EVALUATION OF SELECTED CROP STARCHES AS AN ALTERNATIVE CHEAP GELLING AGENTS FOR MICROPROPAGATION OF SWEET POTATO (*Ipomoea batatas* L.)

BY

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ABSTRACT

Experiments were conducted to investigate cheap gelling agents as substitutes to agar in order to reduce the cost of micropropagation. Starches from cassava, sweet potato, irish potato, wheat, sorghum, maize and rice were tested together with 0.08% (w/v) agar as control and sweet potato cv. Ukerewe was used as test plant. Standard methods of determining physicochemical properties of the starch and Tissue Culture (TC) were used. Nodal explant cultures were initiated in MS (Murashige and Skoog, 1962) media supplemented with 3% sucrose and 0.5mg/l Benzyl-Amino-Purine and gelled by seven starches each at concentrations: 12%, 15%, 18% and 21% (w/v). *In vitro* nodal explants were subcultured individually on fresh media supplemented with 3% sucrose, 5 mg/l gibberellins and solidified by 15% cassava, 11% sweet and irish potato starches. During culture initiation, Cassava, sweet and irish potato starches outperformed the control in influencing the number of leaves, nodes, height and fresh weight of *in vitro* shoots. Of the three starch gelled media, sweet potato starch based media had the highest micropropagation rate of 2058 *in vitro* nodal propagules in 84 days but it was lower than that of the control which produced 3584 *in vitro* nodal propagules during the same time. Highest media and propagule cost reduction of 44% and 67% respectively was achieved by using sweet potato starch instead of agar. Therefore, sweet potato starch has high potential to replace agar due to good plant growth and low cost of the media.

DECLARATION

I RICHARD RAPHAEL MADEGE, do declare to the senate of the Sokoine University of Agriculture that the work presented here is my original work and has not been submitted or concurrently for higher degree or any other award in any other university

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Dedicated to almighty **GOD** who mysteriously kept me healthy throughout this course. To my parents **Mr. and Mrs. Raphael Madege** who laid the foundation of my carrier

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LIST OF ABBREVIATIONS AND SYMBOLS

µm	micrometer
°C	Degrees Celsius
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
ARI	Agricultural Research Institute
BAP	Benzyl Amino Purine
BIOEARN	East African Regional Programme and Research Network for Biotechnology, Biosafety and Biopolicy Development
CIMMYT	International Maize and Wheat Improvement Center
cm	Centimetre
cv	cultivar
CRD	Completely Randomized Design
DMC	Dry Matter Content
DMRT	Duncans multiple range test
et al	And others
EC	Emulsifiable Concentrates
FAO	Food and Agricultural Organisation
g	Gram
GT	Gelation Temperature
H_2O	Water
HCl	Hydrochloric acid
I	Iodine
ISI	International Starch Institute

KPa	Kilopascal
LGC	Least Gelation Concentration
M	Mole
mg	miligram
mm	millimetre
m^{-2}	Per square meter
MARI	Mikocheni agricultural research institute
MAFSC	Ministry of Agriculture, Food Security and Cooperatives
MS	Murashige and Skoog
NPK	Nitrogen, Phosphorus and Potassium
NaOH	Sodium hydroxide
NaOCl	Sodium hypochlorite
rpm	Revolutions per minute
SP	Swelling power
SUA	Sokoine University of Agriculture
s^{-1}	Per second
TC	Tissue Culture
URT	United republic of Tanzania
USDA	United States Department of Agriculture
v/v	Volume to volume ratio
w/v	Weight to volume ratio

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Sweet potato (*Ipomoea batatas* (L.) Lam.), a plant from the family Convolvulaceae, is an important food and cash crop, which is widely grown in tropical, subtropical and warm temperate regions (Burden, 2005; Saranya *et al.*, 2006). It ranks 6th most produced food crop in Africa being surpassed by wheat, rice, corn, irish potato and possibly cassava (Verma, 2004; Tewe, 2004). Similarly in East Africa it is the sixth most important staple food being surpassed by maize, sorghum, rice, irish potato and cassava. It is cultivated extensively in regions surrounding Lake Victoria in Uganda, and western and central regions of Kenya (Ogema *et al.*, 2007). In Tanzania sweet potato is mainly produced in the regions surrounding Lake Victoria, southern highlands and eastern coastal zones (Ndunguru and Kapinga, 2007). In Tanzania sweet potato is mainly produced by women as a food security crop for the household as well as a source of income (Ndunguru and Kapinga, 2007).

Although sweet potato leaves and shoots are edible, the starchy tuberous roots are by far the most important product. Besides starch, roots are rich in dietary fiber, vitamin A, vitamin C, and vitamin B6 (Jegtvig, 2006). Furthermore, sweet potato is used as animal feed.

For the low-income households, sweet potato is a food security crop of choice because it can be easily grown with minimum use of agro-inputs. This is because it has relatively high adaptability to a wide range of agro-ecologies (Scott *et al.*, 2000;

Tewe, 2004). Also sweet potato can stay in the field for a long time, forming field store from which the crop can be harvested in piecemeal.

Despite of the described attributes, smallholder farmers get low yields varying between 5-12 tones/ha, which is by far below the potential yield of 40-60 tones/ha achievable in many tropical soils (Tewe, 2004). The low yield is due to both biotic and abiotic factors. The main biotic factors include the unavailability of high yielding varieties together with the prevalence of diseases especially those of viral origin, which cause yield losses of up to 50%, (Mukasa *et al.*, 2003). This problem is exerbated by vegetative propagation by using stem vines. This propagation system generates a series of challenges such as short shelf life, low propagation rates, and high handling and transport costs (Escobar *et al.*, 2005). Sanitary health is particularly difficult to maintain, and the vines often become the means for disseminating diseases from generation to generation p articularly viral ones by recycling infected planting materials from the previous harvests (Fuglie *et al.*, 1999).

Such challenges can be reduced by using disease free planting materials, which can be produced through Tissue Culture (TC) using meristem tip culture (Manganaris *et al.*, 2003; Helliot *et al.*, 2002). The rapid clonal multiplication through tissue culture is widely used in commercial multiplication of uniform and disease free planting materials for many crops. Using such TC products, productivity of many crops including sweet potato has been increased. For instance, the TC technology was used to produce and multiply commercially, the disease-free planting materials of high yielding Chinese varieties of sweet potato for distribution to farmers (Fuglie *et al.*, 1999). The report concluded that the availability of virus-free planting materials increased average sweet potato yield in China by at least 30%, with little or no change in the use of other inputs.

The advantages of the TC technology have not adequately helped the farmers in developing countries. This is mainly due to high costs of production which makes the TC products commercially unaffordable. The high costs of production in developing countries are mainly caused by the adoption and exclusive use of conventional TC protocols, which involve use of expensive imported consumables (Mehrotra *et al.*, 2007). Therefore research to identify cheap alternative TC protocols is important.

1.2 Problem statement and justification

Tissue culture is accepted worldwide in research as well as commercial production systems as a technique for rapid multiplication of good quality disease free planting materials among other uses (Bhojwan and Razdan, 1996). However, this technology has not been used adequately to address the existing shortage of disease free planting materials of the high yielding varieties of sweet potato, which farmers in Tanzania and other African countries are highly in need of.

It has been established that the main reason for this failure is that, the micro-propagation protocols for many plants do not offer cost effective commercial plant micro-propagation (Puchooa *et al.*, 1999; Kuria *et al.*, 2008). Using the conventional micropropagation protocols for many crops, 35% of the total cost of production is due to TC media (Prakash *et al.*, 2002). Many micropropagation protocols use semisolid TC media and the solidifying (gelling) agent in these media usually is agar.

Agar contributes up to 70% of the total cost of making one liter of TC media (Prakash, 1993). Therefore there is need for identifying cheaper gelling agents if a cost effective micro propagation of sweet potato that helps the smallholder farmers has to be realized. This work was undertaken to evaluate the potential of starch from seven crops as substitute cheap gelling agents for making TC media for micro propagation of sweet potato.

1.3 Objectives

1.3.1 Overall objective

To establish the potential of starch from selected crops as cheap gelling agents that will replace agar to reduce the cost of micro-propagation of sweet potato.

1.3.2 Specific objectives

1. To determine the physical and chemical properties of starches extracted from seven selected crops: cassava, sweet potato, potato, maize, rice, sorghum and wheat.
2. To evaluate the potential of starch from the selected seven sources to support *in vitro* growth of sweet potato.
3. To determine the effect of starch on rate, quality and cost of micropropagation of sweet potato

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Importance and production of sweet potato

Sweet potato (*I. batatas* (L.) Lam.) is a dicotyledonous of the family Convolvulaceae which is the only Ipomoea species of economical importance as a food crop (Wolfe, 1992). It is a perennial plant that is mainly grown for its starchy tuberous roots although the leaves and shoots are also edible. The crop has a wide range of other uses such as animal feed and industrial uses like production of starch and alcohol. According to Zuraida (2003), sweet potato tubers are rich in starch, vitamins and essential mineral salts. The young leaves are rich in protein and are usually processed as vegetables. The yellow and orange flesh varieties represent the least expensive year-round source of dietary vitamin A available to poor families in Southern and East Africa (Ewel and Mutuura, 1994).

Together with other root and tuber crops, sweet potato plays a significant role in the global food system. It is produced and consumed by many of the worlds poorest and most food insecure house holds (Scott *et al.*, 2000). It contributes to the energy and nutrition requirements of more than 2 billion people in developing countries. In Africa sweet potato is a major starch staple with production estimated at 7.5 metric tons, which is about 6% of the world production (Ndunguru and Kapinga, 2007). In East Africa, Uganda has the highest (2 650 000 tones) annual production of sweet potato followed by Tanzania (970 000 tones), Rwanda (908 300 tones) and Burundi (834 400 tones) (USDA, 2005). The crop is one of the three most important root crops alongside cassava and potato produced and consumed throughout the region by majority especially the poor (Zuraida, 2003).

