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Note paper

Use and Evaluation of Commercial Starch and Gum Arabic Blends as Low-cost Gelling Materials for Tissue Culture Banana Propagation

Islam Fath Alrhman Ahmed Elmahi [1], Abdelazim Mohamed Ali[1], and Ibrahim Abdelmaksoud Ibrahim[2]

1Faculty of Agriculture, Nile Valley University, Atbara, Sudan

2 Kaffaa Project, Berber, Sudan

Corresponding author: azimali58@yahoo.com

Abstract

The study was conducted to assess the efficiency of blend of different quantities of commercial starch and Gum Arabic as media solidifying material and banana plantlets healthy growth as a substitute for high cost conventional gelling materials (agar, agarose and gelrite). Nine blends of these materials from them other using some conventional agar were tested. Result indicated that adding 20-40 grams of commercial starch with 0-5 grams of Gum Arabic to one liter of prepared medium component resulted in sufficient gelling results, with reasonable pH and satisfactory explant growth.

Keywords**:** *Low cost alternatives, Gelling agent, Commercial starch, Gum Arabic, Growth efficiency*

استخدام وتقييم خلائط النشا التجاري والصمغ العربي كمواد تصلد منخفضة التكلفة لإكثار الموز بزراعة الأنسجة

إسلام فتح الرحمن أحمد الماحي [1]، عبد العظيم محمد علي [1]، وإبراهيم عبد المقصود إبراهيم [2]

1كلية الزراعة، جامعة وادي النيل، عطبرة، السودان

2مشروع الكفاءة، بربر، السودان

ممثل المؤلفين azimali58@yahoo.com

المستخلص

أُجريت هذه الدراسة لتقييم كفاءة خلط كميات مختلفة من النشا التجاري والصمغ العربي كمواد تجميد للوسط ، ولنمو سليم لنباتات الموز، كبديل لمواد التصاد التقليدية عالية التكلفة (الآجار، الآجاروز، والجيلريت). تم اختبار تسعة خلائط من هذه المواد منها أخرى باستخدام الآجار التقليدي. أشارت النتائج إلى أن إضافة 20-40 غرامًا من النشا التجاري مع 0-5 غرامات من الصمغ العربي إلى لتر واحد من مُكوّن الوسط المُحضر أدى إلى نتائج تصلد كافية، مع درجة حموضة معقولة ونمو مُرضٍ للنباتات.

الكلمات المفتاحية: *بدائل منخفضة التكلفة، مادة التصلد، النشا التجاري، الصمغ العربي، كفاءة النمو*

Introduction

Bananas are one of the most important tropical fruit crops worldwide. They occupy a significant position in global trade, and playing important role in the economies of many countries. They are also highly valued by consumers, particularly due to their sweet taste and distinctive flavor. They are also well-known from compared to other fruits by their year-round availability, as well as their ability to be transported, traded, and stored (Osman and Daffalla, 2015; Saad and Mustafa, 2009).

Bananas are vegetatively propagated; due to such fact the potential for pathogens and parasites transmission with seedlings from contaminated agricultural areas is high. Those interested in growing the crop have found that using tissue culture to provide clean and affordable banana seedlings is the best option.

The high cost of the media components in tissue culture makes seedlings partially expensive for small producers. This has led scientific platforms to search for low-cost options and develop ideas to reduce seedling production costs. The advantages of tissue culture seedling production compared to traditional propagation methods (suckers) in bananas include higher propagation rates, the production of pathogen free, and uniform planting material, and the reduced space required to produce a large number of plants. Tissue culture plantlets are lighter than plants produced by traditional ways, especially if light pots and rooting mixes were used instead, which makes transportation easier. Plantlets are also develop much faster, seedlings grow faster and more vigorously, have a shorter and more consistent production cycle, produce more leaves and offshoots, and are more uniform than traditional plant offshoots. Banana plants produced by tissue culture flower more abundantly and produce a uniform harvest, leading to better marketing. They also offer a 20-50% higher fruit yield across all components of yield, such as the number of hands, the number of fingers per hand, finger length, finger circumference, and pseudo stem circumference (Anonymous, 2004; Rakshi, *et al*., 2017; Wilson and Tenkouano, 2020). The main drawbacks of tissue culture seedling production are its requirement for specialized skills, sophisticated equipment, and high capital expenditures, which are not readily available to farmers. Furthermore, contamination rates can be still as high as 15% compared to scientific laboratories, mostly caused by fungi, yeasts, and bacterial contaminants. Still, tissue cultured plants are often tender and require more care during the first two months after planting. To mitigate these limitations, attention has focused on modifying the composition of the growing medium to reduce costs and produce large quantities of plantlets and seedlings. One way to reduce the costs of in vitro culture in developing countries and make it more affordable is to gradually replace most of the expensive components with lower-cost alternatives without significant loss in intermediate and final yield (Wilson and Tenkouano, 2020).

