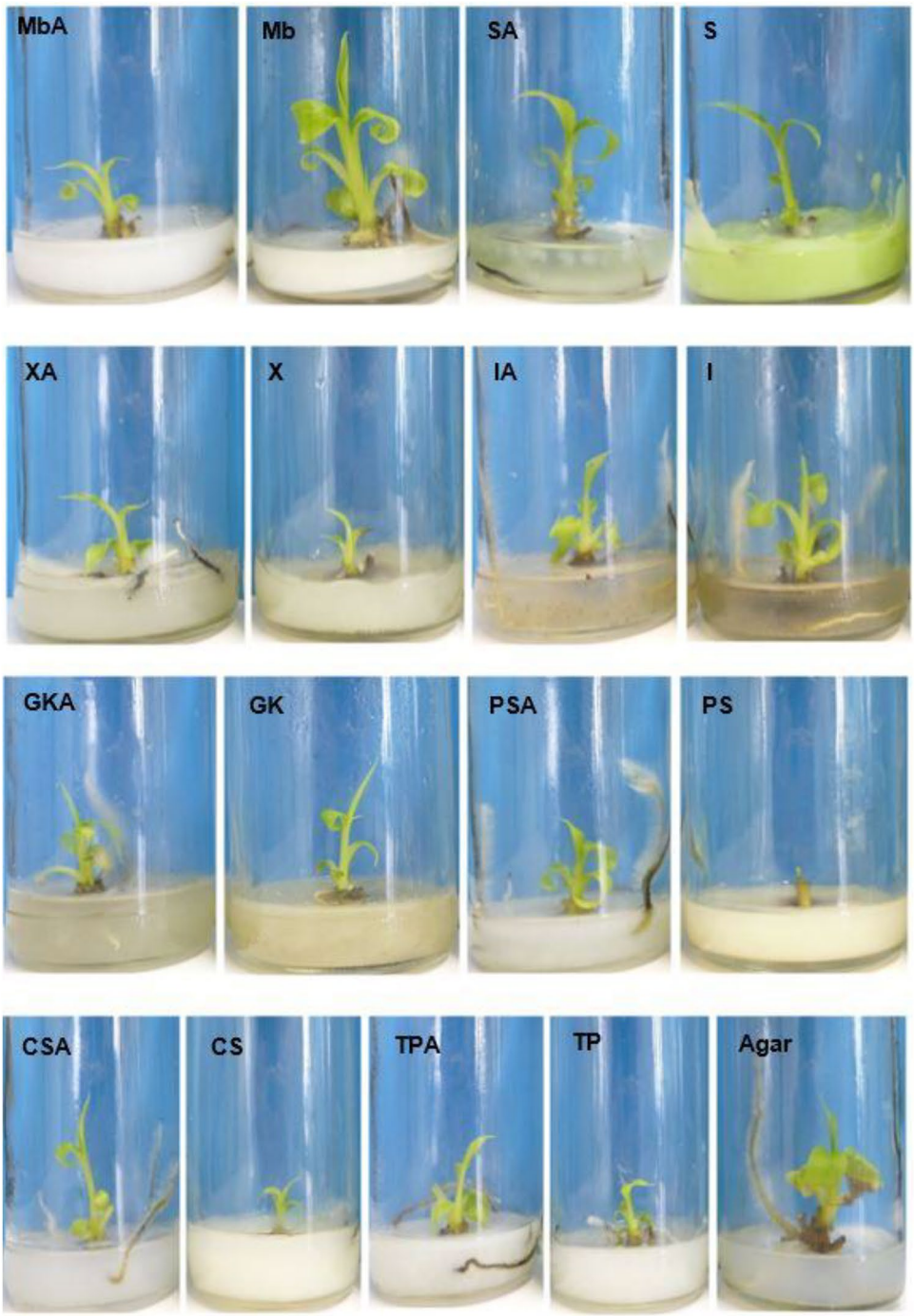

Figure 4. This figure shows explants of Apantu (*Musa* AAB) growing in the different gelling agent's media. The roots of the explants could be seen through the gelling agent with good clarity. Agar (A), mung bean (Mb), mung bean plus agar (MbA), sago (S), sago plus agar (SA), xanthan (X), xanthan plus agar (XA), Isabgol (I), Isabgol plus agar (IA), guar gum (GK), guar gum plus agar (GKA), pear sago (PS), pear sago plus agar (PSA), cassava starch (CS), cassava starch plus agar (CSA), tapioca starch (TP), tapioca starch plus agar (TPA).

