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Suitability of Cassava Starch as a Gelling Agent for the *in vitro* Culture of Banana Plantlets

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ABSTRACT

In this study, the suitability of cassava starch for the *in vitro* culture of banana tissues was investigated. A combination of cassava starch and agar was used as a solidifying agent as against pure cassava starch and pure agar. Three types of media with respect to the inclusion of cassava starch were prepared as; media with 60 g cassava starch, 6 g agar and 2 g agar+40 g cassava starch. Explants were prepared by sterilizing them with 70% ethanol which was followed by 1% sodium hypochlorite+tween 20. They were then cultured at 26°C. The conditions in the culture room were 16 h photoperiod, 3000 lux light intensity and a relative humidity of 60%. Tissues cultured on medium with a combination of cassava starch and agar had the highest fresh weight values of 25 g and this was significantly different from those cultured on the other two media. Dry weight was also better (10 g) for plantlets cultured on a combination of cassava starch and agar, compared to those on agar alone which had dry weight value of 8 g. Medium with agar alone had a water potential of -0.4 MPa and that of medium with a combination of cassava and agar was -0.3 MPa. The growth of plantlets was better on medium with cassava starch and agar combination even though the differences in some of the parameters measured were not significant. This also reflected in the number of roots produced per plantlet and subsequently the percentage survival in the nursery. Cassava starch was thus found to be a suitable substitute for agar in the *in vitro* culture of banana.

Key words: *In vitro*, cassava starch, agar powder, banana

INTRODUCTION

Tissue culture techniques have been important tool in the large scale clonal propagation of crops and crop improvement. It is a technique that has been used to propagate many crops, including those that are known to be difficult to propagate conventionally (Buah *et al*., 2010). The most important and large scale use to which tissue culture techniques have been put is the production of healthy planting materials, on a large scale (Buah *et al*., 2010). To achieve the large scale production of healthy seedlings *in vitro* requires a better understanding of the influence of culture conditions on shoot regeneration and development as well as the preparation of the tissue culture medium.

Media for the *in vitro* culture can either be solid or liquid and in the preparation of the solid medium, agar has been the most frequently used solidifying agent. Afrasiab and Jafar (2011) even though various brands and grades of phytagel, gerlite and gellan gum have been used *in vitro* (Debergh, 1983). One of the most expensive ingredients for the preparation of gelled tissue culture medium is agar which contributes about 70% of the total production cost (Nkere and

Mbanaso, 2009). Due to the large input cost in tissue culture media preparation, many researchers have tried other low cost alternatives. For example, Raghu *et al.* (2007) tried household sugar and tap water as a substitute for laboratory sucrose and double distilled water, respectively. Bhattacharya *et al.* (1994) and Naik and Sarkar (2001) used cheaper gelling agents; sago while Gebre and Sathyanarayana (2001) used commercial cassava and sago as gelling agents for the *in vitro* culture of potato.

Mohammed *et al.* (2009) have also reported that a combination of corn starch and potato starch are efficient gelling agents for the *in vitro* propagation of potato. Maliro and Lameck (2004) worked with cassava flour as a gelling agent while Daud *et al.* (2011) used a combination of corn and potato starch as a gelling agent in the *in vitro* culture of banana. Though tissue culture tools have been in application for many years, most developing countries like Ghana, have not significantly benefited from it partly due to the high cost of consumables like agar (Maliro and Lameck, 2004).

Ghana produces cassava tubers in large quantities all year round and an appreciable proportion of it perishes due to the lack of suitable and efficient storage techniques. When the tubers are processed into gari and other food types, the starch which in most cases is a by-product is discarded. It is therefore worthwhile that cassava starch is looked at as an alternative gelling agent in media preparation to reduce the cost of preparing medium in the laboratory.

This study was therefore carried out to investigate the suitability of cassava starch as an alternative to agar as a gelling agent in the *in vitro* culture of banana.

