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Evaluation cassava, sweet potato and Irish potato starches as cheap alternative gelling agents for micropropagation of sweet potato (*Ipomoea batatas* L.)

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ABSTRACT: Starch extracts from cassava, sweet potato, and Irish potato were tested as cheap alternative gelling agents for micropropagation of sweet potato (Ipomoea batatas L.). Nodal explant cultures were initiated in MS (Murashige and Skoog, 1962) medium supplemented with 3% sucrose and 0.5mg/l Benzyl Amino Purine (BAP) and solidified by either 12% sweet potato, 12% Irish potato, 15% cassava starch or 0.8% agar (w/v). Shoots were multiplied through 3 subcultures in BAP free MS medium with 5mg/l gibberelic acid. The overall quality of shoots in starch based media was slightly lower than in agar medium. In 84 days, average number of propagules produced from one explant was 40 in Irish potato medium, 1312 in cassava, 2058 in sweet potato and 3584 in agar. Despite the low multiplication rate of shoots produced on starch media, the cost per propagules was reduced by about 67%, 44% and 33% for sweet potato, cassava and Irish potato starches, respectively. This result suggests that, although starch based media were not as efficient as agar, , sweet potato and cassava starch may be suitable agar substitutes due to low costs and good quality of propagules.

KEYWORDS: Ipomoea batatas (L.), starch, gelling agent, micropropagation cost.

1 INTRODUCTION

Sweet potato (*Ipomoea batatas* (L.) Lam.) is an important food crop which is mainly grown for its starchy tuberous roots. Besides starch, the roots are rich in dietary fiber, vitamin A, vitamin C, and vitamin B6 [1]. Furthermore, sweet potato is used as animal feed. The crop is widely grown in tropical, subtropical and warm temperate regions[2, 3]. For the low-income households, sweet potato is a food security crop of choice because it can be easily grown with minimum input supply of agro-inputs. However the smallholder farmers get low (5-12 tones/ha) yields which is below the potential yield of 40-60 tones/ha recommended for many tropical soils[4]. The low yield is partly due to continued use of unclean planting materials[5]. Since the crop is vegetatively propagated, the common planting materials are stem vines of sweet potato harvested from farms of previous crop. It has been established that this method generates a series of challenges such as dissemination of diseases, short shelf life, low propagation rates, and high handling and transport costs[5, 6] . These challenges can be addressed by using Tissue Culture (TC) techniques for multiplication of clean planting materials[7]. However, the TC technology is inadequately used by many developing countries mainly due to high costs of production[8]. It has been established that of the total cost of production, more than 30% is due to chemical media of which about 70% is cost of gelling agent [9]. The common gelling agent for many micropropagation laboratories is agar. Previous research on agar substitutes has established that starch from different sources can be used as alternative gelling agent for micropropagation of many crops [10, 11]. However information on the effects of these starches on the micropropagation rate, quality and costs is limited. This work therefore aimed to evaluate starch extracts from selected crops as alternative cheap gelling agent for micropropagation of sweet potato.

2 MATERIALS AND METHODS

2.1 GELLING AGENTS AND TEST PLANT

Starch was extracted from Irish potato (*Solanum tuberosome* L. cv. CAP), sweet potato (*Ipomoea batatas* L.) cv.Ukerewe) and cassava (*Manihot esculanta* Crants cv. kiroba). Agar powder (Technopharmachem Ltd) was used as control gelling agent. The performance of these starches was evaluated using a Tanzanian sweet potato cv. Ukerewe as source of explants

2.2 STARCH EXTRACTION AND TC METHODS

Starch from the test crops was extracted using standard methods for root crops [12, 13]. The extracted starch was used to prepare gelled media for establishing nodal cultures of sweet potatoes. Explants were excised from multinodal shoots harvested from healthy stem vines raised in screen house at Mikocheni Agricultural Research Institute (MARI). Before excising the explants the shoots were surface sterilized by washing thoroughly under tap water for 5 minutes. This was followed by washing in 1 % (v/v) liquid soap supplemented by 1 drop Tween 20 (BDH, UK) before rinsing in distilled water three times. The washed vines were then treated with 70% ethanol for 1 minute followed by rinsing three times using sterile distilled water inside laminar flow hood. The vines were then exposed to 10% commercial bleach (3.5% NaOCl) containing two drops of Tween 20 for 10 minutes followed by 5% of the same for another 10minutes. After this treatment, vines were rinsed with sterile distilled water three times (3-4 minutes each) to remove excess bleach. The surface sterilized shoots were cut into 1 cm nodal explants before being placed on a culture medium.