from MbA, SA (PSA), and the agar (Table 2). However, all treatments in this group did not show symptoms of the phenolic compound problem (toxicity) to explants and further demonstrated considerable shoot growth. The second group was the treatments showing phenolic compound problems (2 to 3), such as S, XA, TPA, and TP (Table 2). All the treatments in this group were significantly different from the agar and the treatments in the first group. This group's multiplication rate of explants showed only 1.0 shoot *per* explant. Explants from S recorded the highest shoot height followed by TPA, XA, and TP (Table 2). Explants in this group had an average number of leave between 2 and 4, which were not significantly different from the first group (Table 2). The explants in this group (S, XA, TPA, and TP) had an average of 2.6 to 3.6 roots *per* explant (Table 2). The third group was the treatments that had the most phenolic compound problems (> 3 times), such as X, GKA, GK, PS, and CS (Table 2). The multiplication rate of the explants

Table 2 The performance and suitability of different media types regarding different parameters such as the mean and standard deviation of number of shoots, the mean and standard deviation of height of explants, the mean and standard deviation of number of leaves, and the mean standard deviation of number of roots of '*Musa* AAB' tissue culture

Treatment number	Code of medium	Phenolic compound problem (point)	Mean ± SD (shoots per explant)	Mean ± SD of shoot height (cm)	Mean ± SD (leaves per explant)	Mean ± SD (roots per explant)
1	MbA	0.0 ± 0.0 a	3.8 ± 0.4 b	15.8 ± 3.2 a	3.2 ± 0.8 abc	2.4 ± 0.9 cdef
2	Mb	0.0 ± 0.0 a	4.0 ± 1.2 b	13.0 ± 2.8 ab	2.4 ± 0.5 cd	3.8 ± 1.3 abc
3	SA	0.0 ± 0.0 a	2.4 ± 0.5 c	15.6 ± 2.1 a	3.2 ± 0.4 abc	2.4 ± 0.9 cdef
4	S	2.0 ± 0.0 c	1.0 ± 0.0 e	14.2 ± 2.7 ab	2.4 ± 0.9 cd	2.8 ± 0.4 abcdef
5	XA	2.6 ± 0.5 de	1.0 ± 0.0 e	10.4 ± 3.5 bcde	3.4 ± 0.5 abc	3.2 ± 0.8 abcde
6	X	3.0 ± 0.0 e	1.0 ± 0.0 e	9.4 ± 1.9 bcde	2.8 ± 0.4 abc	2.4 ± 0.9 cdef
7	IA	0.8 ± 0.4 b	1.0 ± 0.0 e	13.6 ± 1.9 ab	4.0 ± 0.7 a	4.6 ± 1.1 ab
8	I	0.4 ± 0.6 ab	1.8 ± 0.8 d	14.0 ± 1.6 ab	3.8 ± 1.3 ab	4.8 ± 1.3 a
9	GKA	3.0 ± 0.0 e	1.0 ± 0.0 e	11.0 ± 1.6 abcde	2.6 ± 1.1 bcd	2.8 ± 0.4 abcdef
10	GK	3.0 ± 0.0 e	1.0 ± 0.0 e	11.4 ± 2.1 abcd	2.8 ± 0.4 abc	1.6 ± 0.5 def
11	PSA	0.0 ± 0.0 a	1.6 ± 0.5 d	10.6 ± 1.1 bcde	3.2 ± 0.8 abc	1.8 ± 0.8 def
12	PS	4.0 ± 0.0 f	1.0 ± 0.0 e	6.4 ± 2.1 e	0.0 ± 0.0 e	1.0 ± 0.0 f
13	CSA	0.4 ± 0.5 ab	1.0 ± 0.0 e	9.8 ± 2.6 bcde	3.6 ± 1.1 abc	3.6 ± 1.1 abcd
14	CS	3.0 ± 0.0 e	1.0 ± 0.0 e	8.4 ± 2.3 de	1.6 ± 0.5 d	1.2 ± 0.4 ef
15	TPA	2.0 ± 0.0 c	1.0 ± 0.0 e	10.6 ± 1.1 bcde	3.2 ± 0.8 abc	3.6 ± 1.1 abcd
16	TP	2.2 ± 0.4 cd	1.0 ± 0.0 e	8.8 ± 2.4 cde	3.4 ± 0.5 abc	2.6 ± 0.5 cdef
17	Control	0.0 ± 0.0 a	4.6 ± 0.5 a	13.6 ± 2.7 abc	3.2 ± 0.8 abc	1.2 ± 0.4 ef