Many different gelling materials, such as agar, agarose, and gelrite, are marketed under trade names and are used to give culture media the desired solidifying degree. Gelrite, one of the

traditional standard gelling materials, is very expensive, while a common alternative to gelrite, agar, is slightly less expensive. Agar is the most widely used gelling agent in plant tissue culture. Since its introduction over a century ago, it has remained the most widely used solidifying material in culture media for both microbes and plants. The properties of agar that make it the most widely used solidifying material in tissue culture are its stability, reasonable transparency, and resistance to metabolic changes. Due to its high price, attempts have been made to identify suitable alternatives (Jain and Babbar, 2002). Furthermore, although agar was once thought to be biologically inert and non-toxic, its adverse effects, such as embryonic abortion and reduced culture yield, have been reported. On the other hand, gelrite often causes vitrification or over hydration of cultures (Wilson and Tenkouano, 2020). Due to its stability, reasonable transparency, non-toxicity, and metabolic inactivity, agar extracted from red algae has been the most widely used media solidification material. Although the cost of medium preparation constitutes a small portion of the total cost, obtaining guaranteed culture medium components is not easy and expensive, especially agar in plant tissue culture laboratories, which is commonly used for woody plants. Tested alternatives include Isubgol, derived from the seeds of some plants, guar gum, and several types of starches produced from some plants like wheat, cassava, corn and potato, (Fira *et al*., 2013).

Agar, as traditional gelling agent, has said to have several drawbacks that negatively affect growth and differentiation in many cases. Variability among different agar types, causing vitrification, and the presence of impurities and growth-inhibiting compounds limits its use as a propagation medium in tissue culture (Palanyandy *et al*., 2020). Possible cheaper alternatives to agar include various types of starch. Agar has been tested in commercial micro propagation, and a mixture of starch and gelrite has been found to be effective instead of each of them alone. A mixture of washed starch, potato starch, and semolina (2:1:1) has also been used, and it has been found to reduce the cost of the gelling agent by 70-82%.

Despite its drawbacks, which negatively affect growth and differentiation in many cases as stated by Palanyandy et al., (2020), agar forms a gel that liquefies at 60-100°C and solidifies at 45°C. Therefore, agar gels are stable at all possible incubation temperatures. Furthermore, gels do not react with media components and not broken down by plant enzymes and easy to be dispensed at higher temperatures. The gelling ability of agar gel is regulated by the concentration, shape, and pH of the agar powder used in the culture medium.

Several attempts have been made to identify suitable and cheaper alternatives to expensive agar as a solidifying agent for microbial and plant tissue culture media, such as potato starch and gum extracted from some plants. Similar work has been reported for tissue culture of chrysanthemum (Asad UIIah *et al*., 2013; Asad UIIah *et al*., 2015).

Gums represent one of the most abundant raw materials due to their sustainable and bio safe properties. The term gum is used to describe a group of natural polysaccharides that have wide-ranging industrial applications due to their ability to create viscous solutions or stabilize

emulsion systems. Gum exudates are complex polysaccharides produced by tree species in the genera Acacia and Prosopis. Gum exudation is produced under conditions of heat and drought, as part of the normal metabolism of plants or as a result of protective mechanisms against mechanical or microbial injury (Lelon *et al*., 2010).

Gum Arabic is an exudate obtained from the stems and branches of Acacia trees, which are grown in Sudan as a cash crop in agroforestry systems. The international standards used to assess the quality of Gum Arabic in the global market are based on Sudanese gum extracted from the *Acacia senegal* variety Senegal (Ali and Daffalla, 2018).

Gum Arabic is used as an emulsifier and stabilizer in the food and pharmaceutical industries, as well as in other industrial products that utilize gum products, including adhesives, textiles, printing, lithography, paints, paper sizing, and pottery and glass polishing. Its high solubility, high acidity (pH≈4.5), and low viscosity make Gum Arabic a medium thickener rather than a solidifier (Ali and Daffalla, 2018; Lelon et al., 2010). Rheological properties and pH are considered important factors due to their role in regulating the solubility of medium nutrients and their uptake by cultures. The highest rate of branching in Amelanchier canadensis resulted from a medium containing guar gum as a hardener, while the longest shoots resulted from a medium containing starch as a solidifier. Approximately four times the number of sprouts was obtained on media containing guar gum compared to the weaker results found on media hardened with Isubgol, as a comparison of different gelling agents. Different gelling agents were used, including 6.8 g/L 1-1 fibrous agar, 50.0 g/L 1-1 wheat starch, 20.0 g/L 1-1 guar gum, 15 g/L Isubgol, or 50.0 g/L wheat starch mixed with 0.5 g/L Phytagol (Fira *et al*., 2013).