MATERIALS AND METHODS

Plant materials and explant preparation: The study was conducted at the tissue culture laboratory of the University of Cape Coast from July 2012 to February 2013. The banana cultivar, Dwarf Cavendish (Musa accuminata) was used as the explant source. Plant materials were taken from sword suckers that had been grown under good watering regime in the open field at a farmer's farm at Efutu, a village in the Cape Coast Metropolis. The materials were taken early in the morning with earth chisel to separate the sucker from the parent at the point of attachment. Prior to washing the plant material under running tap water, the roots and the top of the shoots were trimmed off. The sheaths that form the pseudostem were carefully removed to reduce the size of the material to about 4 leaf sheaths. They were then sterilized with 70% ethanol for 3 min and washed three times in sterilized distilled water (Buah *et al.*, 2010). More leaf sheaths were then removed aseptically in a clean bench until about two leaves covered the shoot meristem. This process was followed by sterilization with 1% Sodium hypochlorite solution containing a drop of polyoxyethylenesorbitan monolaurate 20 for 5 min with occasional shaking and there after washed three times with sterilized distilled water. Prior to their inoculation on the medium, each shoot tip (about 1 cm) was longitudinally divided into two halves and again sterilized with 1% Sodium hypochlorite (NaClO) as above for 1 min.

Preparation of cassava starch: The method of preparation adopted by Kwoseh *et al.* (2012) was used. Tubers of a cassava variety called Gblemoduade were obtained from a farmer at a village in the Central Region of Ghana. The tubers were peeled, washed and grated. A blender was used to blend 1 kg of the grated cassava into a paste. The paste was then strained into a clean plastic bucket using a cheese cloth and the solution obtained was topped up with 3 L of distilled water. The starch solution was left in the laboratory for 24 h and the supernatant was poured off to obtain a clean starch paste. The starch was then dried for 48 h at room temperature, crushed and stored for future use.

Proximate analysis of starch: Protein, fiber, lipid, carbohydrate were determined by the AOAC (1990) method of analysis.

Media composition: MS medium (Murashige and Skoog, 1962) supplemented with 4.5 mg L^{-1} 6-Benzylaminopurine was used. Three different media were prepared. The difference in the media was the type and amount of gelling agent used to solidify the medium which served as the various treatments and each treatment was replicated 30 times. The culture bottles were arranged in a completely randomized fashion as 60 g cassava starch, 6 g Agar and a combination of Agar 2 g and 40 g cassava starch. About 30 g L^{-1} sucrose was used because it had been the optimal sucrose concentration from previous work with Musa species (Buah *et al*., 2010; Shirani *et al*., 2009). The pH of the media was adjusted to 5.8 before autoclaving for 15 min at 121°C. The explants were inoculated into the various media and kept under a temperature of 26°C, 16 h photoperiod with an intensity of 3000 lux and a relative humidity of 60%. The initial sub culturing was done 4 weeks after initial culture and subsequently at two weeks interval. During subculturing, materials with multiple shoots were separately removed and placed into different vessels. In all, seven subcultures were done during which data was taken.

Determination of media water potential: The isopiestic psychrometer (Tang *et al*., 2002) was used in measuring the water potential of the various media before inoculating them with the explants. Prior to the measurements, the jointed parts of the psychrometer were made airtight by applying vaseline to prevent the escape of water vapor from the samples. The thermocouples were then cleaned with 90% ethanol and rinsed in sterilized distilled water before drying with an air stream. About 0.4 g of each of the media samples were placed one at a time in the chamber and covered with the cylinder containing the thermocouple. A drop of sucrose at a designated concentration was placed at the tip of the thermocouple before inserting it into the cylinder that covers the chamber.

Data was taken from the first subculture on number of shoots, fresh weight (g), shoot height (cm), shoot dry weight (g) and media water potential (MPa), number of shoots and percentage survival.

Statistical analysis: Data was analyzed with Genstat version 7.1 for analysis of variance and Excel 2007 for plotting of graphs (Hilbe, 2007).

RESULTS

The results of the study showed that cassava starch has the potential of substituting commercial agar in the preparation of MS medium for the *in vitro* culture of banana. Some properties of the starch used are shown in Table 1.