During culture initiation single explants were placed into culture bottles (30mm x 100mm) containing 20mls of initiation MS medium [14]. The media was supplemented with 3% (w/v) sucrose and 0.5mg/l Benzyl Amino Purine (BAP). The *in vitro* shoots were subcultured 3 times at 21 days interval. To initiate multiple cultures, two centimeter long nodal explants were excised under sterile environment and subcultured into multiplication media supplemented with 5mg/l giberrelic acid. Subculturing was done by transferring the *in vitro* shoots into fresh medium after every 21 days. Cultures for both initiation and multiplication were incubated at 23± 2^{o}C under a 16 hrs photoperiod with a photosynthetic photon flux density of 40 µmol m^{-2} s^{-1} provided by overhead cool fluorescent lamps (Philips, India 30 Watts).

2.3 DATA COLLECTION AND ANALYSIS

At the end of each cycle the number of leaves per shoot, number of nodal propagules (of about 2cm long), shoot height, number of nodes per shoot and internode length, fresh and dry weight (mg) were recorded. Dry weight (mg) was determined after oven drying at 50^{o}C for 8hrs. Estimates of micropropagation cost were determined based on market prices calculated using the formula [15]

$$\frac{Amount\ used\ in\ culture\ medium\ x\ price\ of\ amount\ bought}{Amount\ bought}$$

Differences between cost of the conventional agar based medium and the starch based alternatives were calculated followed by establishing the their percentages changes in expenditure cost for each gelling agent, overall media and unit production cost of the produced propagules. Multiplication rates were calculated as the difference in mean shoot number between the mean numbers of shoots derived before and after subculture from one culture at the end of each passage. The averages of number of shoots and multiplication rate were also determined for the three subculture passages for each treatment according to procedures reported previously[16]. One way Analysis of variance was calculated using GENSTAT software, version (VSN International Ltd, Hemel Hempstead, UK). Least square differences (LSD) were calculated.

3 RESULTS

3.1 EFFECT OF TYPE OF GELLING AGENT ON IN VITRO GROWTH QUALITY OF SWEET POTATO

3.1.1 THE NUMBER OF PHOTOSYNTHETIC LEAVES PER SHOOT

The number of leaves per *in vitro* shoot in the agar based medium (control) significantly ($P \leq 0.05$) outperformed the starch based media in all stages of culture (Figure 1). Among the three botanical starches tested for culture initiation, no statistical differences ($P \leq 0.05$) in number of leaves were established although sweet potato starch produced seemingly the

highest. However during all the three subcultures, Irish potato starch produced significantly the lowest number of leaves while there was no differences between cassava and sweet potato starch (Figure 1).