In Tanzania, sweet potato is mainly grown in the Lake Victoria zone, Southern and Coastal regions (Kapinga *et al.*, 1995). It ranks 6th most produced crop among food crops after maize, sorghum, rice, irish potato and cassava. It is preferred by the majority resource poor farmers because it can be stored in the field and harvested in piecemeal. Furthermore, growing it is easy since it has a short growing season and is adapted to a wide range of agro-ecologies. It performs relatively well in poor soils where other crops would not produce without application of costly inputs (Smith, 1997). The farming households in Tanzania consider sweet potato as a crop that prevents food shortage before the next harvest of maize or other staple food crops (Kapinga *et al.*, 1995). Sweet potato is grown mainly by rural women near their homes to feed their families and as means for women's entry to cash economy (Kapinga *et al.*, 1995).

2.2 The potential of TC to addressing problems facing sweet potato production

One of the primary objective of micropropagation of sweet potato and other crops is to produce high quality planting materials, which in turn lead to increased productivity. The generated plants must be vigorous and capable of being successfully transplanted in the field, and must have high field survival (Bhojwan and Razdan, 1996). Furthermore, they should be genetically uniform, free from diseases and price competitive to the plants produced through conventional methods.

In trying to achieve such an objective, several workers have reported successful clonal micropropagation of forest trees (Arya *et al.*, 2002), tree crops (Sharma *et al.*, 1999; Kumar *et al.*, 2003), horticultural crops (Schneider, 2005; Baiyeri and Aba, 2005) and field crops. Among the field crops, several workers have reported successful micropropagation of root and tuber crops. Such root and tuber crops include cassava (Smith *et al.*, 1986; Konan *et al.*, 1997), irish potato (Zaman *et al.*, 2001; Kuria *et al.*, 2008) and sweet potato (Elliot, 1968; Fuglie *et al.*, 1999).

Tissue culture has been reported as useful in conservation of genetic resources *in vitro* (Kameswara, 2004), selection of mutants, somatic hybridization and genetic engineering (Smith and Drew, 1990). Furthermore TC technique like cell cultures in bioreactors, allow production of secondary metabolites (Altman, 2000). Moreover, functioning of TC system is essential for successful modern biotechnologies like genetic engineering (Altman, 2000).

2.3 Standard TC procedure for micropropagation of Sweet potato

Micropropagation of sweet potato to produce plantlets, which are ready for transplanting into the field occurs in five phases. According to Altman (2000), the first is called Phase 0 which involves growing mother (stock) plants under hygienic conditions usually in greenhouses. The second is Phase I (culture initiation) which involves the selection of explants, disinfestations and the cultivation under aseptic conditions *in vitro*. The third is Phase II (multiplication phase). This is a rapid regeneration and multiplication of numerous propagules by repeatedly subculturing *in vitro* plants into new media.

The fourth is Phase III (rooting phase). This is the elongation and root induction or development phase meant to induce the establishment of a complete plant *in vitro*. It is the last period in *in vitro* before transferring the plantlets to *ex vitro* conditions. The fifth is Phase IV (acclimatization). In this phase, *in vitro* plantlets are transferred to *ex vitro* condition usually in the green house. It is meant to make the plant adapt to *in vivo* environmental condition (Dewir *et al*., 2005). These phases and the subculture cycles, involve repeated preparations of micro propagation media without recycling. This becomes a very expensive operation if cheap media ingredients are not sought. Consequently the products may remain unaffordable making the technology fail to benefit the smallholder farmers who are the majority in East Africa.

2.4 Commercial utilization of plant micropropagation in developed and developing countries

In the global trade, micropropagation has the highest contribution in the flower industry as means of rapid multiplication of high value genotypes throughout the year (Traud *et al*., 2006). In the developed world, tissue culture and micropropagation have become important elements in the plant propagation industry. It is estimated that, some 50 000 varieties of plants are propagated in the world using tissue culture method, the majority of which are ornamental species. Over 95% of the world's micropropagation is produced by the developed countries where more than 600 business companies are involved. For instance, in German more than 48 million plants (*Phalaenopsis*, *Rhododendron, Fragaria and Gentiana*) were propagated (Traud *et al*., 2006).

In the developing countries especially Africa, although there is a substantial tissue culture and micro propagation research being carried out in almost every country, only few of these countries have reached the take-off stage in using large-scale micro propagation for the development of local and regional market-products (Brink *et al.*, 1998). Similarly, according to Mugoya *et al.* (2006), in East Africa, the research in tissue culture and micro propagation is being targeted to crops like Banana, Cassava, Sweet potato, Potato, Citrus, Coconut, Macadamia and other tree plants. In this report, Kenya appears to be ahead of others as it had successfully used TC for rapid propagation of banana, pyrethrum, sugarcane, citrus, cassava, potatoes, and flowers among others.

Application of micropropagation in other East African countries is insignificant. The situation is partly due to very high prices of TC products that smallholder farmer can not afford. The high prices are due to continued use of expensive micropropagation protocols (Mugoya *et al.*, 2006).

2.5 Cost as a constraint to commercial micro propagation in developing countries

Both national and international organizations agree that TC technology is very relevant to agriculture. However its production cost is an important concern for both research and commercial laboratories (Zimmerman *et al.*, 1995). Its potential can fully be exploited if the problem of high cost of production is satisfactorily solved (Savangikar, 2002). The production cost mainly originates from electricity, labour and consumables.

Despite of high costs of production, TC has become a relevant and affordable technology for propagation of plants with inefficient conventional propagation systems especially ornamentals because, their trade command high unit value (Savangikar, 2002). However in developing countries like Tanzania, the potential of TC in increasing agricultural production and generating rural employment can not be maximized because the cost of micro propagated plants is unaffordable by majority of smallholder farmers. This is well illustrated in India where in the last decade about 90 micro propagation units were established and 32 were closed almost during the same time mainly due to high costs of production (Mascarenhas, 1999).

While TC systems in developed countries are mainly constrained by labour cost (60-70%), those in the third world are mainly constrained by high costs of electricity (50%) and consumables (30%). Of the consumables, media chemicals cost about 15% of micro-plant production (Prakash, 1993). Of the medium components, the gelling agents such as agar contribute 70% of the costs. Other ingredients like salts, sugar and growth regulators have minimal influence on production cost.

2.6 Gelling agents

2.6.1 Agar

Agar is the most common gelling agent used by majority of commercial micropropagation, because its cost is lower than the agarose, phytagel, and gelrite (Puchooa *et al.*, 1999; Zimmerman *et al.*, 1995). Agar is a natural polysaccharide made up of two major molecules, agarose and agaropectin. The former is composed of alternating D-galactose and 3, 6-anhydro-L-galactose with side chains of 6-methyl-D-galactose residues while the later is like agarose but additionally contains sulfate ester side chains and D-glucuronic acid. It is extracted from various species of seaweed particularly the Gelidialea (e.g. *Pterocladia* and *Gelidium*) and Gracilariales (e.g. *Gracilaria*) (Lucyszyn *et al.*, 2006). When agar is mixed with liquid, it forms a gel at around 30°C that melts at about 90°C and resolidifies at about 45°C. This has high clarity, optimal strength and does not react with any components of the medium and it is not digested by enzymes from the plant tissue (Scholten and Pierik, 1998).

Despite of these good qualities, like any other gelling agent, the type of agar used can influence the growth and development of the explants in culture. Hence although agar is considered biologically inert with highest purity levels, some workers have reported presence of impurities and differences in type and quality from batch to batch has been associated with adverse effects like inhibition of growth, hyperhydricity, necrosis and cytotoxic effects (Puchooa *et al.*, 1999; Te-Chato *et al.*, 2005; Lucyszyn *et al.*, 2006). Therefore the choice of a suitable agar should be based on its quality. The quality parameters which are useful to determine the right agar brand for microbiological and plant tissue culture include color (White to light cream), Particle Size (95% passes 60 mesh sieve), Moisture (12% or less), Gel Strength (800-1150 g/cm2 for Nikan, 1.5% sol), Gelling Point (32-38°C for 1.5% sol), Melting Point (80-90°C for 1.5% sol), pH in Gel (6.1-6.9 for 1.5%

sol), Turbidity (6 NTU or less for 1.5% sol), Ash (4.5% or less) and Solubility in boiling water.

2.6.2. Gelrite

Gelrite consists of a polysaccharide produced by the bacterium *Pseudomonas elodea*. Medium solidified with Gelrite has the advantage of being clearer than agar-solidified medium. Consequently contamination is easily detected at an early stage (Scholten and Pierik, 1998). Gelrite contain inorganic ions as impurities and requires more stirring than agar when added to media. Unlike agar, Gelrite cannot be reheated and gelled successfully.

One limitation of gelrite is that the concentration of divalent cations such as calcium and magnesium ions must be within the range of 4-8 mM/liter (Johnson, 2008). Concentrations of these two ions either less than or greater than this range result in the media not gelling. Gelrite may also produce hyperhydric plants when used at low concentrations.

2.6.3 Phytagel

Phytagel is an agar substitute produced from a bacterial substrate composed of glucuronic acid, rhamnose and glucose (Johnson, 2008). It produces a clear, colorless, high-strength gel, which aids in detection of microbial contamination. It is used at a concentration of 1.5-2.5 g/L. To prevent clumping, phytagel should be added to rapidly stirring culture medium which is at room temperature. Hyperhydricity may also be a problem with this gelling agent (Te Chato *et al.*, 2005). The selection of a gelling agent for specific plants is generally empirical. For unknown reasons, tissues of some species grow more vigorously on one gelling agent than on another. Another major consideration is the degree of hyperhydricity induced in a species by the different gelling agents. One potential way to overcome this is to combine agar and either gelrite or phytagel in the medium.