Table 1: Percentage protein, fibre, lipid and carbohydrate content of Gblemoduade

Parameters	Values
Protein	0.61
Fibre	2.81
Lipid	1.26
Carbohydrate	60.71

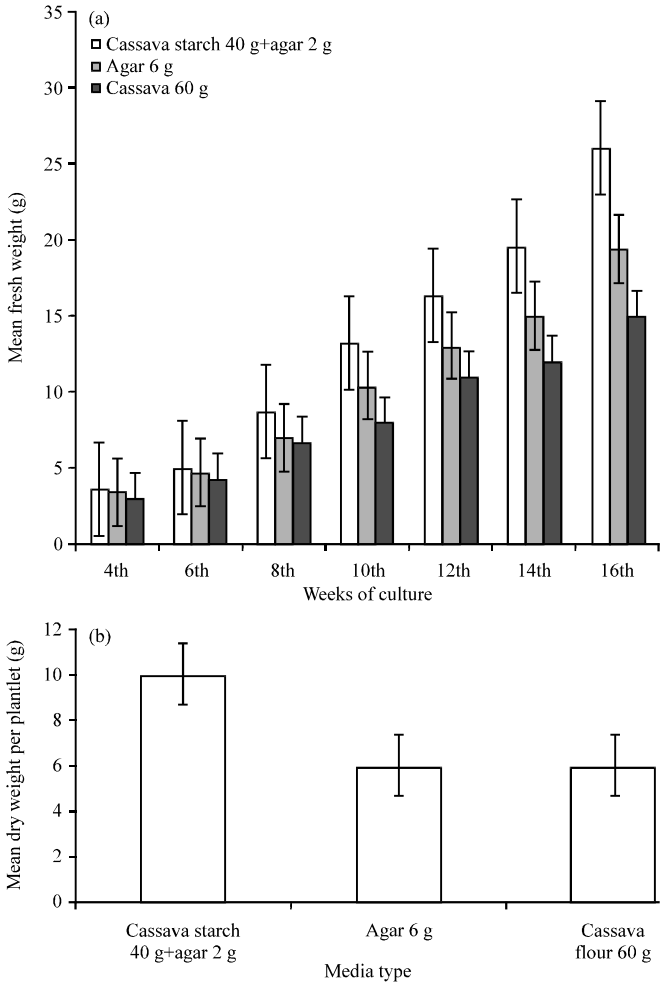

Fig. 1(a-b): Mean (a) Fresh weight and (b) Dry weight of plantlets cultured on media supplemented with agar and cassava flour for 16 weeks

A combination of 40 g cassava starch+2 g agar gave the best results at the end of 16 weeks of culture. This was evident in the fresh weight of the plantlets. Plantlets which were cultured on a combination of cassava starch and agar had the highest fresh weight of 26 g at 16 weeks compared to 20 and 15 g for agar and cassava starch, respectively (Fig. 1a).

The difference in fresh weight among the various treatments was significant. Even though agar supplemented medium appeared better than cassava starch, the growth of plantlets on the cassava starch supplemented medium was appreciable.

The results of the dry weight followed a similar pattern as that of the fresh weight (Fig. 1b) with a combination of cassava and agar supplemented medium producing plantlets of high dry weight compared to the two other treatments.

The number of shoots per plantlet however were high on plantlets cultured on agar powder compared to the other gelling agents and the differences were significantly different (Fig. 2).

The height of plantlets started showing clear increases from the 10th week through to the 16th week. As indicated in Fig. 3, the superiority of agar+cassava starch supplemented medium was

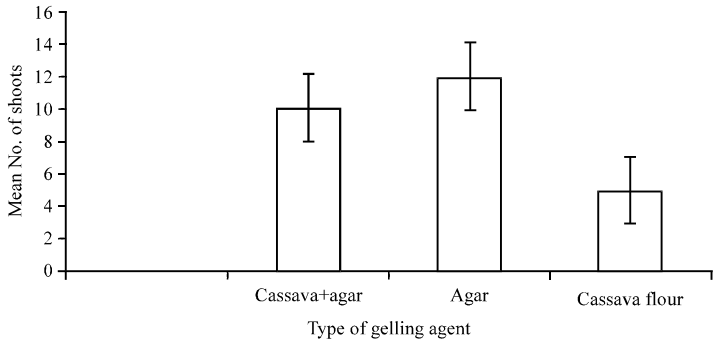

Fig. 2: No. of shoots per tissue cultured on different gelling agents for 16 weeks