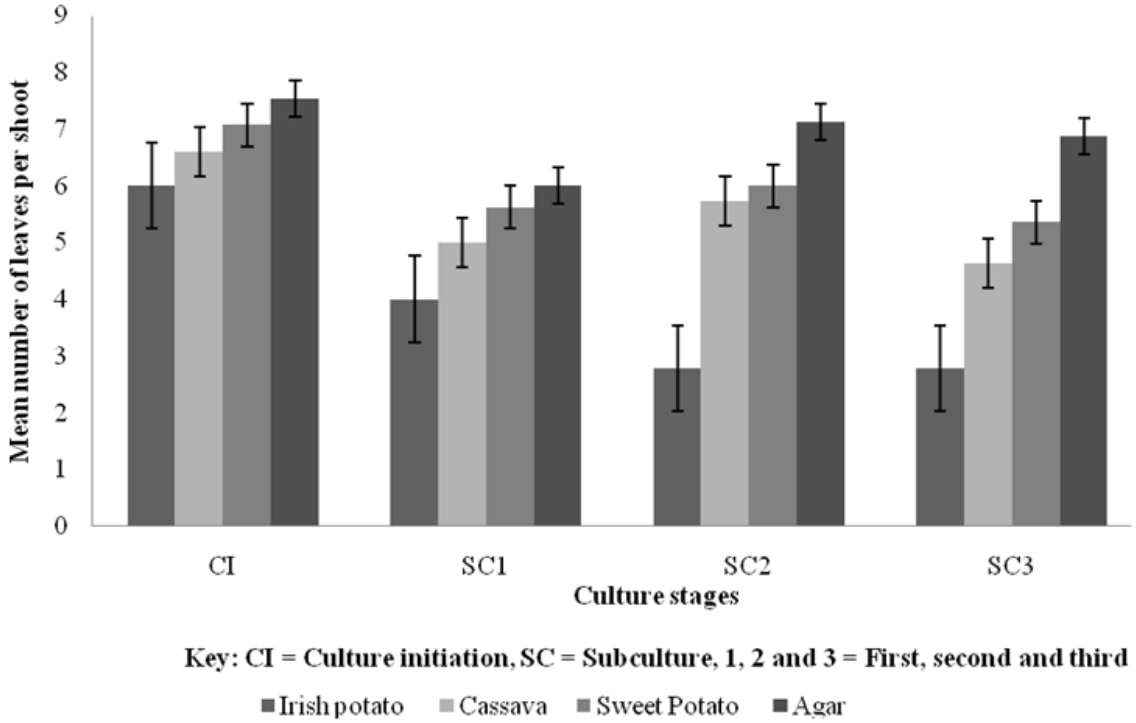

Figure 1: Effect of type of gelling agent on number of photosynthetic leaves per shoot atdifferent stages of in vitro culture. Vertical error bars represent standard error and means with overlapping error bars are not significantly different at 5%

3.1.2 FRESH WEIGHT OF IN VITRO SHOOTS

Fresh weight of in vitro shoots in the TC medium solidified by different sources of gelling agents varied significantly ($P \leq 0.05$) in culture initiation and the three subsequent subcultures (Figure 2). The control medium produced shoots with highest fresh weight in all culture cycles (Figure 2). The Irish potato starch gelled media produced the lowest fresh weight. In all cycles of the tissues culture, the fresh weight of in citro shoots in Sweet potato and cassava starch based medium were statistically ($P \leq 0.05$) the same.