2.7 The need for cheaper agar substitutes

Agar is reported by many workers that it is the most expensive component of tissue culture media (Zimmerman *et al.*, 1995; Puchooa *et al.*, 1999; Naik and Sarkar, 2001; Kuria *et al.*, 2008). Hence, the cost of commercial micropropagation can be highly reduced if cheap alternatives to the high quality expensive purified agar are used. Moreover, over reliance on agar as the only gelling agent could result to over exploitation of it from its sources. In recognition of the above two facts, research to find out suitable agar substitutes has been emphasized by many TC practitioners. As a result, there is a number of agar alternatives already tested by many researchers for TC of different crops. These include starches from various crops and plant gums (Pierik, 1989, Nagamori and Kobayashi, 2001), Methylcellulose and Alginate (Adaoha and Roscoe, 1982) and Isobgol (Jain and Babbar, 2005).

The sources from which starch has been tested as substitute gelling agent include; maize and potato starch (Ibrahim *et al.*, 2005), sago starch (Naik and Sarkar, 2001), cassava starch (Gebre and Sathyanarayana, 2001; Kuria *et al.*, 2008) and cassava flour (Maliro and Lameck, 2004). Others are starches from barley, rice and wheat (Prakash *et al.*, 2004; Srathongjai *et al.*, 2007). The majority of those who worked in using starch as gelling agents, show that, *in vitro* plant growth was higher in starch gelled medium than the control treatment (Agar gelled medium), others record growth lower than the agar based media while some improvement in performance is observed when starch is mixed with agar at certain ratios (Maliro and Lameck, 2004; Kuria *et al.*, 2008).

2.8 Sources and availability of starch

Starch is a biopolymer carbohydrate, which is composed of anhydroglucose units and is the major storage energy in various plants in nature. It is largely found in cereal grain seeds of corn, wheat, rice, sorghum and millet. Tubers like potato and roots like cassava, sweet potato and arrowroot have large reserve of starch. Legume seeds of peas, beans and lentils are other minor sources of starch. In addition, starch is also found in fruits like green bananas, unripe apples and green tomatoes. Trunks and leaves of trees such as sago palm and tobacco respectively do have some amount of starch (Chen *et al.*, 2006a). However, in the order of importance worldwide, corn is the largest source of starch followed by wheat, rice, potato, cassava and sweet potato. These crops are grown almost in every part of the globe saving as staple food and have starch as the largest proportion of their Dry Matter Content (DMC). For instance, the DMC of corn has 62%, 8% and 4% of starch, protein and lipids respectively (ISI, 2007).

2.9 Composition and physicochemical properties of starch

Starch is a polysaccharide composed of 20-30% amylose and 70-80% amylopectin whose actual concentrations depend on genotype and environmental factors

(Shimelis *et al.*, 2006). Reports indicate the amylose contents of different crops as: waxy rice (0%), non waxy rice (25%), high amylose maize (70%), maize (28%), cassava (17%), waxy sorghum (0%), sorghum (28%), wheat (26%), sweet potato (18%), potato (20%) and sago (26%) (Satin, 2005; Beta and Corket, 2001).

Amylose is a biopolymer made up of linear chain of D-glucose units linked by α-1→4 bonds with average molecular masses of up to 2 X 10^6 although few (3-0.5%) have α-1→6 linkages (branches) (Chen, 2003). Unlike amylose, amylopectin is a highly branched polymer consisting of relatively short segments of D-glucopyranose residues (20-25%) linked by α-D-(1,4)-bonds, connected by α-D-(1,6)-glucosidic linkages with molecular masses ranging from 10 X10^6 to 500 X 10^6 (Herero-Martinez *et al.*, 2004). As compared to amylose, the amylopectin structure is more complex since 4-5% of the total linkages form branches. Amylopectin structure consists of three type chains: the C chain, which carries the sole reducing group in the molecule to which the B chains are attached while the terminal A chain is attached to B chain.

Naturally, starch exists in tightly and radially packed dehydrated granular forms with original specific size, distribution, shapes and dimensions. For instance the granular size of maize is 2-30 µm, wheat is 1-45 µm, potato is 5-100 µm, cassava is 3-28 µm, sorghum is 3-27 µm, sweet potato is 4-40 µm, sago is 15-50 µm and waxy rice is 2-13 µm (Satin, 2005). The granular and molecular structures, the ratio of amylose to amylopectin and their respective degree of polymerization play an important role in determining the physicochemical properties of starch and ultimately its functional properties in various applications (Satin, 5005).

These functional properties include chemical composition (fat, protein, phosphorus, Ash and Fiber contents), solubility, swelling power, water binding capacity, gel strength and viscosity and gelation temperature and concentration (Herero-Martinez *et al.*, 2004). Different workers have reported the physicochemical and pasting properties of cassava starch (Niba *et al.*, 2002; Benesi, 2005; Rai, 2004), sweet potato (Chen, 2003), sorghum (Beta and Corket, 2001), maize (Sandhu and Singh, 2006; King, 2005) potato (Rai, 2004), rice (Chatakanonda *et al.*, 2000; Spigno and Faveri, 2003) and wheat (King, 2005; Sasaki, 2005). Since different botanical starch types have different properties, they are used in various applications as gelling agent, film former, thickener, adhesive, textural stabilizer, glazing agent, foam strengthener, dusting agent, crystallization inhibitor, flow aid and molding medium (Satin, 2005; Chen *et al*, 2006b).

It is recommended that, starch as gelling agent for cost effective tissue culture should be evaluated for clarity, gel strength, biological growth of plants along with other physiochemical properties of the product (Zallie, 1998). A formulation which allows free movement of water to avail other nutritional contents to the explant is desirable (Mehrotra *et al.*, 2007). In addition the gel strength of the chosen formulation should be good enough to hold the explant upright. Therefore to make proper prediction of the suitable starch types, their physicochemical properties should be analyzed. Such properties include purity, granule size, distribution and shape, gel strength, swelling power or water binding capacity and gelation temperature.

2.10 Important physicochemical properties of starch as gelling agents for tissue culture

2.10.1 Starch purity

For the purpose of TC, starch extracts with high starch purity and low content of other components (e.g. protein, fat, ash, fiber) are highly desirable. Attaining high purity depends on the efficiency of the used extraction procedure to eliminate the impurities. According to Chen (2003), high levels of lipid content may result into low clarity of the starch gel as with cereal starches. Furthermore lipids suppress starch granule swelling. The report also pointed out that, high levels of phosphate ester groups give amylopectin slight negative charge, resulting into some repulsion that may contribute to the rapid swelling of starch granules in warm water. In addition, it leads to high viscosity, high clarity and low rate of retrogradation. Moreover, Shimelis *et al.* (2006) indicated that protein forms protein-amylose complex which was associated with the decrease in swelling power.

2.10.2 Starch swelling power (SP)

According to Shimelis *et al.* (2006), swelling power is a measure of the hydration capacity of starch. It is expressed as the weight of centrifuged swollen granules, divided by the weight of the original dry starch used to make the paste. The granule size, distribution and shape determines its swelling functionality with granules being generally either larger or lenticular (lens-like, A-starch) or smaller and spherical (B-starch) with less swelling power. Work by Rai (2004) revealed that potato starch held slightly more water (10.44 g H_2O gK1) than that of tapioca (10.06 g H_2O gK1) and corn (7.92 g H_2O gK1) starches. This variation was associated with the difference in the degree of the engagement of hydroxyl groups to form hydrogen and covalent bonds between starch chains. This difference has an implication on swelling power and ultimately on the degree of availability of water and the dissolved mineral nutrients for plant growth *in vitro*.

2.10.3. Starch gel formation

2.10.3.1 Least gelation concentration

A gel is formed when cooked paste of starch cools without agitation such that intermolecular bonds are formed both within and between the swollen starch granules and their fragments. Studies on corn, potato and cassava revealed differences between and within botanical starch types on the minimum concentration required to form gel (Rai 2004). It was noted that potato starch gelled at lower (4.0%, w/v) concentration followed by corn (6.0%, w/v) and cassava (8.0%, w/v) starches. This observation can be explained in terms of the amylose linear fraction that readily set up into a solid gel.

Since amylose content is higher in corn and potato they form gel at concentration lower than that of cassava starch. This is explained by the fact that amylose forms gel due to the straight chains that orient themselves in a parallel alignment so that a large number of hydroxyl groups along the chain are in close proximity to those on adjacent chains resulting in to gel formation or gelation while the alignment is inhibited in case of amylopectin due to its branched structure (Rai, 2004)

2.10.3.2 Gelatinization Temperature (GT)

Mackil *et al.* (1996) defined GT as a temperature at which the starch granule begin to swell irreversibly in hot water as it undergoes gelatinization. The starch granules when heated in water gradually absorb water and swell, causing the mixture to thicken. With continued heating, the swollen granules fragment, the mixture becomes less thick, and the amylose and amylopectin become soluble in the hot mixture. Once gelatinized the granules cannot be recreated and the starch behaves as a mixture of amylose and amylopectin. Because of the larger size of the swollen granules compared to the original size of amylose and amylopectin, the viscosity of the swollen granule mixture is much higher than the viscosity of the amylose/amylopectin mixture. Starches from different plant sources vary in their gelatinization temperatures, rate of gelatinization, maximum viscosity, clarity of the gelatinized mixture, and ability to form a solid gel on cooling.

2.10.3.3. Texture of starch gel

The texture of heat-gelatinized starch is variable. According to Rai (2004) some gelatinized starch mixtures have a smooth creamy texture, while others are more pastelike. Some starches form gels after cooking and cooling. Such starch gels may lack stability and slowly exude water through the gel surface, the process called syneresis. Although amylose is soluble in hot starch gel, it tends to become insoluble in the cooled mixture and this phenomenon is called retrogradation occurring when the amylose chains bind together in helical and double helical coils. Retrogradation affects the texture of TC media but lowers the digestibility of the product hence the media become resistant to digestion by plant enzymes.