Several starch sources (cassava, rice, corn, and potato starch) were tested as solidifying agents compared to agar. They found that 60 grams of l-1 cassava starch-agar mixture added to Murshigi and Ascog's medium resulted in the highest number of regenerated seedlings. Corn starch or potato starch-agar mixtures were also highly effective for micro propagation of potato (Daud *et al*., 2011). By studying the effect of gels such as agar, cassava starch, their combinations, and liquid media on fresh weight, nodule number, and seedling survival in potato seedlings, Correa et al. found that the number of nodules was significantly lower on gel media containing agar, and that the fresh weight was higher on the medium.

From a study of the effect of gels such as agar, cassava starch, their mixtures, and liquid media on the fresh weight, tuber number, and survival rate of potato seedlings, Kuria *et al*. (2008) found that the number of tubers was significantly lower on agar-containing gel media and that the fresh weight was higher on liquid media, while the survival rate was higher on gel media. In all studies considered, media mixed with 10% cassava starch proved to be the most effective in culture performance (Kuria *et al*., 2008). Three banana genotypes (tetraploid hybrids PITA 14 (AAAB) and BITA 3 (AAAB) and the cooking banana (ABB) Cardaba genome) were grown on gelrite and compared with the use of an alternative gelling agent made from cassava starch in

three steps of micro propagation initiation, propagation, and regeneration. The number of buds revealed in BITA 3, PITA 14, and Cardaba did not differ significantly between gelrite and starch as a gelling agents during shoot initiation. During both the growth and regeneration stages, there were no significant differences in the effect of gelrite and cassava starch on the number of buds produced by BITA 3 and Cardaba. However, the banana hybrid PITA 14 produced significantly more buds in gelite than in cassava starch at both stages. Cardaba had significantly fewer buds than the hybrid at all stages (Wilson and Tenkouano, 2020). In another study, bananas were found to be affected by the type and concentration of solidifying agent during tissue culture, primarily due to their effect on the variation in the physical properties of the medium. Plant growth and proliferation were higher on solidified media containing 0.9 g L-1 gelrite compared to those on media containing 4–8 g L-1 agar or 2–6 g L-1 gellan gum. Most shoots cultured on 0.7 g L-1 gelrite or 4 g L-1 agar and on liquid media showed poor growth and proliferation due to replication. High concentrations of gellan gum (6 g/L and 8 g/L agar) did not support shoot growth, which was explained by reduced water and mineral salt uptake (Buah *et al*., 1999).

In some recent study Ebile, *et al*. (2022) indicated that some substrates, such as xanthan, had good gelling properties, but their cost was too high (5.98 Euro per liter) to be considered low-cost. Other such as cassava starch, did not have suitable gelling properties; however, the cost was low (0.99 Euro per liter). They also indicated that two other gelling alternative, mung bean, and Isabgol, had suitable gelling properties with less than one euro cost.

Objectives of this study attempted to evaluate effectiveness of different blend concentrations of commercial starch and Gum Arabic as safe and low cost alternative for medium gelling materials in banana micro propagation

Materials and Methods

Experimental site

This experiment was conducted in the tissue culture laboratory of Al Rajhi Kaffaa Company, 5 km from Berber. Berber is located in the center of River Nile State, on the eastern bank of the Nile River, parallel to the course of the Nile. The locality is between latitudes 17.40 and 18.30 and longitudes 32.20 and 34.20. It is bordered by Atbara Locality and to the north by Abu Hamad Locality.

Plant Source:

Grand Nain banana offshoots were brought from the University of Gezira farm, east of Wad Medani, to the tissue culture laboratories of the Kafaa Project in Berber.

Experimental Methods:

The plants were washed for cleaning under running water, and the corms were brought to the company's tissue culture laboratory. They were cleaned and surface sterilized with sodium

hypochlorite, followed by pre cultivation treatments and incubation as stated in the steps bellow. Regenerating Shoots were subsequently taken for further studies according to the proposed experimentation treatments

In the first stage, Murashige and Skoog's (1962) medium salts were used as concentrated solutions. Gelrite was added at a rate of 2 grams per liter as a medium solidifying agent. The pH of the medium was adjusted to 5.8 using both potassium hydroxide and hydrochloric acid. Sucrose was used as a carbon source, and a growth regulator was added. Finally, 30 ml of the medium was poured into 250 ml containers and covered with Teflon caps. The containers were placed in an autoclave at 121°C and 15 psi for 30 minutes to sterilize the medium. The containers were then incubated for 4 days in a dark room at 27°C and examined for contamination before planting. The plant parts were then planted in the culture medium under a sterilization cabinet (hood). The cultures were then incubated at 27°C in complete darkness and transferred to a fresh medium every month for three months. They were then transferred to the multiplication medium specified for each experimental treatment.