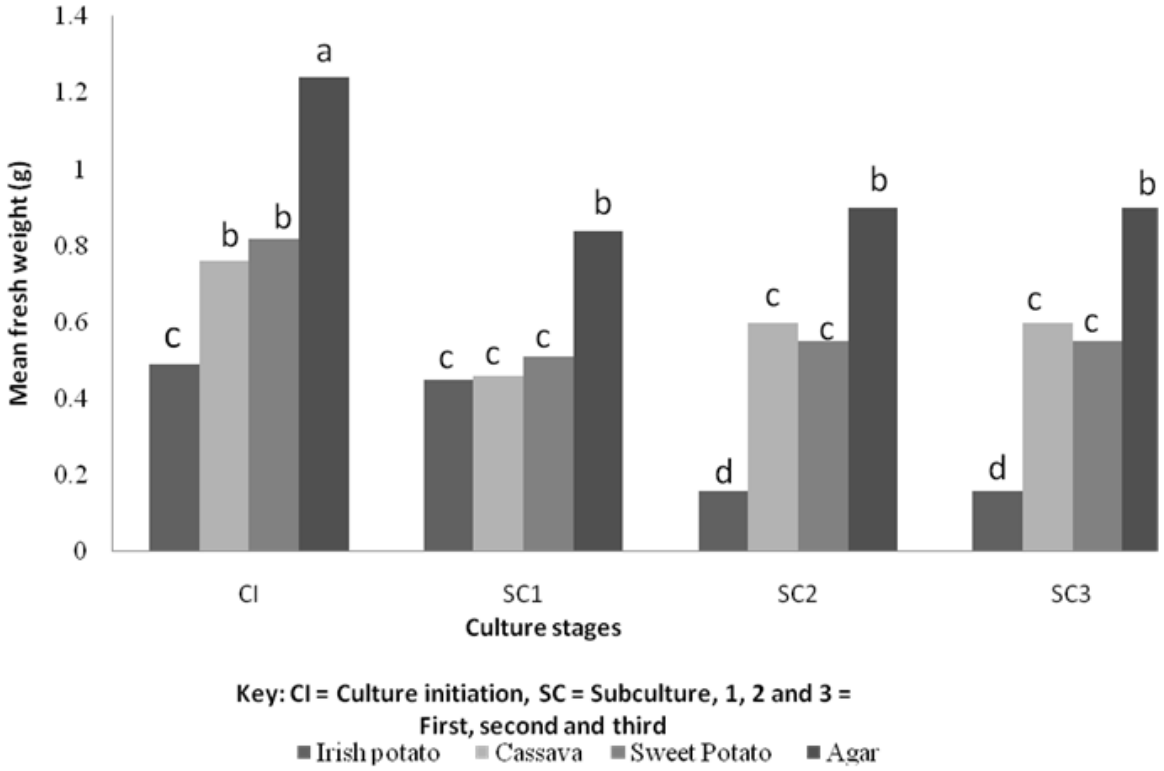

Figure 2: Effect of type of gelling agent on fresh weight of in vitro shoots at different stages of culture. Bars headed with same letters represent means which are not significantly different (P ≤ 0.05)

3.1.3 DRY MATTER CONTENT OF IN VITRO SHOOTS

Differences of Dry Matter Content (DMC) of shoots proliferating in TC medium gelled different agents were significant (P ≤ 0.05) in all TC cycles. During the four culture stages, agar solidified media produced shoots with the highest (10%) DMC except at the second subculture where the cassava starch solidified media slightly outperformed the control by 0.24%. During the three subcultures, Irish potato starch gelled media recorded the lowest performance varying between 7.66% and 7.54 % DMC. Except during culture initiation, the DMC in sweet potato and cassava did not differ significantly (P ≤ 0.05) (Figure 3).

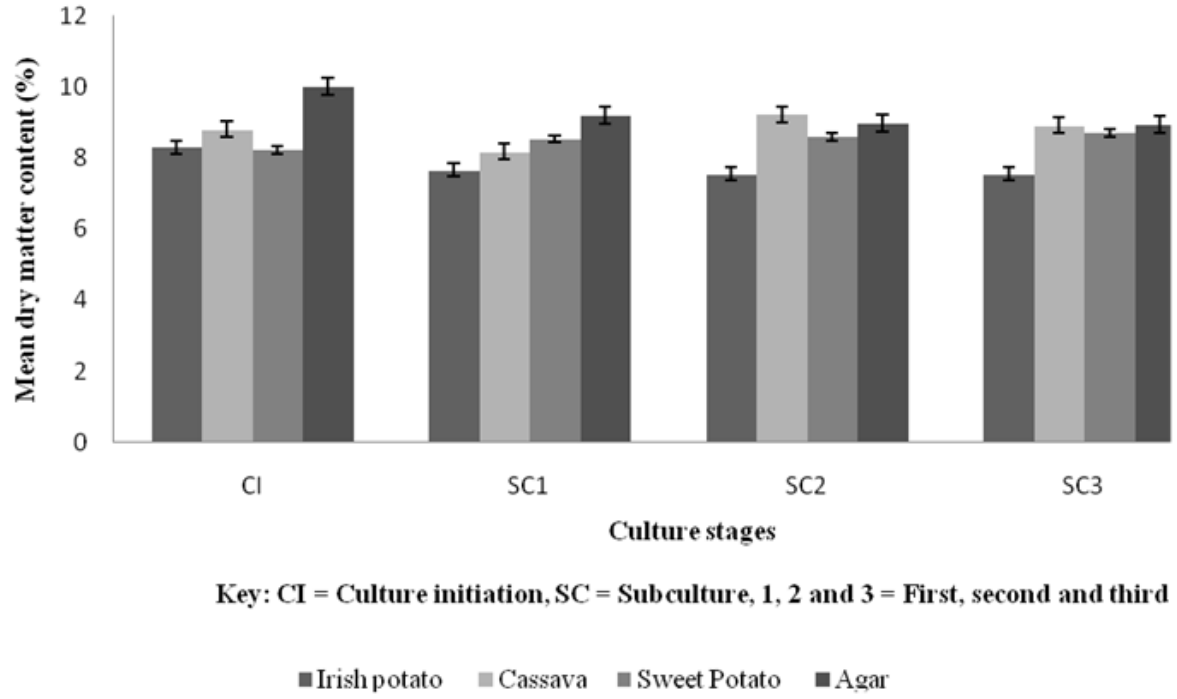

Figure 3: Effect of type of gelling agents on dry matter content of in vitro shoots at different stages of culture. . Vertical bars represent standard error and means with overlapping bars are not significantly different at 5%