*__Note:__ Values represent mean ± standard error. For each treatment, values followed by the same *letter* within a *column* are not significantly different at $p \leq 0.05$. *A*, agar; *Mb*, mung bean; *MbA*, mung bean plus agar; *S*, sago; *SA*, sago plus agar; *X*, xanthan; *XA*, xanthan plus agar; *I*, Isabgol; *IA*, Isabgol plus agar; *GK*, guar gum; *GKA*, guar gum plus agar; *PS*, pear sago; *PSA*, pear sago plus agar; *CS*, cassava starch; *CSA*, cassava starch plus agar; *TP*, tapioca starch; *TPA*, tapioca starch plus agar

in this group showed only 1.0 shoot *per* explant with an average height of 6.4 to 11.4 cm and an average number of leaves between 0 to 3 leaves and 1 to less than three roots *per* explant. Treatments in this group were the most unsuitable for the plant to grow. Furthermore, the texture of the medium was either too hard or too gluey; the consequence was that it triggered a lot of phenolic compound problems making it even more toxic for the plants. The indicator for non-toxicity was the average number of shoots *per* explant. For the model plant in this study, plantain (Apantu), the alternative gelling agents Mb (4.0) and MbA (3.8), produced the highest numbers of shoots *per* explants after the agar (4.6) indicating the lowest phenolic compound problems in (Table 2).

Phenolic compound problem (toxicity) according to the response of Apem The results for the model explants of plantain (Apem) were also categorized into three groups. The first group was the treatments with no or minor phenolic compound problems (< 1.0 times), such as MbA, Mb, SA, PSA, I, and CSA. All treatments in this group were not significantly different from the agar (0.0 times) at $p \leq 0.05$ (Table 3). The multiplication rate of explants in this group showed only 1.0 shoot *per* explant except for PSA with 1.2 shoots *per* explant (Table 3). Explants from Mb and I recorded the highest average shoot height followed by the control, MbA, SA, and IA (Table 3). Explants from Isabgol (I) had the highest average number of leaves but were not significantly different from Mb; however, both treatments were significantly different from other treatments in the same group. Explants from MbA showed the lowest number of roots at 1.4 roots *per* explant, which was between 50 and 65% lower compared to other treatments in the same group. The second group was the treatments with some phenolic compound problems (2.0 to 3.0 times), such as GKA, CS, XA, IA, TPA, and TP. All treatments in this group were significantly different from the agar and the first group. This group's multiplication rate of explants showed only 1.0 shoot *per* explant, an average height of 9 to 10.8 cm, average leaves of between 1.8 and 3.2, and an average number of roots between 2.4 and 3 *per* explant (Table 3). The third group was the treatments with the most phenolic compound problems (> 3.0 times), such as S, X, GK, and PS. This group's multiplication rate of explant showed only 1.0 shoot *per* explant, an average height of 6.0 to 11.6 cm, and an average number of leaves between 0.8 to 2 and 1.4 to 2.4 roots *per* explant. The alternative gelling agent PSA had the highest number of shoots *per* explant (Table 3).