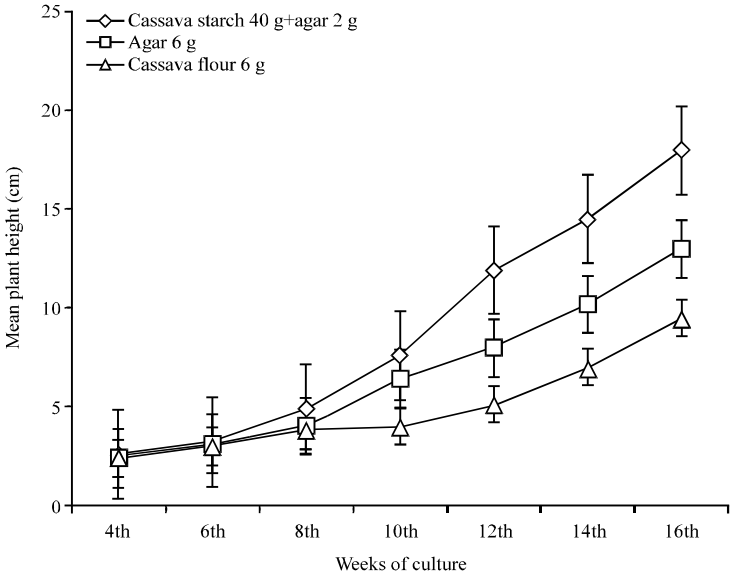

Fig. 3: Mean height of plantlets cultured on media supplemented with agar and cassava flour for 16 weeks

evident in the height of plantlets on the various media. Whilst plantlets cultured on a combination of agar and cassava starch attained an average height of 18 cm, those on agar and cassava starch alone had 12 and 9 cm, respectively and these differences were significant.

Measurement of the water potential of the various media confirmed that the different solidifying agents used had an effect on the media water potential. This measurement was important since the water potential of a medium has a relationship with the growth of explants. From Fig. 4, it could be seen that medium solidified with agar+cassava starch had a water potential of -0.3 MPa whilst medium solidified with agar had a water potential of -0.4 MPa. The highest water potential -0.2 MPa was recorded from the cassava starch supplemented medium.

Plantlets were hardened and transplanted to the field to determine their percentage survival 8 weeks after nursery. In terms of percentage survival, plantlets cultured on a combination of agar and cassava starch performed as well as those cultured on agar powder medium, all having 97%