3.2 EFFECT OF STARCH TYPE ON MICROPROPAGATION FACTORS

The heights of *in vitro* shoots produced in different types of media were significantly different ($P < 0.05$). During culture initiation, agar gelled medium had the tallest (10.98cm) shoots while Irish potato starch medium had the shortest (6.55cm) (Table 1). No differences ($P \leq 0.05$) in shoot height were established between cassava and sweet potato during culture initiation. Number of nodes per in vitro shoot varied significantly at 5% level of significance. Each treatment produced shoots with different number of nodes whereas agar produced the highest mean (8.73) while Irish potato produced the lowest (5.40). No statistical differences of the length of internodes were established between treatments during culture initiation. However the potential propagules produced in TC medium which were solidifies by the different gelling agents varied significantly at 5% level. Agar based medium produced the highest (7.9) numbers while the lowest (5.4) were observed in Irish potato starch.

Agar based medium produced shoot with highest height in all subculture cycles whereby during the first, second and third subcultures agar based medium produced shoots with heights of 9.3cm, 10.3cm and 9.6cm respectively. On the other hand, Irish potato produced the lowest values of shoot heights of 5.3cm, 3.02cm and 3.03cm during first, second and third subcultures respectively. Similar patterns of results were observed for the number of nodes per shoot and propagules produced except the internode lenghth for which the differences were not significant ($P \leq 0.05$) (Table 1).

Table 1: Effect of starch types on the number of shoots, shoot height, number of nodes/explant and internode length of sweet potato microplants during culture initiation and multiplication stages

Culture stage	Gelling agent (w/v)	Shoot height (cm)	Nodes/ shoot	Internode length(cm)	Propagule/ shoot
Culture initiation	Irish potato (12%)	6.55±0.20a	5.40±0.13a	1.08±0.10a	5.40±0.13a
	Cassava (15%)	8.03±0.27b	6.13±0.13b	1.32±0.03a	6.33±0.12b
	Sweet potato (12%)	8.42±0.22b	7.73±0.12c	1.25±0.03a	7.33±0.16c
	Agar (0.08%)	10.98±0.38c	8.73±0.42d	1.26±0.04a	7.90±0.30d
First subculture	Irish potato (11%)	5.33±1.07a	4.00±0.71a	1.3±0.07a	4.01±0.95a
	Cassava (15%)	6.24±0.36a	5.33±0.23b	1.35±0.32a	5.67±0.29b
	Sweet potato (11%)	7.26±0.32b	6.00±0.71b	1.23±0.05a	6.63±0.37c
	Agar (0.08%)	9.30±0.27c	6.93±0.23c	1.35±0.32a	7.6±0.27d
Second subculture	Irish potato (11%)	3.02±0.55a	2.56±0.50a	1.21±0.10a	2.25±0.53a
	Cassava (15%)	7.33±0.36b	6.46±0.41b	1.2±0.04a	6.55±0.41b
	Sweet potato (11%)	7.87±0.23b	6.91±0.21b	1.14±0.01a	6.91b±0.21c
	Agar (0.08%)	10.29±0.21c	8.20±0.20c	1.30±0.02a	7.47±0.26c
Third subculture	Irish potato (11%)	3.03±0.55a	2.22±0.40a	1.40±0.09a	2.33±0.44a
	Cassava (15%)	6.86±0.36b	4.73±0.36b	1.50±0.12a	5.55±0.67b
	Sweet potato (11%)	7.43±0.41b	5.73±0.27c	1.30±0.05a	6.09±0.55b
	Agar (0.08%)	9.56±0.09c	8.00±0.24d	1.20±0.03a	8.2±0.28c

Means followed by the same letter within the column are not significantly different at $P < 0.05$.

3.3 MICRO PROPAGATION RATE

It was established that the control treatment had the highest micropropagation rate whereby in 84 days of culture the control produced 3584 propagules(Figure 4). Within this time, second highest propagation rate was observed in sweet potato starch based medium (2058 propagules), Cassava starch (1312 propagules) and Irish potato (32 propagules) (Figure 4)