Table 3 The performance and suitability of different media types regarding different parameters such as the mean and standard deviation of number of shoots, the mean and standard deviation of height of explants, the mean and standard deviation of number of leaves, and the mean standard deviation of number of roots of '*Musa* AAAB' tissue culture

Treatment number	Code of medium	Phenolic compound problem (point)	Mean ± SD (shoot per explant)	Mean ± SD Shoot height of explant (cm)	Mean ± SD (leave per explant)	Mean ± SD (root per explant)
1	MbA	0.4 ± 0.5 a	1.0 ± 0.0 b	11.8 ± 3.0 abc	2.6 ± 0.5 def	1.4 ± 0.5 b
2	Mb	0.0 ± 0.0 a	1.0 ± 0.0 b	14.4 ± 1.7 a	4.0 ± 0.0 abc	3.2 ± 1.3 ab
3	SA	0.0 ± 0.0 a	1.0 ± 0.0 b	10.8 ± 2.3 abcd	2.4 ± 0.9 defg	3.0 ± 1.2 ab
4	S	3.4 ± 0.5 d	1.0 ± 0.0 b	11.6 ± 2.1 abc	1.8 ± 1.6 fgh	2.0 ± 1.0 ab
5	XA	3.0 ± 0.0 cd	1.0 ± 0.0 b	10.0 ± 1.6 abcde	1.8 ± 0.8 fgh	2.8 ± 1.1 ab
6	X	3.4 ± 0.5 d	1.0 ± 0.0 b	6.6 ± 1.3 de	0.8 ± 0.8 hi	2.4 ± 2.1 ab
7	IA	2.2 ± 0.4 b	1.0 ± 0.0 b	10.8 ± 2.3 abcd	2.8 ± 1.1 cdef	2.6 ± 0.5 ab
8	I	0.6 ± 0.5 a	1.0 ± 0.0 b	14.4 ± 1.5 a	4.6 ± 0.5 a	4.0 ± 1.4 a
9	GKA	2.6 ± 0.9 c	1.0 ± 0.0 b	9.2 ± 2.2 bcde	2.2 ± 1.3 defg	2.8 ± 1.1 ab
10	GK	3.2 ± 0.8 d	1.0 ± 0.0 b	8.8 ± 2.8 cde	1.2 ± 0.8 ghi	2.4 ± 0.5 ab
11	PSA	0.0 ± 0.0 a	1.2 ± 0.4 a	11.4 ± 4.0 abc	3.4 ± 1.1 abcd	2.8 ± 0.8 ab
12	PS	4.0 ± 0.5 e	1.0 ± 0.0 b	6.0 ± 1.6 e	2.0 ± 1.6 i	1.4 ± 1.1 b
13	CSA	0.0 ± 0.0 a	1.0 ± 0.0 b	10.2 ± 1.6 abcde	4.2 ± 1.1 ab	3.2 ± 0.8 ab
14	CS	2.6 ± 0.5 c	1.0 ± 0.0 b	9.2 ± 2.2 bcde	2.0 ± 1.6 efgh	2.4 ± 0.5 ab
15	TPA	2.0 ± 0.0 b	1.0 ± 0.0 b	9.0 ± 1.2 bcde	3.2 ± 0.8 bcde	2.8 ± 1.1 ab
16	TP	2.0 ± 0.0 b	1.0 ± 0.0 b	10.0 ± 1.6 abcde	3.2 ± 1.3 bcde	3.0 ± 0.7 ab
17	Control	0.0 ± 0.0 a	1.0 ± 0.0 b	13.6 ± 2.1 ab	3.0 ± 0.7 bcdef	3.0 ± 0.0 ab

*Values represent mean ± standard error. For each treatment, values followed by the same *letter* within a *column* are not significantly different at $p \leq 0.05$. *A*, agar; *Mb*, mung bean; *MbA*, mung bean plus agar; *S*, sago; *SA*, sago plus agar; *X*, xanthan; *XA*, xanthan plus agar; *I*, Isabgol; *IA*, Isabgol plus agar; *GK*, guar gum; *GKA*, guar gum plus agar; *PS*, pear sago; *PSA*, pear sago plus agar; *CS*, cassava starch; *CSA*, cassava starch plus agar; *TP*, tapioca starch; *TPA*, tapioca starch plus agar

Evaluation of suitable alternative gelling agent The toxicity of the two model plants was included in the evaluation (Table 4). The toxicity results are similar for all the alternative gelling agents except S (sago starch 220 g) because the phenolic compound problem was an issue with this gelling agent. A gelling agent could be suitable but not low-cost, or low-cost and not suitable. A gelling agent such as IA met all three suitable gelling properties criteria, but the cost was above one Euro; therefore, it was not an excellent low-cost alternative gelling agent. Other gelling agents, such as MbA, SA, and PSA, met two of the suitable properties of the criteria but were unsuitable because their costs were above one Euro, as shown in Table 4. It is shown that gelling agents, such as TP and CS, with a cost of less than one euro did not meet two of the suitable properties of the criteria (Table 4). Mb and I were the gelling agents that met at least two of the suitable properties of the criteria and still had their cost below 1 Euro (Table 4).