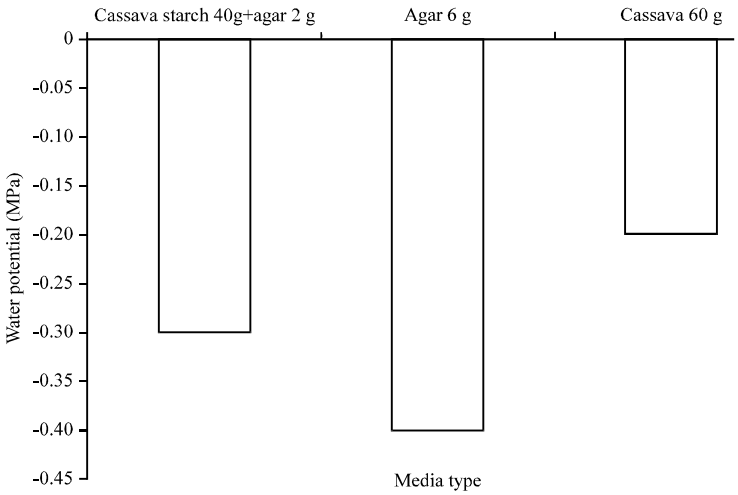

Fig. 4: Water potential of media supplemented with agar and cassava starch

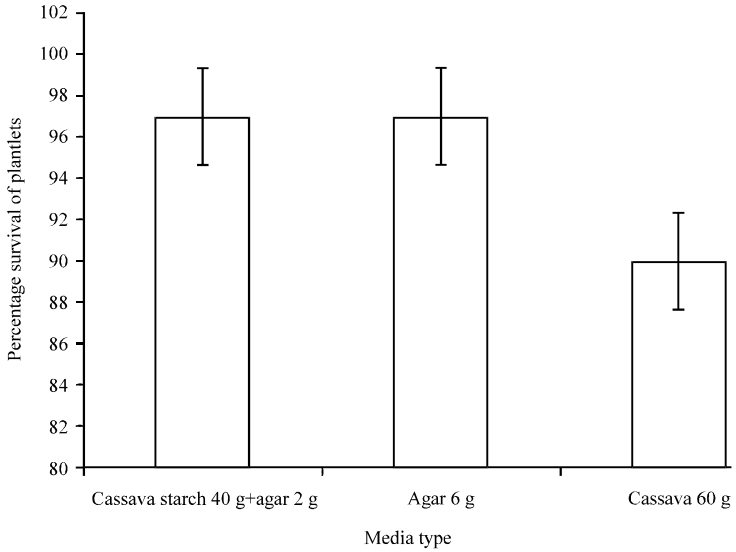

Fig. 5: Percentage survival of plantlets cultured on different media 8 weeks after nursery

survival as shown in Fig. 5. Plantlets that were cultured on cassava starch medium had a 90% survival rate which was significantly different from the two other media.

Roots are very important for the survival of plantlets in the nursery. Plantlets that were cultured on the cassava starch+agar supplemented medium had more roots than those cultured on the other two media. The difference between the cassava starch+agar medium and the two other media was significant (Fig. 6).

DISCUSSION

The growth and development of plantlets *in vitro*, depends on many factors. Among these factors is the movement of water from the medium into the tissues and this largely depends on the type and concentration of gelling agent used. This study assessed the suitability of cassava starch

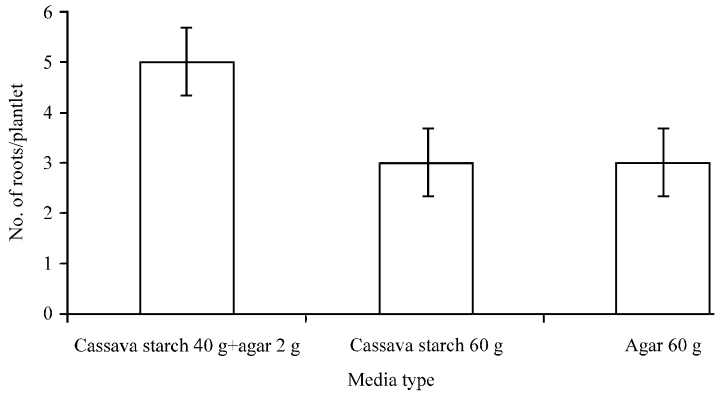

Fig. 6: No. of roots per plantlet cultured on media supplemented with agar and cassava starch for 16 weeks

as a gelling agent for the *in vitro* culture of banana. All the gelling types that were used supported the growth of banana plantlets with a combination of cassava starch and agar offering the best support.

Plantlets that were cultured on a combination of cassava starch and agar produced the highest mean number of shoots 12 shoots/explants in 16 weeks with those on a combination of cassava starch and agar having 10 shoots/explants. Maliro and Lameck (2004) and Mbanaso (2008) have used cassava and agar combination *in vitro* and their results have been collaborated by the findings of this research. Cassava flour acts as an additional carbon source and adds other ionic supplements to the medium which most likely led to improved cell growth and morphogenesis (Onwueme, 1982). Cassava starch has the ability to form hydrogen bonds which results in increased water holding capacity a reason which has been offered by Lupano and Gonzalez (1999). It is likely that these attributes of cassava starch contributed to the high number of shoot recorded on Cassava starch+agar combination in this work. Kuria *et al.* (2008) used a combination of cassava starch and agar to solidify medium and reported that the combination of 8% cassava starch and 0.25% agar powder provided the same firmness as medium solidified with 0.8% agar. The results in Table 1 showed that the amount of protein and lipid in the starch were compared to Nuwamanya *et al.* (2010) results. The presence of protein and lipid helps in the pasting properties and lipids have also been known to improve on the textural properties of starch leading to viscosity stability hence improving the starch quality (Tukomane *et al.*, 2007).