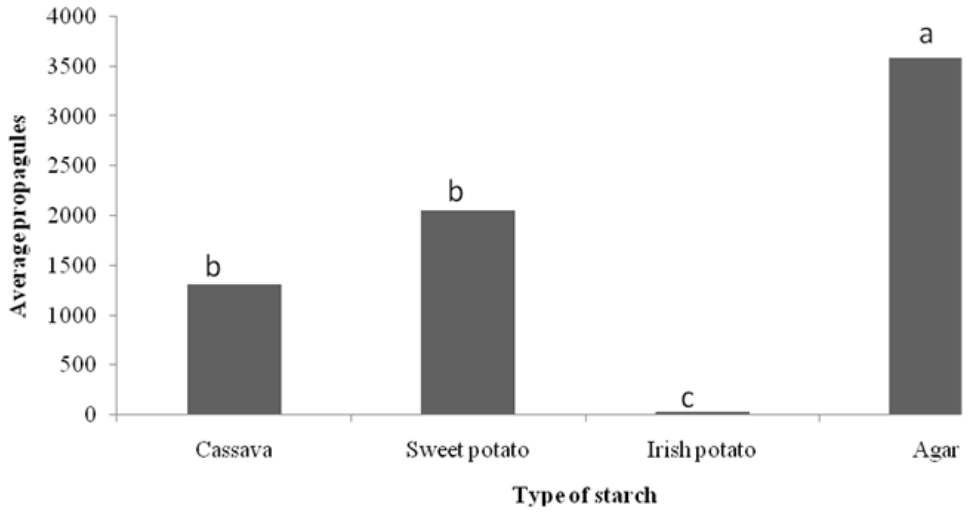

Figure 4; The effect of type of gelling agent on the propagules produced. Bars headed by the same letters are not significantly different at 5%

3.4 EFFECT OF TYPE OF GELLING AGENT ON MICROPROPAGATION COST

The total cost of sweet potato starch (12%) to make one liter of TC medium was significantly ($P \leq 0.05$) the lowest (TZS 275) followed by cassava starch (TZS 420) . Agar, had the highest cost (TZS 894) followed by Irish potato starch (TZS 616) (Table 2). The results were equally implied in the total cost of medial for one litter whereby the agar and irish potato starch

based media were equally (P = ≤ 0.05) expensive and highest followed by cassava while media gelled by sweet potato was least expensive (TZS 763) (Table 2)

Table 2; The effect of type of gelling agent on media cost

Gelling agent	Conc. (%w/v)	Cost of gelling agent per liter (TSh/l)	Media cost / l (TSh/l)
Cassava	15	420c	883b
Sweet potato	12	275d	763c
Irish potato	12	672b	1135a
Agar	0.08	894a	1357a

With reference to the control, Figure 5 shows the magnitude of change in the expenditure and production cost of media and propagules respectively which result from using starch as alternative gelling agents. it was established that expenditure cost of media to produce a unit of propagule in Irish potato, sweet potato and cassava was reduced by 99%, 82% and 80% respectively. Comparison of this reduction to the propagation rate in each starch based media the production cost of propagules in Irish potato, Sweet potato and cassava was reduced by 33%, 67% and 44% respectively (Figure 5).

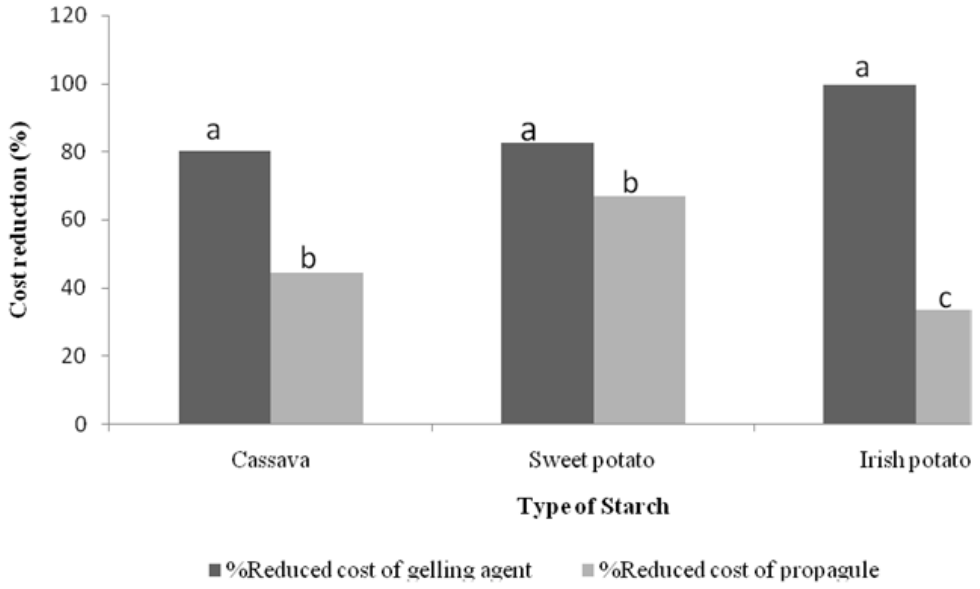

Figure 5; Unit cost reduction resulting from using botanical starch as gelling agents in Tissue culture

