THE USE OF CASSAVA STARCH FOR *IN VITRO* REGENERATION AND PROPAGATION OF SWEET POTATO PLANTLETS

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Abstract
Cassava starch was used as a gelling agent for *in vitro* regeneration and propagation of sweet potato plantlets. Shoot height, leaves and node increases of 10.7±2.5 cm, 15.5±1.4 and 14.5±1.6 were obtained from TMS 98/0505, TMS 92/0057 and TMS 92/00057 starch-gelled media compared to 13.6±2.7 cm, 19±2.1 and 17.7±2.0 from gelrite-gelled media respectively after 8 weeks of culture. Growth on TMS 30572-starch gelled medium was generally poor compared to other starch-gelled media and gelrite-gelled medium throughout the 8 weeks of culture. The most probable reasons for the superior performance of gelrite-gelled medium over cassava-starch gelled media could be attributed to the purity and lesser concentration of gelrite in the growth medium. Besides these advantages, availability and the reduced cost of cassava-starch gelled medium is considerable and attractive for its adoption in the sub-Saharan Africa.

Key words: cassava starch; gelrite; *in vitro* propagation

Introduction

Cassava (*Manihot esculenta*) is uniquely suited for food security and can yield starch more efficiently than any other crop (Thro *et al*., 1999). Cassavas is a tuberous root crop that contains up to 30% of starch and are low in proteins, high in soluble carbohydrates and fats, thus extraction and purification of cassava starch is relatively simple and straight forward. It is estimated that about 12 t. of high quality starch per ha could be produced in a single harvest (Cock *et al*., 1979). Starch is one of the most common carbohydrate polymers in nature. It is widely distributed throughout the plant kingdom and functions as a major source of carbon and energy for most organisms**.** In cold water, starch absorbs water reversibly and swells slightly. In hot water, irreversible swelling occurs, producing gelatinization. Hence, the property of forming thick pastes or gels is the bases of many starch uses. Comparatively, the gel stability of cassava starch is much higher compared to cereal starches and generally cassava starch is available and cheaper than corn, yam, sweet potato and potato starches.

Agar, the gelling agent of choice for culture media is widely employed for both plants and microbial cultures. There exist levels and composition of impurities in the existing conventional gelling agents like phytagel, noble agar, agar, gelatin and gelrite. Phytagel and gelrite are purer and more clarity than agar. They are also used in lesser amount per liter than agar to obtain the same consistency (Lima *et al*., 2012). Although these gelling agents are generally good in cultures, recent investigations have cast doubts about their biological inertness and their non-toxic nature (Babbar and Jain, 1998). For example, gelrite was reported to induce hyperhydricity on regenerated shoots (Pasqualetto *et al*., 1988). Inhibitory effects of agar resulting in embryo abortion in anther culture of *Nicotiana tabacum* have been reported by Kohlenbach and Wernicke, in 1978. Henderson and Kinnersley, (1988) also reported that embryo formation from barely anthers was greater on media gelled with starch than on agar-solidified media. They also observed that the frequency of plantlet production from anthers cultured on media with barely starch was five times higher than that from anthers on agar-solidified media.

Medium composition is a prerequisite for a successful micropropagation and conservation. Medium ingredients such as agar, gelrite and noble agar used as gelling agents are imported and considerably expensive in sub-Saharan Africa due to import duties and weak currencies necessitating the search for local alternatives. The use of sago as a cheaper gelling agent has been described by Bhattacharya *et al*., 1994 and Naik and Sarkar (2001). Gebre and Sathyanarayana (2001) investigated the use of cassava and sago starches for *in vitro* propagation of potato (*Solanum tuberosum* L.). Maliro and Lamerck (2004) successfully improved the gelling quality of cassava flour with some agar. Twiwari and Rahimbaev (1992) also worked on barely starch as a gelling agent. Similar investigations have been carried out with guar gum (Babbar *et al*., 2005), isubgol (Jain-Raina and Babbar 2011; Saglam and Cifici, 2010), ispaghol (Hussain Shah *et al*., 2003; Jain *et al*., 1997), gum katira (Jain and Babbar 2002), and locust bean gum (Gonclaves and Romana, 2005). Other researchers have also reported the use of isubgol in micropropagation of commercially important plants such as woad plant, banana, tumeric, blueberry and tobacco (Ozel et al., 2008; Fira et al., 2008; Atici *et al*., 2008, Agrawal *et al*., 2010; Saglam and Cifici 2010), while Chitta and Aolemla, (2013) have successfully cultured *Cymbidium iridioides* on low cost substrate.