In terms of shoot fresh weight, dry weight, height, number of roots and percentage survival, a combination of cassava starch and agar as gelling agent performed better than the two other gelling agents. However the difference between the cassava-agar combination and the agar supplemented medium was not significant.

Various researchers have used cassava as an alternative gelling gent and have reported the feasibility of using cassava as a substitute for agar. Gebre and Sathynarayana (2001), Mbanaso *et al.* (2001), Umeh and Uguru (2013) as well as Daud *et al.* (2011) have all confirmed that cassava powder alone or its combination with agar is a suitable substiute as a geling agent. Daud *et al.* (2011) has reported that agar improves cassava gel by its improvement of the organic and inorganic composition of the medium while maintaining a good osmotic concentration and this has also been reported by Gebre and Sathyanarayana (2001) and Anoop and Chauhan (2011).

Even though Gebre and Sathyanarayana (2001) used Tapioca, their reasons for improved gel properties of tapioca+agar could be true for cassava starch since tapioca and cassava are very similar.

Water potential of the various media most likely played a role in the differences in the parameters considered in this study. From previous study by Buah *et al.* (2011), banana plantlets performed better on medium with water potential of -0.3 MPa. The cassava starch+agar supplemented medium in this study had a medium water potential of -0.3 MPa whiles cassava alone had water potential of -0.2 MPa and agar with water potential of -0.4 MPa. Where a medium has low water potential, the flow of water into the tissues is affected and this affects leaf conductance and photosynthetic activity. Ramesh *et al.* (2014) and Tang *et al.* (2002) have all given similar reasons and further said that shoot fresh and dry weight are also affected by low water potential. This could explain why plantlets on cassava+agar supplemented medium had better growth parameters (fresh and dry weights, number of roots and height of shoot) compared to the other two. It was evident that the addition of agar to cassava starch improves upon the medium characteristics as shown in the growth of plantlets on medium solidified with only cassava starch which did not perform as well as those on medium solidified with cassava starch+agar combined.

The low growth performance of shoots on medium solidified with cassava alone collaborated the results of other researchers. Gotea *et al.* (2012) reported that cassava supplemented medium loses its consistency after 2 weeks and this causes tissues to sink. This could be due to a drop in the pH of the medium as a result of autoclaving of the medium. Maliro and Lameck (2004) and Lupano and Gonzalez (1999) reported similarly in results. Amylose content of starch have been reported to affect the retrogradation properties of starch which can also affect pasting properties. Charles *et al.* (2004) and Novelo-Cen and Betancur-Ancona (2005) reported that increased amylose content of starch leads to increased retrogradation. During *in vitro* culture, it is possible that the starch will be hydrolysed, breaking down the amylose and the amylopectin in the starch and this can affect the stability of the medium thus making it watery and the plant suffering from hyperhydricity (Czuchajowska *et al.*, 1991; Amaka *et al.*, 2013). The growth of plantlets on cassava starch and agar supplemented medium was better that those cultured on medium with agar alone. Though the difference in some cases were not significant, it is worth stating. Previous research (Buah *et al.*, 2011) showed that agar contained other chemical elements which does not make it inert as has been reported and this could possibly affect to a limited extent, the growth of plantlets. Debergh (1983) have confirmed that the chemical inhibitors in agar could affect the growth of tissues cultured on them.

CONCLUSION

The results of this study has shown that cassava starch can serve as a substitute for agar powder in the *in vitro* culture of banana tissues.

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