Discussion

Due to the fragile nature of the food system, it is critical to find an appropriate low-cost gelling agent to mitigate food insecurity through the rapid propagation of critically endangered crops in rural areas. In this study, the current experimental model using plantain demonstrated that some locally available starch-based substrates in many developing countries could be used as an appropriate low-cost gelling agent in tissue culture propagation. Some of the substrates used as gelling agents in the study had suitable gelling properties, but the cost was considered high for the region. Other substrates tested were cheap, but their gelling properties were not good *per* the criteria set. However, in line with the research question, the study found two substrates (mung bean starch (Mb) and Isabgol (I)) with both having suitable gelling properties and reasonably low cost. This is in contrast to previous research that focused only on low cost (Kodym and Zapata-Arias 2001; Agrawal *et al*. 2010; Gitonga *et al*. 2011) or only on suitable gelling properties (Daud *et al*. 2011; Raina and Babbar 2011; Singh and Kaur 2011). The findings from the present study have demonstrated a huge potential to employ some commonly available materials within reach of smallholder farmers for media preparation. The practical implication of the present findings was that many rural communities in developing countries can now use tissue culture technology to propagate indigenous crops that are difficult to propagate due to changes in land use and eating habits making them endangered, as reported by Keller *et al*. (2005).

Table 4 Suitability of the gelling agent's properties and the suitability of the cost of the model explants

Treatment number	Code of the medium	Texture	Clearly	Phenolic compound problems (Apantu)	Phenolic compound problems (Apem)	Suitable medium	The cost In Euro L^{-1}
1	MbA	Suitable	Unsuitable	Suitable	Suitable	Suitable	2.66
2	Mb	Suitable	Unsuitable	Suitable	Suitable	Suitable	0.78
3	SA	Suitable	Unsuitable	Suitable	Suitable	Suitable	3.15
4	S	Unsuitable	Unsuitable	Suitable	Unsuitable	Unsuitable	3.22
5	XA	Suitable	Unsuitable	Unsuitable	Unsuitable	Unsuitable	5.36
6	X	Unsuitable	Unsuitable	Unsuitable	Unsuitable	Unsuitable	5.98
7	IA	Suitable	Suitable	Suitable	Suitable	Suitable	2.92
8	I	Unsuitable	Suitable	Suitable	Suitable	Suitable	0.82
9	GKA	Unsuitable	Suitable	Unsuitable	Unsuitable	Unsuitable	2.88
10	GK	Unsuitable	Unsuitable	Unsuitable	Unsuitable	Unsuitable	1.03
11	PSA	Suitable	Unsuitable	Suitable	Suitable	Suitable	2.89
12	PS	Unsuitable	Unsuitable	Unsuitable	Unsuitable	Unsuitable	2.19
13	CSA	Suitable	Unsuitable	Suitable	Suitable	Suitable	2.53
14	CS	Unsuitable	Unsuitable	Unsuitable	Unsuitable	Unsuitable	0.99
15	TPA	Suitable	Unsuitable	Suitable	Suitable	Suitable	2.85
16	TP	Unsuitable	Unsuitable	Suitable	Suitable	Unsuitable	0.78
17	Agar	Suitable	Suitable	Suitable	Suitable	Suitable	4.74

*A, agar; *Mb*, mung bean; *MbA*, mung bean plus agar; *S*, sago; *SA*, sago plus agar; *X*, xanthan; *XA*, xanthan plus agar; *I*, Isabgol; *IA*, Isabgol plus agar; *GK*, guar gum; *GKA*, guar gum plus agar; *PS*, pear sago; *PSA*, pear sago plus agar; *CS*, cassava starch; *CSA*, cassava starch plus agar; *TP*, tapioca starch; *TPA*, tapioca starch plus agar