2.10.3.4. Strength of starch gel

Gel strength is highly influenced by the amylose-amylopectin ratio. Correlation analysis done by Mackill *et al.* (1996) revealed that, the higher the amylose content of rice, the lower is the swelling power and the smaller is the gel strength for the same starch concentration. Also increasing amylose concentration decreases gel stickiness but increases gel firmness. To a certain extent, however, a smaller swelling power due to high amylose content can be counteracted by a larger granule size. Some amylopectin (e.g. potato) has phosphate groups attached to some hydroxyl groups, which increase its hydrophilicity and swelling power. In general, the ability of a starch to form a gel along with relative firmness of the gel is related to a starch's molecular make-up. Amylose, the linear D-glucose homopolymer tends to associate tightly in solution into linear bundles and is therefore primarily responsible for gel formation. Amylopectin, on the other hand, is a branched glucose polymer and much larger than amylose, resulting in greater immobilization of water (viscosity) but no gel formation. Therefore, it can be concluded that the higher the amylose content, the firmer the gel and the faster the rate of gel formation.

2.11. Production and utilization of starch

The world production of starch is estimated to more than 800 000 000 tonnes produced from corn (75 %), potato (7.6%), wheat (9%), cassava (4.2%) rice (0.7%) and the rest other sources. In Europe, about 7.7 million tones of starch are produced annually from corn (49%), wheat (29%) and potato starch (22%) (Sandhu and Singh, 2006). In East Africa, maize, rice, sorghum, wheat, potato, cassava and Sweet potatoes are major crops producing starch. However, the market for other industrial uses is growing.

Up to 2002, Uganda had approximately 1500 Mt annual consumption of starch. A recent survey by Steven and co-workers (Steve, B. K., Personal communication 2008) conducted in three East African countries (Tanzania, Kenya and Uganda) revealed that starch is increasingly being used in laundry, pharmaceuticals, food industries, textile mills, paper mills, wood processing and cardboard making. Most of the starch used is from corn and mixtures imported from outside these countries mainly from India and Europe.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental sites

Starch profiling to determine the physicochemical properties of the selected botanical starch types was conducted at the National Crop Resources Research Institute (NaCRRI), Namulonge, Uganda. The Tissue Culture experiments were conducted at Mikocheni Agricultural Research Institute (MARI), Dar es Salaam, Tanzania.

3.2 Materials

3.2.1 Gelling agents and test plant

Starches which were extracted from seven commonly grown crops were used as gelling agents. These included maize (*Zea mays* L cv. stuka) and wheat (*Triticum aestivum* L. cv. riziki) which were bought from the Agricultural Research Institute (ARI)-Seliani, Arusha, Tanzania. Others were sorghum (*Sorghum bicolor* L cv. macia) and rice (*Oriza sativa* L cv. saro 5) which were bought from ARI-Ilonga and Cholima research station respectively both in Morogoro, Tanzania. Starch was also extracted from fresh tuberous roots of cassava (*Manihot esculanta* Crants, cv. kiroba), irish potato (*Solanum tuberosome* L cv. CAP) and sweet potato (*Ipomoea batatas* L. cv. unknown) which were bought from the Morogoro market. The agar powder (Technopharmachem Ltd) was provided by MARI. The test plant was sweet potato (*I. batatas* L cv. ukerewe) that was obtained from the sweet potato germplasm collection at ARI-Kibaha, Coast region, Tanzania.

3.3 Methods

3.3.1 Starch extraction

3.3.1.1 Extraction of starch from maize

The alkali method described by Seetharaman and White (2004) was used with little modification. The maize sample weighing 2kg was winnowed and washed using tape water to remove contaminants before taking the sample to a grinding mill for course grinding to remove the pericarp and germ. The sample was then rewashed and steeped in 1% (w/v) NaOH solution at 45°C water bath for 24 hrs to loosen the endosperm. The steeped grains were washed in distilled water before wet grinding (1:1 w/v) in commercial blender. The slurry was filtered through triple cheese muslin cloth and the remaining materials were re-washed, re-filtered and the remaining grains were subjected to repeated grinding and filtration. The filtrate was left to settle (sediment) at room temperature for 8hrs before the supernatant was drained off. The remaining sediments were oven dried at 30°C for 24 hrs. The dried starch in form of pellets was milled using a laboratory hummer mill (3 000 rpm) to obtain a fine powder.

3.3.1.2 Extraction of starch from sorghum

The alkali method described by Beta and Corket (2001) was used with little modification. The sample weighing 2 kg was winnowed and washed using tape water to remove contaminants before it was steeped in 0.25% (w/v) NaOH solution respectively at 45°C water bath for 24 hrs to loosen the endosperm. The steeped grains were washed using distilled water and wet ground (1:1 w/v) in commercial blender. Other procedures after grinding were the same as those described in section 3.3.1.1 for maize.

3.3.1.3 Extraction of Starch from Rice and Wheat

Extraction of starch from rice and wheat followed the model developed by Al-Hakkak (2006) with some modifications. Rice and wheat grains were winnowed and washed to remove foreign materials before they were air dried. The samples were ground into flour using a commercial hummer mill (3 500 rpm) before the flour was manually mixed with water in 1:1 (w/v), flour to water ratio to form coherent dough. The dough was then incubated at room temperature for 4hrs to allow separation of starch granules from other grain components. The dough was kneaded under distilled water to release the starch into water. About 10 washing rounds were done to ensure starch is removed from the dough matrix remaining with protein in form of gluten. The slurry was washed through filtering using a series of sieves (500 μm, 200 μm, 120 μm and 75 μm). All other treatments of the filtrate to form the final starch powder were the same as described in section 3.3.1.1 for maize

3.3.1.4 Extraction of starch from cassava, sweet potato and irish potato

Extraction from Cassava, Sweet potato and Potato was carried out using a modified method described by Benesi (2005) and Riley *et al.* (2006). Fresh tubers and roots each weighing 20 kg were washed and peeled before grating using a motorized grater (2 500 rpm). The pulp was stirred for 2 minutes and filtered using a triple cheese (muslin) cloth. The filtrate was allowed to stand to facilitate starch

sedimentation for 8hrs before the supernatant was decanted and discarded while the remaining sediments were air dried and ground using a laboratory hummer mill (3 000 rpm).

3.3.2 Establishment of explants source plants

About ten vines each with a length of 10cm were collected for planting in pots in the screen house at MARI. The vines were planted in two liter pots containing forest soil. A liquid fertilizer 20% (w/v) Agrofeed (NPK 20:20:20) was applied fortnightly to boost the growth. Insect pests were controlled by applying 25% Sumethion (50 EC) also fortnightly.

3.3.3 Tissue culture procedure

3.3.3.1 Preparation of the MS (Murashige and Skoog, 1962) medium

Tissue Culture (TC) media for culture initiation were prepared based on full strength of Murashige and Skoog (1962) recipe of inorganic and organic nutrients (Appendix 1). The initiation media were supplemented with 3% (w/v) sucrose and 0.5 mg/l Benzyl Amino Purine (BAP). The multiplication medium was supplemented with 5 mg/l Giberrelic acid (GA_3) without BAP. While the pH of agar gelled medium was adjusted to 5.7 before adding the gelling agent, the starch gelled medium was adjusted to the same pH after adding the gelling agent. The pH was adjusted while stirring using magnetic stirrer. The adjustment was done using 1M NaOH and 0.1M HCl. The TC media were dispensed into culture bottles (30 mm x 100 mm) before sterilizing at 121°C and 100 kPa for 20 minutes. Each culture bottle contained 20 ml of the medium.

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3.3.3.2 Explant preparation, inoculation and incubation

Healthy and rapidly growing stem vines of about 5cm long were harvested from the parent stock and trimmed by cutting the leaves 1cm from the attachment. The vines were then washed thoroughly under tape water to reduce surface contaminants. The vines were further washed using distilled water with 1 % (v/v) liquid soap with 1 drop of Twin 20 followed by rinsing them three times using distilled water. The washed vines were then transferred into a sterile environment in the laminar flow hood where they were washed in 70% ethanol for 1 minute followed by rinsing three times using sterile distilled water. Lastly, the vines were disinfected by exposing to 10% commercial Jik (3.5% NaOCl) containing two drops of Twin 20 for 10minutes then to 5% of the same for another 10 minutes before rinsing them four times using sterile distilled water.

The surface sterilized vines were kept in sterile distilled water to avoid desiccation. One centimeter long single node explants with axillary buds were isolated from the surface sterilized stem vines under aseptic condition in the laminar flow hood. The excised explants were inoculated into a culture bottle (30 mm X 100 mm) containing 20 ml MS medium solidified by different gelling agents. These were incubated at 23± 2°C under a 16 hours photoperiod with a photosynthetic photon flux density of 40 μmol m^{-2} s^{-1} provided by overhead cool fluorescent lamps (Philips, India 30 Watts) for 21 days.

3.3.4 Determination of physicochemical properties of starch extracts

3.3.4.1. Determination of starch content

The starch content of triplicate samples of each of the seven extracts was determined according to (Bhagya *et al.*, 2006). A total of 100 mg of each starch extract was defatted by boiling in 30 ml of 80% ethanol for 10 minutes and the residue collected was dried at 70°C for 4 hours. To the residue 10 ml of 52% $HClO_4$ was added to digest the starch for 15 minutes at 28°C of the water bath and made up to 25 ml with distilled water after which the mixture was filtered through Whatman No.1.

The Dubois method (Dubois *et al.*, 1956) was employed to determine the total sugars. About 20 µl of the filtrate was made up to 1 ml with distilled water and 1 ml of 5% phenol followed by 5 ml 36 N H_2SO_4 was added. Pure glucose (Sigma) (20 µ – 100 µ) was taken as standard from which a standard curve was established. Absorbance of the solution was read at 490 nm using a spectrophotometer (Altrospec 2000 by Pharmacia biotech). The amount of sugar contained in each sample of starch extract was calculated from the standard curve and the respective starch content was calculated as total sugar multiplied by 0.9.