Although the gelling quality of cassava starch has been investigated for *in vitro* multiplication of crops like yam, ginger, and cassava, its use for *in vitro* propagation of sweet potato may not have been reported. Sweet potato (*Ipomoea batatas* L. Lam) is among the world's most important versatile and underexploited root crops. It is an important food crop after

cassava, rice and yam in the tropics. It is cultivated mainly for its storage roots and is probably the food crop that produces the most energy per unit area. Its high-energy value exceeds even that of potato, cassava and other known tubers (Ubalua and Okoroafor, 2013). Based on its calorific yields, nutritional value, adaptability, versatility and vegetative reproduction, sweet potato could in future contribute significantly to world agriculture. We now report our successful *in vitro* regeneration and propagation of a local sweet potato cultivar (UMUSP/3) using processed cassava starch as an alternative gelling agent.

Materials and Methods

Extraction of starch milk

Peeled and clean cassava (NR 8082; TMS 92/0057; TME 419; TMS 98/0505; TMS 30572), roots were disintegrated with a grater. The pulps were sieved on a muslin cloth, and flushed with clean water until the starch stream ceases. The crude starch milks were kept in a refrigerator overnight to discourage microbial growth and degradation. The supernatants were decanted leaving the precipitated starch. The precipitated starches were purified with three litres of clean water equivalent to three times the volume of the crude starch yielding a clean starch cake. They starches were dried at room temperature and pulverized with a milling machine (Thomas Wiley Mill-model Ed-5, Philadelphia, USA, 1982) and stored in bottles. Preservation was in a refrigerator (Haier Thermocool, model HRF-350, 2001).

Media preparation

The complete Murashige and Skoog media with vitamins were brought to pH 5.8 before adding the starches and the gelling agents. Gelrite was dissolved in the medium by heating while thick slurries of the starches were first made with 20 ml of the medium to be gelled. The remaining portion of the medium was heated to a temperature of 75 ± 5^0C and the corresponding starch slurry stirred vigorously into it. Ten (10) ml of each of the medium were dispensed into clean test tubes, sealed and autoclaved at a temperature of 121^0C for 15 minutes.

Establishment of cultures

Apical shoot-bud explants were aseptically excised in a laminar airflow cabinet from proliferating shoot cultures of a local sweet potato cultivar (UMUSP/3) after eight weeks of growth in MS basal medium. The shoot-buds were seeded singly into sterile test tubes containing 10 ml of previously sterilized media. The test tubes were sealed properly with parafilm and clearly labeled before growing the cultures at a temperature of 28 ± 2^0C for 16 h photoperiod and 8 h darkness at light intensity of 2000-3000 lux. In all the experiments, 8 plantlets were cultured in each medium (one plantlet per test tube) with 10 replicates per treatment. After 8 weeks, shoot height, number of nodes, number of leaves, rates of survival of the plantlets in the various media and colour were assessed and determined.

Statistical analysis

Completely randomized design (CRD) was used for the experiments. Each treatment was repeated three times and data were obtained every 2 weeks for a period of 8 weeks after the beginning of the treatments. Analysis of variance (ANOVA) was used to compare the number of leaves, nodes and shoot height in all the media investigated. Means were separated using DMRT test at 5% level of significance.

Results and Discussion

The study compared the potentials of gelrite and cassava starch gelled media on *in vitro* shoot regeneration and propagation using sweet potato (*Ipomea batatas* L. Lam) apical dome cuttings as explants. Expectedly, the explants cultured on gelrite and the starch saturated media performed differently. Among the cassava starches and the control (gelrite) investigated, gelrite was observed to have the most significant support on regeneration and growth of the sweet potato plantlets (Table 1). Consequently, the regenerants grown in gelrite-gelled medium produced the highest shoot height of 13.6±2.7 cm compared to 10.7±2.5 cm produced by TMS 98/0505 starch-gelled medium after 8 weeks in culture (Table 1).

Leaf development and growth almost followed a similar pattern as the highest number of leaves recorded in gelrite-gelled medium was 17.4±2.1 compared to 15.5±1.4 produced by TMS 92/0057 starch-gelled medium (Table 2) after 8 weeks in culture. Although there was a significant difference between gelrite-saturated medium and the starch-saturated media in supporting leaf growth and development, TMS 92/0057-starch medium compared favourable with gelrite-gelled medium after 8 weeks of culture (Table 2) implying that TMS 92/0057 could substitute gelrite as a gelling agent in *in vitro* regeneration and multiplication of sweet potato plantlets.