The results from both genotypes, Apantu and Apem, were similar. However, the phenolic compound problems impact with sago as an alternative gelling agent using Apantu and Apem showed different results. In this case, Apem had more phenolic compound problems than Apantu and less multiplication rate. The response of the two plantain cultivars in terms of phenolic compound problems to the various gelling agents demonstrated that Apem had more phenolic compound problems than Apantu and less multiplication rate when cultured on media with sago as a gelling agent. This difference in phenolic response between Apem and Apantu could be attributed to the genotypic differences that exist between the two cultivars. While Apem is French plantain, Apantu is a False Horn (Dadzie and Wainwright 1995), and the difference in variety might affect the multiplication rate of the explants. The low multiplication rate of Apem compared to Apantu found in the present study was in line with studies by Mensah *et al*. (2017), which indicated that the Apantu genotype multiplication rate and even the resulting suckers were better than the Apem genotype. The generalizability of this study was limited because the investigation just focused on one crop to assess the gelling agents. Thus, different plants with food security implications in developing countries, such as cocoyam and indigenous vegetables, should be used to confirm the suitability of these gelling agents.

One of the key pillars of food security and a major cause of food insecurity in many developing countries is economic access to food (money to buy food) (Jiao *et al*. 2012). Therefore, it is vital that when introducing new technology, such as tissue culture, the cost should be affordable to increase the adoption rate in poor communities. With the cost of the gelling agent making over 70% of the cost of tissue culture media, according to Gour and Kant (2011), this study sought to find a cheaper gelling agent as a tissue culture media component for growers in developing countries. Except for X and XA, all evaluated substrates were cheaper than agar, which is in agreement with previous reports that also concluded that starch, as a gelling agent, was cheaper than agar (Kuria *et al*. 2008). However, none of the starch substrates demonstrated the potential to be used routinely as agar due to starch-based gelling agents' weak solidification properties (Kuria *et al*. 2008). This to a large extent justifies why starch is frequently used either in combination with other solidifying agents like agar or agarose (Henderson and Kinnersley 1988; Jain-Raina and Babbar 2011), hence one of the reasons why the present study adopted the approach of combining some of the substrates with agar in preparation of some treatments.

The findings from the current study indicated that the combination of the different substrates with 50% of agar in the media as gelling agent produced positive results in plant response. However, the cost of the substrates with 50% agar was 1 to 4 times higher than the substrates alone but cheaper than agar alone. These results were supported by the findings of Gonçalves and Romano (2005), which demonstrated that starch-based substrates, such as locust gum, could be used

in combination with agar in a culture medium for *Ceratonia silique* L. propagation. Moreover, a mixture of xanthan gum: Agar (6:4) could serve as an ideal replacement for agar (Raina and Babbar 2011). In an attempt to multiply two pear cultivars, Zimmerman *et al*. (1995) demonstrated that starch and gel rite mixture was easy to prepare, and the cost was 10 to 15% cheaper than that of agar alone. The findings from the present study revealed that most of the 16 treatments were cheaper than agar (control) except xanthan (X) and xanthan mixed with agar (XA) with the cost of both treatments being more expensive than the agar. In addition to its high cost, the texture of the media with xanthan (X) was extremely gluey and unsuitable for growing the explants. The texture was improved after mixing with agar (XA); consequently, the cost of the mixture was even more expensive than agar alone. This outcome was in sharp contrast to the findings of Jain and Babbar (2006), who reported that xanthan was three times cheaper than agar. This variation in xanthan and agar costs could possibly be associated with the location where they were purchased. This study further found substrates, such as Mb, I, and TP, to be 4 to 6 times cheaper than agar. These substrates were less than one Euro L^{-1} and were in line with the study's hypothesis to find a low-cost gelling agent that is affordable for developing countries. The results of the present study support the finding of Bhattacharya *et al*. (1994), which showed Isabgol (I) to be a low-cost gelling agent for the propagation of plantlets of chrysanthemum (*Dendranthema grandiflora* Tzvelev). Other gelling agents that were tested, such as MbA, SA, S, IA, GKA, PSA, PS, CSA, and TPA, were 0.3 to 1.4 times cheaper than agar (control).