3.3.4.2 Determination of the amylose content

A triplicate sample, which weighed 100 mg of starch was defatted according to the standard AOAC methods as in Riley *et al.* (2006) with modification. About 1ml of absolute (99.9%) ethanol was added to the sample followed by votexing for 1 minute before leaving the mixture to settle for 30 minutes after which ethanol was decanted off. To this mixture, 9.0 ml of 1M NaCl was added and the volume was made up to 100 ml with distilled water. From this mixture, 5.0 ml of an aliquot was transferred to a volumetric flask containing 25.0 ml of water. To this volume 19.0 ml of 1M Acetic acid and 1.0 ml of 0.2% Iodine solution were added making the volume up to 50 ml with distilled water. Absorbance of the resulting solution at 620 nm for amylose was read using a spectrophotometer (Altrospec 2000 by Pharmacia biotech). Standard amylose in varying concentrations was used to construct a standard curve from which the concentrations of amylose in different samples of starch were calculated based on Lambert and Beer's equation represented as; A = ebc, where A is absorbance, e is the molar absorptivity with units of L mol^{-1} cm^{-1}, b is the path length of the sample and c is the concentration of the compound in solution, expressed in mol L-1(Lehninger *et al.*, 1992)

3.3.4.3 Determination of swelling powers of the starches

The swelling power of the starch granules was determined according to Leach *et al.* (1959). Firstly the centrifuge tubes were weighed (W1). Secondly, a triplicate sample weighing 0.1 g of starch (W2) from each of the seven sources were put in pre-weighed tubes and incubated in a water bath at 60°C and another same amount at 30°C both for 30 minutes. After incubation, the samples were centrifuged at 7 000 rpm for 20 minutes followed by decanting the supernatant before weighing the wet sample (W3). Swelling power was determined as the percentage change in the weight of samples of starch calculated using the formula:

Percentage Swelling Power = W3- (W1+W2)/W2 X 100

3.3.4.4 Estimation of protein concentration

Proteins were estimated using the Bradford method (Nobel, 2000). The standard procedures were used to prepare a Bradford reagent and stored in brown glass bottle. Starch samples each weighing 5 g in triplicate were mixed with 10ml of distilled water from which 0.5 ml was drawn to be mixed with 0.5 ml of de-Ionized water followed by adding 5ml of Bradford reagent. This mixture was well mixed by vortexing for 1 minute and thereafter left to settle for 5 minutes. After settling, absorbance at 595 nm was read from spectrophotometer (Ultraspec 2000 by Pharmacia biotech). The protein concentration was then estimated from a standard curve, which was prepared using a standard protein (Bovine serum albumin).

3.3.4.5 Determination of crude fat content

Crude fat content of the samples was determined using Soxhlet apparatus as described by Nagur *et al.* (1992). In this method, 2 g of starch (W2) in triplicate samples were put in a pre-weighed (W1) filter paper (Whatman No 1) after which they were taken into the soxhlet system for lipid extraction using chloroform and hexane mixed in 1:1 ratio. The remaining fat free sample was dried and re-weighed (W3) then fat content was calculated as a percentage change by the formula;

Percentage Fat content = [W1+W2] – W3)/W1 X 100

3.3.4.6 Determination of properties of starch and starch gels: Least Gelation Concentration (LGC), gel strength, colour and clarity.

A total of 63 formulations of TC Media gelled by the 7 types of starch each at 9 concentration levels: 6%, 9%, 12%, 15%, 18%, 21%, 24%, 27% and 30% (w/v) were prepared using standard procedures for media preparations. Three people experienced in preparing TC medium were requested to score each treatment for the degree of gel firmness on a 7 point scale (0-1 = liquid, 2-3 = weak semi solid, 4-5 = stable semi solid, 6-7 = solid) modified from method described by Lee and Chung (1989). Colour of starch powder was determined using a colour checker (Whistler, 1985)

The starch powders from the seven sources were then used as gelling agents each at 12% (w/v) making starch gelled medium from which the gel colour, clarity and firmness before and after autoclaving were scored. The gel clarity was scored using a four point scale (1 = Transparent, 2 = Semi transparent, 3 = Semi opaque and 4 = Opaque).

3.3.5 Evaluation of the potential of different starch extracts and concentrations to support *in vitro* growth of nodal explants

A total of seven starch extracts namely rice, maize, sorghum, wheat, irish potato, sweet potato and cassava were used to solidify the TC media. The TC media were solidified by starches each at four concentrations: 12%, 15%, 18% and 21% (w/v). The TC media gelled by agar powder (Technopharmachem Ltd) at a concentration of 0.08% was used as a control. One nodal explant was cultured in each culture bottle containing 20 ml of TC media. The culture bottles containing the experimental media in which nodal explants were inoculated were replicated 15 times for each treatment. From each regenerating nodal explants, the number of leaves and nodes and height of each shoot were recorded weekly for three consecutive weeks and the fresh weight was measured at 21st day of incubation. The height was measured using a 30 cm rule while the fresh weight was measured using an analytical balance.

3.3.6 Determination of the effect of type and concentration of gelling agent on survival of *in vitro* nodal explants in multiplication media subculture

Based on the preliminary observations of the experiment described in 3.3.2, only three starch extracts (cassava, sweet potato and irish potato) were chosen for further micropropagation experiments. In this experiment, at least 1cm long nodal explants from *in vitro* cultures were cultured in TC media, which were solidified by four types of gelling agents namely starch from cassava, sweet potato and irish potato and agar powder. Each type of gelling agent was applied in four concentrations whereby both sweet potato and irish potato starches were applied at concentrations of 9%, 10%, 11% and 12% while cassava starch was applied at concentrations of 13%, 14%, 15% and 16%. TC media gelled by Agar powder at a concentration of 0.08% was used as control.

About 20 ml of TC media were dispensed in culture bottle (30 mm x 100 mm) in which a nodal explant was planted. The culture bottle containing the nodal explant was replicated 15 times for each treatment. After seven days of incubation, the survival of the nodal explants were recorded in terms of the number of regenerating nodal explants of each treatment and computed in survival percentages.

3.3.7 Determination of the effect of type of gelling agent on quality, rate and cost of micropropagation of sweet potato

Based on the preliminary observations of the experiment described in 3.3.2, the starch extracts from wheat, sorghum, rice and maize were eliminated from further micropropagation experiments. For the purpose of this experiment, the surface sterilized nodal explants with an axillary bud, which were at least 1cm long were cultured individually into culture bottles containing 20 ml of initiation media.

The initiation media were solidified by 15% cassava and 12% for both irish and sweet potato starches. After three weeks (21 days) the *in vitro* shoots regenerated from culture initiation were excised into 1-1.5 cm nodal propagules, which were transferred into multiplication media. The multiplication phase was conducted in three subcultures done at 21 days interval. After every subculture a freshly prepared multiplication media was used for the next subculture.

After 21 days of every subculture, the number of leaves per shoot, fresh and dry weights were recorded to determine the quality of propagules produced. Furthermore, the shoot height (mm), number of nodes per shoot and the number of 1-1.5 cm long nodal propagules produced from each shoot were recorded to determine the micropropagation rate.

The costs associated with procurement and processing of starch based and agar gelling agents were recorded for determination of the cost effectiveness of using each gelling agents. The cost items involved were procurement costs of agar, the crops from which starch was extracted, chemical and labour costs of extraction.

3.3.8 Experimental design and data analysis

A completely Randomized Design (CRD) was used for both single factor (Section 3.3.2 and 3.3.4) and two factor (3.3.3) experiments. Variance analysis was calculated using GENSTAT discovery edition 3 (Buysse *et al.*, 2008) to discover the significance of treatment effects based on the statistical models $Yij = \mu + Ai + Bj + (AB)ij + \varepsilon ij$ for experiments with two experimental factors and $Yij = \mu + Ai + \varepsilon ij$ where Yij is the jth observation in treatment i. The μ is the overall mean, Ai is the ith treatment effect, (type of gelling agent), Bj is the jth treatment effect, (concentration), (AB)ij is the interaction effect of the two factors, and εij is random error component. The means which showed significant differences were separated and ranked using Duncans Multiple Range Test (DMRT) in a general linear model.

The number of nodal propagules produced after every culture stage was considered as its multiplication factor. Hence based on the multiplication factors of the different stages of culture, the number of propagules that can be produced during the period of culture was estimated by using the formula: $N = FC \times FS_1 \times FS_2 \times ....FS_n$ where by N = total number of propagules, FC = multiplication factor for culture initiation, FS_1 = Multiplication factor for first subculture, FS_2 = Multiplication factor for second subculture and FS_n = Multiplication factor for the nth subculture (Mendes *et al.*, 1999). The costs for gelling agents and the produced propagules were estimated using the field cost principle established by CIMMYT (1988).

CHAPTER FOUR

4.0 RESULTS

4.1 Physicochemical properties of the starch extracts

4.1.1 Starch purity

There were significant variations in the amount of pure starch among the treatments (P = 0.05). Among the seven samples of starch extracts, the highest (81.49%) amount of pure starch was recorded on cassava and the lowest (29.32%) on irish potato (Table 1). Other starch extracts with relatively high content of pure starch were sweet potato (77.51%), rice (75.63%) and wheat (72.35%).

Table 1: Purity of starch extracted from cassava, sweet potato, irish potato, rice, maize, sorghum and wheat on dry basis.

Starch extract	Starch (%)	Protein (%)	Fat (%)
Cassava	81.49±0.79d	3.60±0.67a	0.26±0.16a
Sweet potato	77.51±1.85cd	9.90±0.14cd	1.94±0.05b
Irish Potato	29.32±3.89a	8.23±0.31bc	4.78±0.49c
Rice	75.63±3.58cd	22.86±1.60e	0.65±0.03a
Wheat	72.35±1.07bcd	10.75±1.47cd	6.54±0.22d
Sorghum	67.35±2.21bc	6.36±0.45b	1.83±0.09b
Maize	62.81±7.50b	12.08±0.06d	0.46±0.18a

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

It was also observed that irish potato starch had high oxidation (Browning) during air-drying of wet extracts (Plate 1).