The media components and their concentration were shown in Table (1). Solidifying materials used were starch + Gum Arabic.

Table (1) Concentrations of added starch, gum and agar as safe gelling agents

Treatment	MS salt strength	Sucrose concentration g/L	Benzyl adenine concentration mg/L	Added starch in grams	Added gum in grams	Added agar in grams
1	3/4 MS	30	5	8	1	1
2	3/4 MS	30	5	9	1	0
3	3/4 MS	30	5	10	0	0
4	3/4 MS	30	5	20	5	0
5	3/4 MS	30	5	20	0	0
6	3/4 MS	30	5	40	5	0
7	3/4 MS	30	5	40	0	0
8	3/4 MS	30	5	80	5	0
9	3/4 MS	30	5	80	0	0

Results and discussion

Table (2) shows the growth efficiency, degree of solidify of the medium and its degree of reaction (pH) when starch and gum were added to the medium, when it contained Morshigi salts, growth regulators, and sugar. The results showed that media Nos. 1, 2, and 3, from which a full liter was prepared, had varying degrees of liquidity, with 1 and 2 being completely liquid. Media 3 was slightly firm, but after incubating the medium for four days, it was observed that media 3

had become completely liquefied. As for media Nos. 4, 5, 6, 7, 8, and 9, a quarter liter (250 ml) of each was prepared. The higher the amount of starch added, the higher the pH reading. The addition of gum also leads to some cohesion between the components of the culture medium.

Regarding growth efficiency

Readings taken after four weeks of cultivation in solidified media ranged from poor, good to average. The best readings resulted from the addition of 20 grams of starch. It was noted that media containing high levels of starch increased the degree of solidifying and reduced growth efficiency. Kuria *et al*., (2008) indicated that the starch addition should not exceed 10%. The results obtained differ somewhat from those obtained by Wilson and Tenkouano (2020) on banana hybrids and are somewhat similar to the results of Kuria *et al*. (2008) on potatoes. Daud *et al*., (2011) and Fira et al., (2013) also achieved good performance using some gums and starch as additives to the medium as gelling agents.

From the above it can be concluded that some physical, chemical and biological attributes of the culture media can be adjusted to be suitable for use according to their capability of supporting plantlets growth and their parts development and differentiation. Such findings are similar to what have been reported by Wilson and Tenkouano, (2020). Partially firmness and viciousness is desirable in the medium to let plantlets float with normal respirations while its partially liquefied properties can help plantlets to absorb water and required nutrients and later to develop penetrating roots deep inside the media.

Capability of forming firm rheological phase is a factor of different conditions; the most prominent of it is medium pH and incubation room temperature. Gums remain highly viscous in high concentration regardless of change in incubating room temperature and with relatively low pH values. However it is chemically inert material. Commercial starch as solidifying gelling materials has some drastic impact on plantlets development if exceeds more than 10-15 % concentration although having good dispensing qualities in higher temperatures and excellent solidification properties at incubating temperatures. Different blending concentration of these materials tried to decrease adverse and makes use of good properties of the two materials.

Wilson and Tenkouano, (2020) reported different response of different banana type to the same culture media. That may indicates the expected reaction of different genetic combination to the tested medium blend

Table (2) The Degree of solidifying as an effect of using mixture of commercial starch and gum as gelling agents with or without agar

Treatment	Ms Salt strength	Sucrose concentration g/L	Benzyl adenine concentration mg/L	Added starch in grams	Added gum in grams	Added agar in grams	Solidifying degree *	Medea pH	Growth efficiency **
1	3/4 MS	30	5	8	1	1	3	5	-
2	3/4 MS	30	5	9	1	0	1	5	-
3	3/4 MS	30	5	10	0	0	1	5	-
4	3/4 MS	30	5	20	5	0	5	5.7	3.6
5	3/4 MS	30	5	20	0	0	6	5.75	3.6
6	3/4 MS	30	5	40	5	0	6	5.74	3.2
7	3/4 MS	30	5	40	0	0	8	5.75	2.4
8	3/4 MS	30	5	80	5	0	8	5.69	3.2
9	3/4 MS	30	5	80	0	0	8	5.70	2.2

*Solidifying degree; 1-5 Liquid, 5-7 Liquid gel, 8-10 Solid gel

**Growth efficiency as described by Bottino, (1981); 1 Death, 2 Weak (Living tissue without growth) 3 Average (growth and doubling not completed)

4 Good (vigorous growth and doubling)

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