As Patil *et al*. (2012) described, the physical appearance and clarity of gelling agents depend on suspended particulate matter in the media. A visual inspection determined the gelling agents' clarity under a white background (Kumar *et al*. 2019). Four gelling agents (IA, I, SA, and GKA) out of 16 treatments employed had suitable clarity to agar. This made it possible to examine the roots' development of the explants and, most importantly, contaminants and phenolic compound problems coming from the explants in the course of the experiment. Three out of four gelling agents identified with suitable clarity had agar added to them. The addition of agar was the reason for the clarity of the media. Isabgol (I) showed the best overall clarity, which aligns with previous studies of Isabgol (I) being used as a gelling agent (Jain *et al*. 1997). It is worth mentioning that the other gelling agents with agar addition that had suitable clarity were more expensive to use as low-cost alternative gelling agents. Unfortunately, the other treatments had poor clarity making it difficult to consider a suitable alternative to agar.

Stable support for the explants to grow is vital to the success of plant tissue culture operation, and a suitable gelling agent should offer a semi-solid and stable structure within the media culture (Sanchez-Cardozo *et al*. 2019). When the media is too soft, the explants will fall to the side as they grow. When the media is viscous, it can support the explants to grow upright. However, when the mixture is hard and gluey, it becomes challenging to manipulate the explants, and the explants have difficulties absorbing nutrients from the media. The 16 treatments from the present study were assessed and classified into four groups. The first group was described as soft but viscous, and they could support the growth of the explants in the media. These treatments included Mb, MbA, SA, IA, PSA, CSA, and TPA. The second group of treatments included GKA and GK (guar gum), and they were classified as too soft to support the explants to grow upright. However, other studies have used guar gum as a gelling agent for *in vitro* seed germination of *Linum usitatissimum* and *Brassica juncea* (Babbar *et al*. 2005). This means that how soft a gelling agent could be is relative to the type of explants being propagated. In the present study, the plantain explants could not stay upright.

The third group of treatments included XA, X, I, and S. These were gluey when used making it difficult to dispense the media into the culture bottles. The fourth group of gelling agents was classified as gluey and hard (high solidification properties). These included PS, CS, and TP. Explants from these treatments had poor plant growth responses (average number of leaves and roots), which could be attributable to poor absorption of nutrients by the explants in the media. The results agree with Scholten and Pierik (1998) and Gonçalves and Romano (2005) whose studies demonstrated that the gelling agent might influence the availability of mineral salts and microelements uptake. Moreover, the gelling agents PS, CS, and TP, which quickly solidified and are gluey, showed explants growing, producing more phenolic compounds, and resulting in poor plant growth and development. Phenolic compounds protect plants and prevent nutrient uptake by the same plants (Chikezie 2012).

The concentration of starch in the alternative gelling agent affects the softness of the media. Some studies have shown that by increasing the concentration of starch by up to 10%, the softness problem in some of the alternative gelling agents could be overcome (Henderson and Kinnersley 1988; Khan *et al*. 2012). A similar trend was observed by Daud *et al*. (2011), who found that a 6% increase in cassava flour starch concentration gave adequate support for shoot regeneration from the stem segment of *Celosia* sp. micro-propagation. Also, Kuria *et al*. (2008) have reported that an increase in cassava starch concentration by 10% in the media gave the best result in potato micropropagation. However, in the present study, as the starch concentration increased, more of the starch settled at the bottom of the media and proved more difficult to dispense. Again, studies by Naik and Sarkar (2001) have reported that an 8% concentration of sago starch is firm enough for potato tissue culture propagation. Findings from the present study showed that 22% sago starch concentration,

25% of pear sago, and 25% of cassava were appropriate for a firm media. The starch concentration was more than 2.5 times that reported by Naik and Sarkar (2001) for the media to be firm enough for plantain micro-propagation. Probably the source of starch could have also been different in terms of purity, and the preparation process in the factory could also be a factor. This is supported by studies on the comparative properties of some commercial starches (Ratnayake and Jackson 2003). They reported that the processing methods employed for starch extraction, even the raw material source, affected the obtained starch quality.