Table 1: Mean shoot height produced by the *in vitro* sweet potato cultivar (UMUSP/3) raised in media gelled with gelrite and cassava starches.

	Mean shoot height (cm)			
Gelling agents	2 weeks	4 weeks	6 weeks	8 weeks
NR 8082	2.27±0.4^{b}	4.41±0.5^{b}	9.27±0.5^{a}	10.3±1.6^{c}
TMS 92/0057	2.22±0.1^{b}	4.54±0.3^{a}	9.04±0.4^{a}	10.0±1.4^{c}
TME 419	2.09±0.2^{b}	3.92±0.1^{bc}	5.98±0.3^{b}	7.5±0.8^{d}
TMS 98/0505	1.77±0.3^{bc}	4.8±0.2^{a}	8.56±0.7^{a}	10.7±2.5^{b}
TMS 30572	2.69±0.1^{a}	4.63±0.5^{a}	4.04±0.1^{c}	4.2±0.04^{e}
Gelrite	2.5±0.2^{a}	4.73±0.1^{a}	8.95±0.5^{a}	13.6±2.7^{a}

Key: Values represents mean ± standard errors for three replications for each treatment. Same letters are not significantly different at p≤0.05.

Table 2: Mean number of leaves produced by the *in vitro* sweet potato cultivar (UMUSP/3) raised in media gelled with gelrite and cassava starches.

	Mean number of leaves			
Gelling agents	**2 weeks**	**4 weeks**	**6 weeks**	**8 weeks**
NR 8082	1.6±0.03^{b}	4.9±0.2^{c}	10.6±0.7^{b}	14.0±2.1^{c}
TMS 92/0057	2.6±0.1^{a}	4.5±0.3^{c}	11.9±1.4^{a}	15.5±1.4^{b}
TME 419	2.5±0.3^{a}	4.2±0.4^{d}	10.2±1.6^{c}	11.1±1.8^{d}
TMS 98/0505	2.6±0.2^{a}	5.5±0.2^{bc}	10.0±0.4^{c}	13.8±5.5^{c}
TMS 30572	2.7±0.4^{a}	5.3±0.4^{c}	6.3±0.3^{d}	6.6±0.6^{e}
Gelrite	2.7±0.04^{a}	7.4±0.5^{a}	11.6±0.9^{a}	17.4±2.1^{a}

Key: Values represents mean ± standard errors for three replications for each treatment. Same letters are not significantly different at p≤0.05.

The superiority of gelrite-gelled medium was further demonstrated in the patterns of node development and growth (Table 3) after 8 weeks of culture. In the gelrite-gelled medium, highest number of nodes (17.7±2.8) was observed in contrast to 14.5±1.6 for TMS 92/0057 (Table 3).

Table 3: Mean number of nodes produced by the *in vitro* sweet potato cultivar (UMUSP/3) raised in media gelled with agar, gelrite and new cassava starches.

	Mean number of nodes			
Gelling agents	**2 weeks**	**4 weeks**	**6 weeks**	**8 weeks**
NR 8082	3.0±0.1^{a}	4.3±0.3^{d}	9.6±1.0^{c}	13.0±2.3^{c}
TMS 92/0057	2.6±0.3^{a}	4.3±0.2^{d}	10.9±2.1^{b}	14.5±1.6^{b}
TME 419	2.3±0.2^{b}	4.0±0.1^{d}	9.0±0.8^{d}	10.3±2.3^{d}
TMS 98/0505	2.5±0.04^{ab}	4.5±0.5^{c}	9.0±0.7^{d}	12.9±1.8^{c}
TMS 30572	2.7±0.3^{a}	4.5±0.2^{c}	5.6±0.2^{e}	6.1±0.5^{e}
Gelrite	1.7±0.3^{bc}	6.8±0.3^{a}	11.8±0.9^{a}	17.3±2.3^{a}

Key: Values represents mean ± standard errors for three replications for each treatment. Same letters are not significantly different at p≤0.05.

Taken together, the superiority of the control (gelrite-gelled medium) over the starches were evident in all the growth parameters investigated after 8 weeks of culture. Thus, the maximum values observed for shoot height, average number of leaves and nodes were (13.6±2.7 cm), (17.4±2.1) and (17.7±2.3) for gelrite after 8 weeks of the sweet potato plantlets in culture, against TMS 98/0505, TMS 92/0057 and TMS 92/0057 that recorded an average of 10.7±1.5 cm in shoot height, 15.5±1.4 leaves and 14.5±1.6 nodes after 8 weeks in culture. The observed superiority may be ascribed to the inherent purity of the control (gelrite). It is therefore suggestive that gelrite is less inhibitory than the starches to explant regeneration and *in vitro* propagation under the conditions of these experiments. Probable reasons for the variability in the performances of the plantlets between the gelling agents include limited diffusion of nutrients, lateral diffusion of water and impurities (Nairn *et al*., 1995 and Puchooa *et al*., 1999).