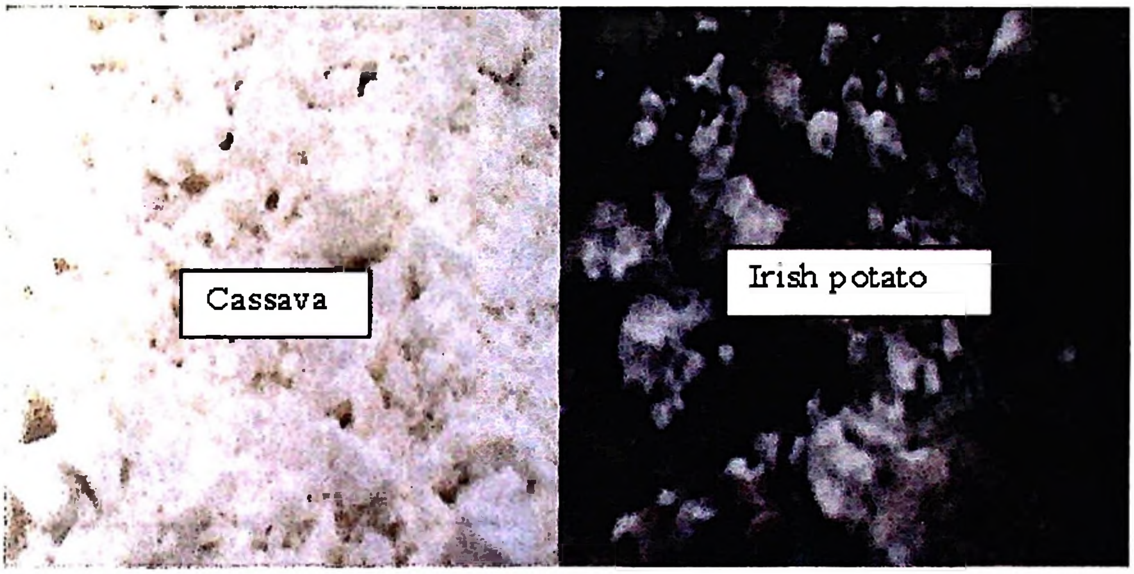


Plate 1: Browning of irish potato starch extract during air drying

Significant differences among the starch extracts for the protein content were observed (P = 0.05). The starch extract from rice had the highest protein content (22.86%) and that of cassava had the lowest (3.6%) (Table 1).

At 5% level of significance, the amounts of fats in the seven starch extracts were significantly different. The highest amount of fat (6.54%) was recorded in starch extracted from wheat while the lowest (0.26%) was recorded in the extract from cassava.

4.1.2 Amylose content

The seven starch extracts had significantly different (P = 0.05) amounts of amylose. The starch extract from rice was found to have the highest amylose content (31.12%) while the cassava starch extracts had the lowest (20.75% (Figure 1).

Generally, the results show that the extracts from roots and tuber crops had amylose content of 20.75 - 23.42% which was lower than the extracts from cereal crops (23.73 - 31.12%) (Figure 1).

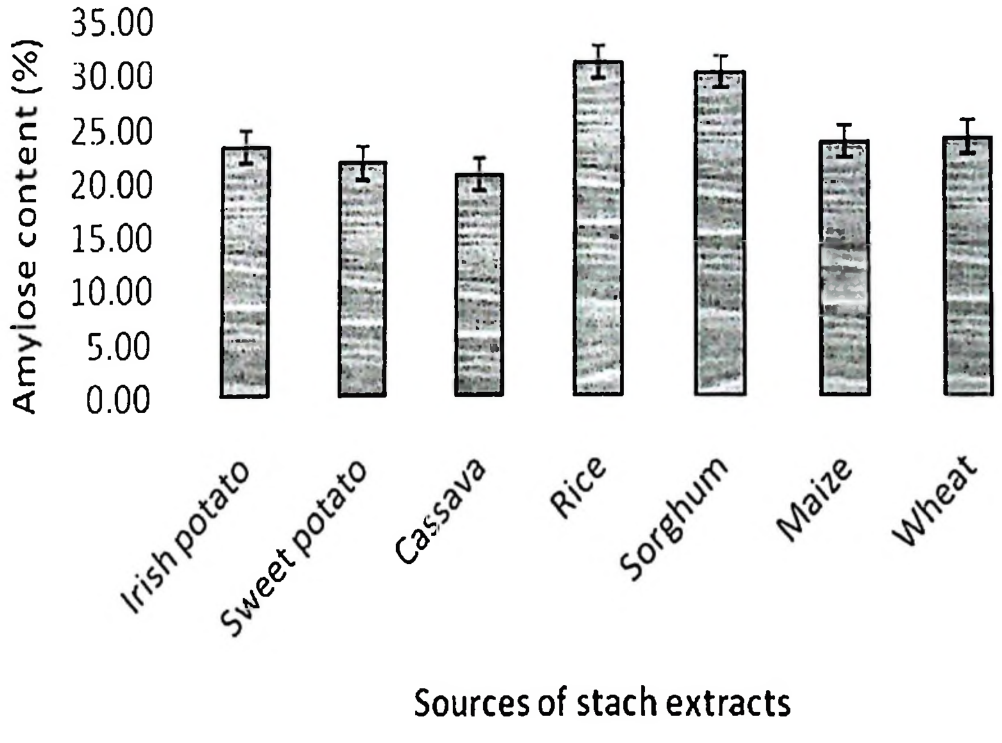


Figure 1: Amylose content of starch extracted from cassava, sweet potato, irish potato, rice, maize, sorghum and wheat.

4.1.3 Swelling power

4.1.3.1 Effect of source of starch on swelling power

There were differences in swelling power of the starch granules of different starch extracts (Figure 2). The starch extracted from wheat had the highest swelling power (105.1%) while irish potato starch had the lowest (52.1%). With exception of wheat starch, the swelling power of all other cereal starches was lower than that of starch from root crops (Cassava and sweet potato).

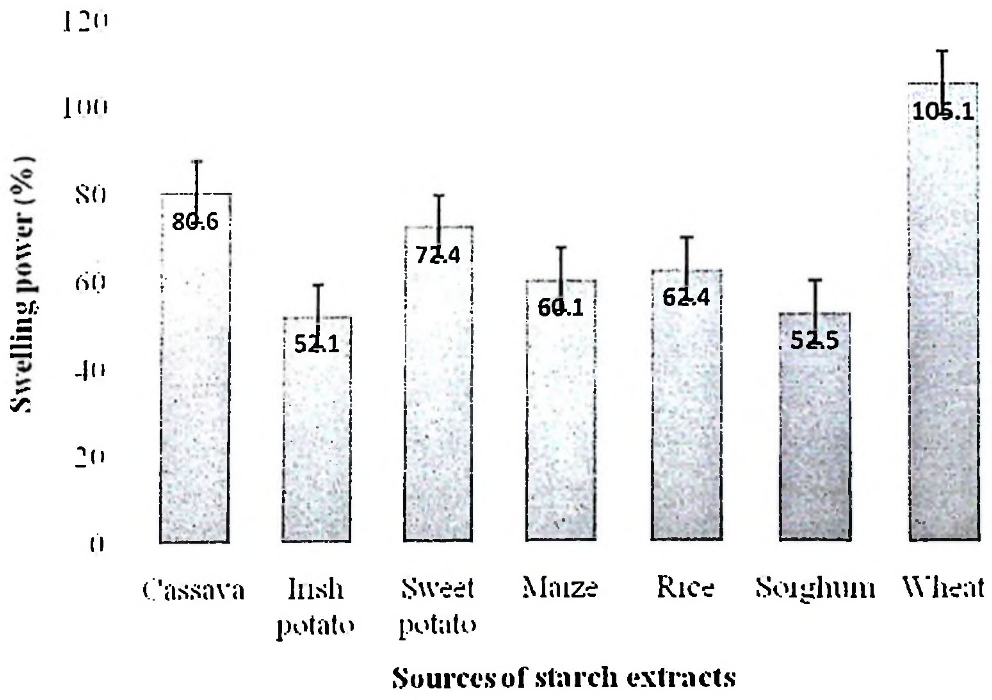


Figure 2: Swelling power profiles of starch extracted from cassava, sweet potato, irish potato, rice, maize, sorghum and wheat.

4.1.3.2 The Effect of incubation temperature on swelling power

The swelling power varied with incubation temperature (Figure 3). The results showed that, the swelling power of starch in all extracts increased with increasing temperature except the starch from maize. The highest increase was observed in wheat starch whereby a temperature change from 30°C to 60°C increased the swelling by 140.72% while with the same change of incubation temperature, the swelling power of sorghum increased by only 15.31% (Figure 3).