One of the main challenges of banana and plantain tissue culture is that phenolics result in poor response from the explants (Chikezie 2012). The study categorized different treatments into groups according to their phenolic compound problems based on toxic wound exudates. The first group was the treatment with no phenolic compound problems, which means non-toxic to explants. Treatments in this group included MbA, Mb, SA, I, IA, PSA, and CSA, which showed similar growth characteristics as that of the control. Additionally, the explants exhibited a high multiplication rate. Considering phenolic compound problems alone, alternative gelling agents from this first group are the most suitable to use for the low-cost banana and plantain tissue culture. The second group was the treatment that had some phenolic compound problems. Explants survived but could not grow as those in the first group. This group's alternative gelling agents included S, XA, TPA, and TP. The growth parameters assessed were significantly different from agar and the treatment in the first group. The third group was the treatment with many phenolic compound problems. Explants in this group showed a lot of phenolic compound problems and even the death of some explants including X, GKA, GK, PS, and CS. The second and third groups produced the least number of shoots *per* explants. However, some of the explants in these groups, such as TPA, had a relatively good average height. The results indicated that the more shoots or multiplication rate of an explant, the smaller the average leaves *per* explant, height of explants, and root *per* explant. This could possibly be ascribed to competition by the shoots from the explants. Some authors have also reported that the high multiplication rate of thidiazuron (TDZ) as a growth hormone could largely inhibit shoot elongation (Huetteman and Preece 1993; Castillo *et al*. 2015).

The findings from the present study revealed that the use of 8% of Mb starch as a gelling agent served as an appropriate amount for media preparation in banana and plantain. These media could reduce the cost of gelling agents by six times compared to agar (control). Mb was cheap and had no problem with pH measurement, dispensing medium, and no phenolic compound problems for the explant. Although, mung bean (Mb), after autoclaving the medium, had layers of some water on top. Some studies have shown that this layer of water could result in hyperhydricity, which is a physiological malformation that results in excessive hydration, low lignification, impaired stomatal function, and reduced mechanical strength caused by waterlogging; *in vitro* plants respond similarly to those subjected to flooding stress (Rojas-Martínez *et al*. 2010). However, our study did not find waterlogging stress problems in the explants. Moreover, the findings from the present study demonstrated that using mung bean (Mb) as an alternative gelling agent resulted in explants that grew taller than agar. This could possibly result from high carbohydrate concentration derived from starch-degradation of starch-based gelling agents and other mineral elements that might be made available in the free form after autoclaving mung bean. These results are similar to Ozel *et al*. (2008), who reported that mung bean media autoclaved resulted in osmotic or metabolic effects on the culture. Mung bean had two suitable properties (texture and toxicity), and the cost was 0.78 Euro L^{-1}, making it the least expensive.

Isabgol was another suitable alternative gelling agent revealed by the present study with a cost of 0.82 Euro L^{-1}. Three percent of Isabgol alone could reduce the cost of a gelling agent by 82.7% compared to the control. However, Isabgol (I) and Isabgol in combination with agar (IA) after dissolving in water become viscous and thus challenging to adjust the pH and dispense the medium. These results are in agreement with Jain *et al*. (1997), who reported that Isabgol (I) had a higher melting point (70.6 °C), which necessitates adjusting the pH and dispensing quicker during preparation. Some of these gelling agents, such as IA, had all suitable properties under consideration; however, the cost was above one Euro, but NGOs could use them (Muyanga 2009). All the other alternative gelling agents had a cost *per* liter above one Euro and were not considered low-cost. Because the type of gelling agent to be used depends on the kind of culture, it is not easy to get a gelling agent that will go for all cultures. The results reiterate Jain and Babbra's (2002) ideas about gelling agents, which pointed out that finding a universally acceptable alternative gelling agent is not expected.

Conclusions

Using plantain as a model plant to assess the gelling properties of 16 starch-based substrates and compare their costs, this study established that low-cost alternative gelling agents with suitable gelling properties are available for developing countries. The results confirmed that mung bean (Mb) and Isabgol (I) are alternative suitable low-cost gelling agents for agar. Therefore, this means that smallholder banana and plantain farmers in Sub-Sahara Africa and worldwide can undertake tissue culture operations in their communities with minimum cost.

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I declare that this work has been composed by all the authors and has not been submitted for consideration in any other journal. Except where states otherwise by reference or acknowledgment, the work presented is entirely all authors.

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Declarations

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