4 DISCUSSIONS

Comparison between treatments on their influence on components of micropropagation rate show that, there was persistent superiority of the control (agar) over the three starch based treatments in both culture initiation and subculture cycles. These components include shoot height, number of nodes per shoot, internode length (cm) and propagules/shoot . Such superiority may be due do the characteristic nature of agar gelled media to maintain stable gel firmness which provides good contact of explants on the media and anchor it upright throughout the culture period.

Furthermore, agar solidifies the TC media in a manner that, it does not restrict movement of water, nutrients and growth regulators within the matrix and from the matrix to the explants as also reported by other authors[17, 18] . Naturally, agar produces transparent gels of TC media which could be another reason contributing to its superiority as the growing shoots are well exposed to light for maximum photosynthetic and photoperiodic functions.

Lowest response on shoot height, number of nodes per shoot, internode length and number of propagules per shoot were observed in TC media solidified by Irish potato starch extract. This result might be caused by its high gel firmness and clarity of gels formed by Irish potato starch. Unlike agar gels, Irish potato starch at 12% was more stiff and opaque. The

stiffness might have caused restricted movement of water and mineral solutes within the media matrix and across cell walls of the explants cells. The poor clarity might have reduced the amount and quality of light available for the cultures. Another reason could be due to characteristic tendency of Irish potato starch to undergo rapid degradation due to oxidation of phenolic compounds as soon as they are exposed to light. Therefore, other compounds included in the extract may have played an inhibitory role to somatic cell division (mitosis) of the explants and the general growth and development of the shoots *in vitro*.

The ability of a TC media to promote rapid and vigorous growth in terms of shoot height has an implication on the rate of micropropagation. This is because the height of a shoot had strong bearing with the number of nodes which determines the number of nodal propagules produced for further subculture and rooting. This observation agrees with a report in the literature[19]. Moreover the height of shoot determined the size of nodal propagule and the *in vitro* plantlet for adaptation in *ex vitro* environment also reported elsewhere [16].

Of the three sources of starch, sweet potato caused the highest multiplication rate. This is centrally to other findings which have established that cassava starch has the greatest potential of being agar substitute [10, 11, 20]

The *in vitro* shoots regenerated from starch based media had fewer photosynthetic leaves per shoot as well as lower fresh weight and dry matter content than those of agar gelled media . The low leaf formation and accumulation of biomass suggest that starch based media did not provide adequate contact between the medium and the explants thus limiting the uptake of water, nutrients and the growth regulator from the culture medium for growth and development. Such inferior growth performance of plants on starch based TC media was also been reported previously [21]

Among the three starches based media, sweet potato and cassava starch gelled media had higher ability to provide nutritional support to the explants than Irish potato starch gelled media. The low performance in Irish potato starch medium can be associated to presence of large amounts of non starch impurities such as protein, fats, phosphorous and other organic compounds which may have played inhibitory roles on growth and organogenesis[20]. Also the improved performance on cassava and sweet potato starch medium can be explained by the fact that root starches may have provided an additional carbon source thus enhancing cell division and growth, a finding which has been documented in previous publications[10]. It is also possible that growth may have been promoted by ionic compound present in starch such as carbohydrates [22] Although the micropropagation quality and rate on starch based media are lower than on agar gelled media, a significant reduction in cost of making 1 litre of media of cassava and sweet potato starched justifies its continued improvement for use in tissue culture. More importantly the justification of starch based medium as alternative to convetional method is further uplifted by the great reduction in production cost per propagule.

5 CONCLUSION

Although, *in vitro* shoots produced from starch solidified medium have low micropropagation quality and rate, sweet potato and cassava starch can have a potential of being agar substitutes in commercial micropropagation of sweet potatoes due to reduction of production costs per propagule

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