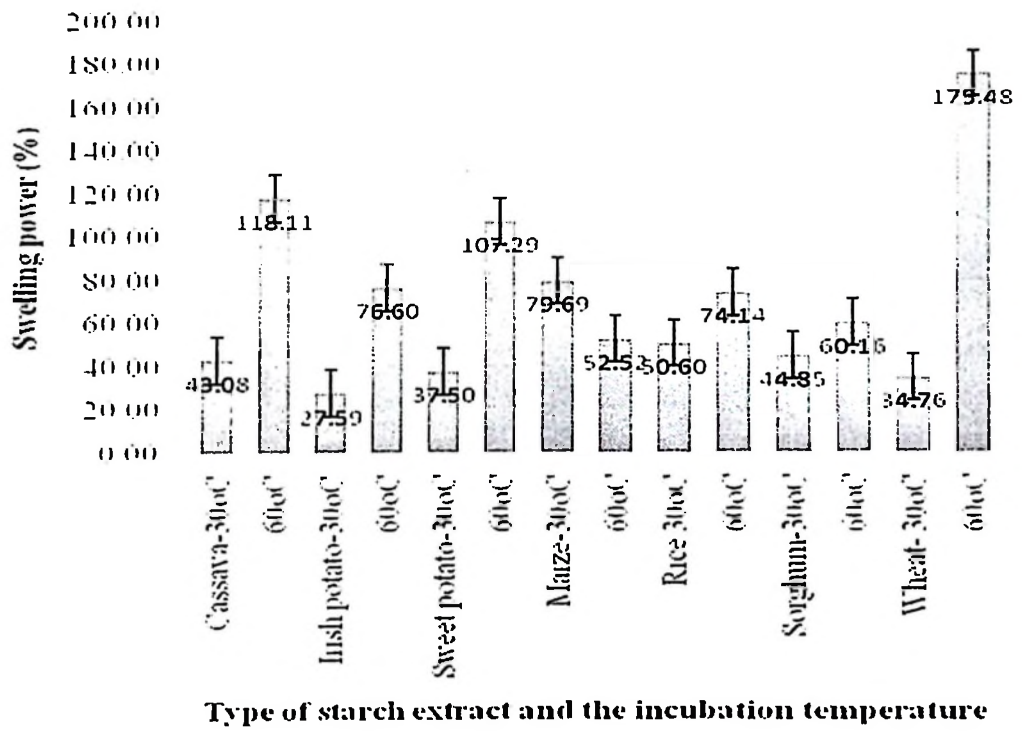


Figure 3: Swelling power profiles of starch extracts at different incubation temperatures.

Unlike the cereal starches, starch extracts of cassava and sweet potato also as the incubation temperature increased, the swelling power increased by 75.03% and 69.79% respectively.

4.1.4 Acidity of starch extracts (pH)

The pH of the starch extract varied with the crop from which they were extracted (Figure 4). Sorghum starch had the lowest pH of 4.57 while the highest (6.92) was recorded in Irish potato starch. Generally the pH of cereal starch extracts was lower than those of root and tuber starches.

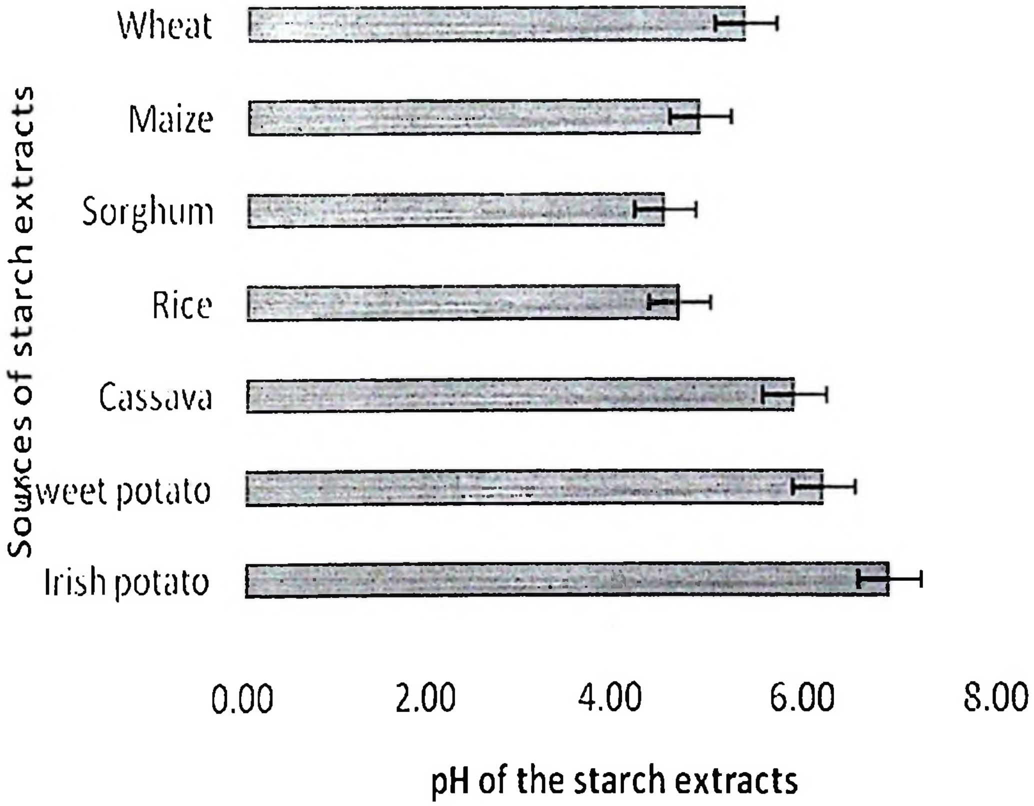


Figure 4: pH of starch extracted from cassava, sweet potato, irish potato, rice, maize, sorghum and wheat

4.2 Properties of starch gels for making solid TC media

4.2.1 Gelation concentration and gel firmness of TC medium

4.2.1.1 Gelation concentration

The results for gelation concentration are shown in Figure 5. The minimum concentration with which starches formed a semisolid gel varied with the source from which they were extracted. The results indicate that, to achieve a semisolid level of gel firmness, rice starch required the highest (21%) concentration while sweet potato and maize required and irish potato and maize required the lowest (12%). On the other hand, the starch from cassava, sorghum, rice and wheat required at least 15% concentration (w/v) to form a semisolid gel.

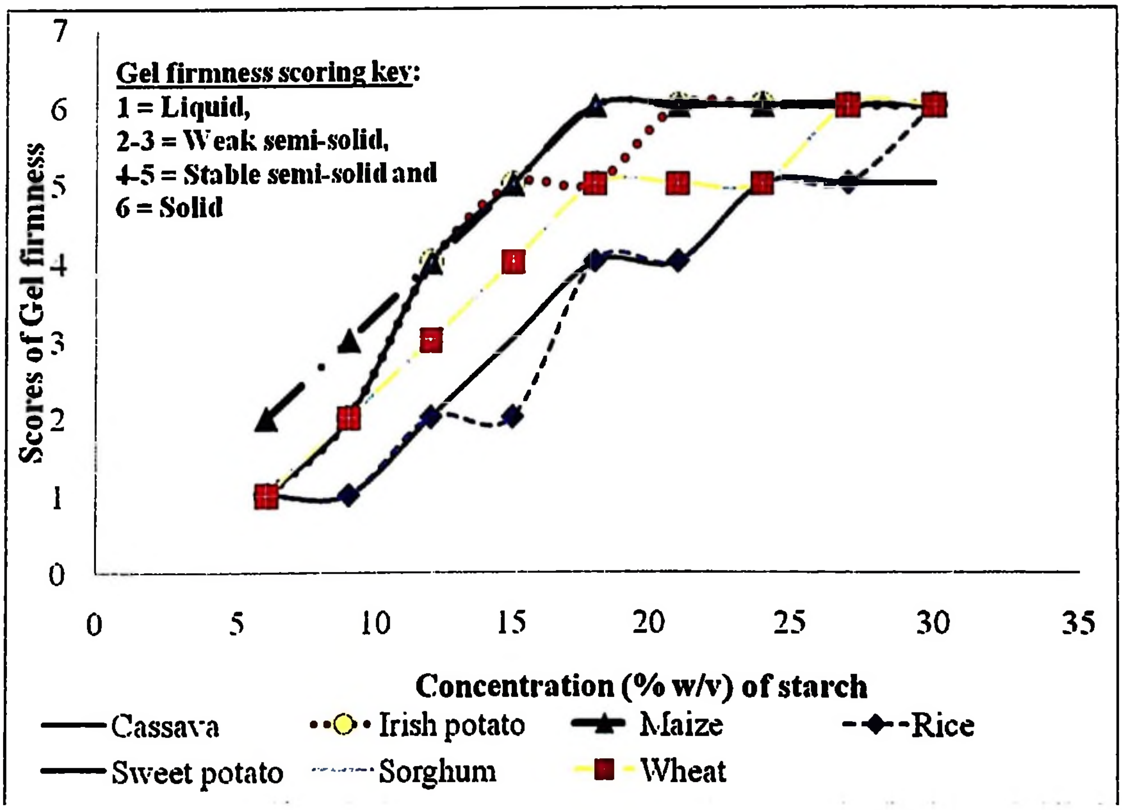


Figure 5: Gelation concentration and gel firmness of the cassava, sweet potato, irish potato, rice, maize, sorghum and wheat starch gels

4.2.1.2 Gel firmness

The gel firmness of TC media, which were solidified by all starch types increased with increasing concentration (Figure 5). Hence, starch extracted from maize, irish potato and sweet potato formed stable semisolid and solid TC medium at concentration below 13%. Sorghum and wheat starches required higher concentration (15%w/v) to form a stable semisolid gel while cassava and rice required the highest (18%) to form the same.

4.2.2 Colour and clarity of Starch gelled TC media

4.2.2.1 Gel colour

The different types of powdery gelling agents had different colours ranging from white to dark gray (Plate 2 A). During preparation of the starch gelled TC media, the results in Plate 2 indicate that the colour of powdery gelling agent changed with heat treatment during the preparatory stages of cooking at about 60°C to make starch gel and autoclaving at 121°C to sterilize the TC media (Plate 2 B and C).