Growth and survival of the apical dome in the cassava starch-gelled media and that of the controls were also compared. Survival ratio was observed to correlate with the performance of the plantlets in the media. The survival percentage of the plantlets in TMS 98/30572 starch-grown medium decreased in line with the poor performance of the plantlets grown in the medium. Impressively, all the media investigated supported a 100% survival rate of the plantlets except that of the TMS 30572 starch-gelled medium that recorded 75% survival rate, suggesting that TMS 30572 may be more inhibitory compared to the other gelling agents.

Gel strength is often an important factor for any gelling agent. In this study, we observed drowning at 70g/l cassava starch concentration, but when the concentration was increased to 96g/l, adequate support and orientation of the plantlets was achieved. This is in contrast to 60g/l cassava starch concentration reported by Daud *et al*. (2011) and Karim *et al*. (2003) for *in vitro* propagation of *Celosia* sp., *Chrysanthemum mortifolium* respectively, but close to 100g/l reported by Daud *et al*. (2011) for *in vitro* potato propagation. These findings were further corroborated by Gebre and Sathyanarayana (2001) and

Nene and Sheila (1994) in their reports on successful shoot regeneration on cassava starch gelled media of concentration lower than 140g/l, and a value higher than 80g/l concentration respectively. These assertions confirmed our present report of 96g/l of cassava starch gelled media used in this study which is between 80g/l and 140g/l reported by Nene and Sheila (1994) and Gebre and Sathyanarayana (2001) respectively. Although the studied cassava starches supported regeneration and *in vitro* propagation of the plantlets, our results also demonstrated the superiority of gelrite-gelled medium over cassava starches as a gelling agent in *in vitro* regeneration and propagation of sweet potato plantlets. We observed that growth and development of the *in vitro* plantlets were influenced by the nutrient composition of the media, quality of the media and physical consistency. It therefore appears that the viscosity of the media and their components played a crucial role in plantlet regeneration and growth. Hence the observed variations in the performances of the various media could be ascribed to the differences in viscosity of the respective gels due to their biochemical and structural differences which are expected to affect the diffusion of the nutrients throughout the medium, resulting in quantitative variations in shoot induction (Ozel *et al*., 2008). *In vitro* growth of plantlets is as a result of interaction between explants and medium, which is dependent upon the gel quality. Thus, a change in the concentration of the gelling agent affects nutrient availability as well as the overall nutrient diffusion in the medium. A small quantity of gelrite (2 g/l) was required to obtain the same gel strength compared to 96g/l for cassava starch.

However, a major advantage of the cassava starches used in our experiments revolves on their availability and the reduced cost of the medium. Besides its cheapness, availability and sustainability of cultures, gelrite-gelled medium proved to be the most efficient for regeneration and *in vitro* propagation of the plantlets and are of better clarity and are easier to dispense. Moreover, beyond 6 weeks in culture, plantlets grown in starch-based media gradually turned light yellow in contrast to plantlets in gelrite-based medium that remained greenish and robust. We also observed softening of the starch-gelled media during the entire period of culture, indicating that the cassava starches were metabolized by the plantlets during culture. In addition, during autoclaving cassava starch yields sugars which produces osmotic or metabolic effect on cultures. Therefore, as starch and their products are not biologically inert, their widespread use in plant tissue culture may be limited (Khan et al., 2012). Generally, gelling agents are a rich source of impurities and constitutes a major source of variation in cultures. But gelrite is widely known to be purer. Scholten and Pierik, (1998) suggested that inorganic compounds in gelling agents and the dynamics of the interaction of gelling agent-medium-tissue play a major role during tissue growth *in vitro*. Therefore, for comprehensive understanding and explanation of the performances of the respective gelling agents, further work on detailed chemical and physical analyses becomes imperative. Conclusively, although cassava starch is readily available in the tropics and about seventy times cheaper than agar, its poor clarity, low gel formation, difficulties in dispensing and the fact that it is not biologically inert may preclude its widespread adoption as an alternative gelling agent.

Acknowledgements
We are grateful to the management of the National Root Crops Research Institute (NRCRI) Umudike, Umuahia, Abia State for their financial support.

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