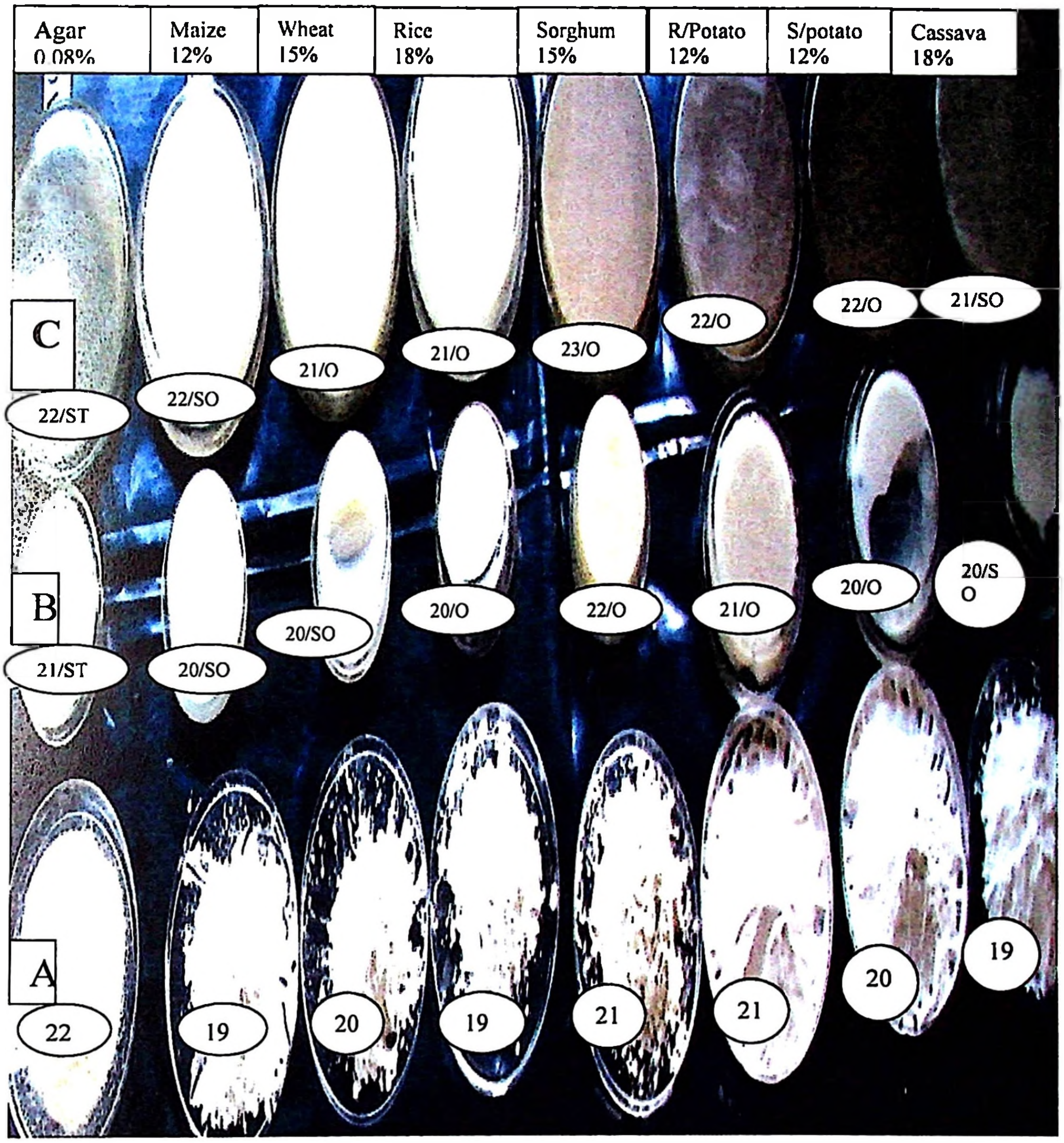


Plate 2: Colour and clarity of unautoclaved and autoclaved gels of selected crop starches

KEY; **A-Powdery gelling agents, B-Unautoclaved gels, C=Autoclaved gels, 19=White, 20=Light-gray, 21=Light medium-gray, 22=Medium-gray, 23=Dark-gray, ST=semi transparent, SO=semi opaque, O=opaque.**

4.2.2.2 Gel clarity

The clarity of starch gels, which were used to make semisolid TC media varied with the type of gelling agent solidifying it (Plate 2). Unlike the control gelling agent (agar) whose gel was semi transparent, all of the starch gels were opaque except those of cassava and maize, which were semi-opaque.

It was also observed that, unlike agar gelled media, once semisolid starch gel were prepared, they did not become liquid on autoclaving. Furthermore, it was noted that, the starch gelled medium had a tendency of forming thin film, which was sticking on the wall of culture vessel as presented in Plate 3.

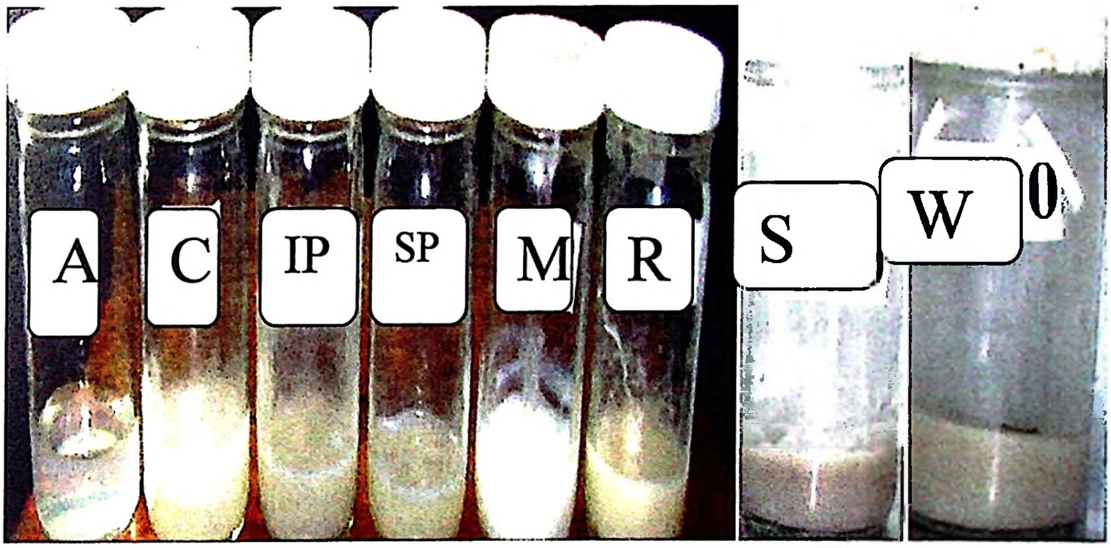


Plate 3: Thin film of opaque and semi-opaque starch gelled media sticking on the walls of culture bottles.

KEY: A=Agar, C=Cassava, IP=Irish potato, SP=Sweet potato, M=Maize, R=Rice, S=Sorghum and W=Wheat.

4.3 The potential of starch from selected crops as gelling agents to support *in vitro* growth of sweet potato (*I. batatas* L,)

4.3.1 Effect of source of starch on *in vitro* growth characteristics under culture initiation

4.3.1.1 Number of leaves per shoot

Number of leaves per shoot in TC media gelled by different starch extracts were significantly different (p = 0.05). The results showed that the mean number of leaves of a 21 days old shoot under culture initiation varied from 0.55 to 5.31 (Table 2). The number of leaves per shoot of *in vitro* shoots which were produced on cassava starch media (5.31) outperformed the control (4.85).

Table 2: The effect of type of gelling agent on number of green leaves and nodes per shoot, height and fresh weight of *in vitro* shoots at 21st day of culture initiation

Gelling agent	Leaves/shoot	Nodes/explant	Shoot height (cm)	Fresh weight (g)
Agar(8g/l)	4.85±0.07f	6.40±0.07g	8.74±0.12e	0.74±0.01h
Sweet potato	4.55±0.07d	4.60±0.08e	5.83±0.13d	0.50±0.01f
Cassava	5.31±0.07e	5.17±0.08f	6.04±0.13d	0.52±0.01g
Irish potato	4.06±0.08c	4.35±0.08d	5.77±0.13d	0.48±0.01e
Wheat	1.73±0.08b	1.73±0.08c	2.49±0.14c	0.18±0.01d
Maize	1.65±0.07b	1.60±0.07c	1.36±0.12b	0.14±0.01c
Rice	1.55±0.07b	0.70±0.07b	1.18±0.12b	0.10±0.01b
Sorghum	0.55±0.07a	0.00±0.07a	0.68±0.12a	0.04±0.01a

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

4.3.1.2 Number of nodes per shoot

Significant differences ($P = 0.05$) were observed on the number of nodes per shoot among the treatments. The number of nodes per shoot for the shoots regenerated from TC media gelled by the 7 starch types the agar based TC media ranged from 0 to 6.4 (Table 2). Agar (the control) outperformed the starches by producing 6.4 nodes per shoot. The responses recorded in TC media gelled by cassava (5.17), sweet potato (4.60) and irish potato (4.35) starches were comparable to that of the control. In contrast, the responses observed in TC media gelled by cereal starches ranged from 0 to 1.73 nodes per shoot, which was very low.

4.3.1.3 Shoot height

The mean heights (cm) of the *in vitro* shoots from different starch gelled media were significantly different ($P = 0.05$) and lower than the control. The values varied from 0.68 cm for sorghum starch gelled media to 8.74 cm of the agar gelled media. Among the media, which were solidified by starch, the best range (5.77 - 6.04 cm) of shoot height was observed in root/tuber starch gelled media as opposed to cereal starch gelled media (0.68 – 2.49 cm). Of the three root/tuber starch gelled media, highest shoot height (6.04 cm) was recorded on TC media gelled by cassava starch while the lowest was observed on irish potato (5.77) starch (Table 2).

4.3.1.4 Shoot fresh weight

Different types of gelling agents influenced the average fresh weight of *in vitro* shoots significantly ($p = 0.05$). The fresh weight varied from 0.04 g to 0.74 g. Agar gelled medium produced shoots with the highest (0.74 g) mean fresh weight and the shoots with least (0.04g) fresh weight were produced on sorghum starch media. As was observed with number of leaves and nodes per shoot and the shoot height in Table 2, the superiority of TC media gelled by starches from cassava, sweet potato and irish potato over the other starches was also observed in the ability to promote biomass accumulation (fresh weight) (Table 2).

4.3.2 The effect of concentration of gelling agent on growth of *in vitro* shoots

The effect of concentration on the number of leaves per shoot was significant (P = 0.05). Highest number of leaves per shoot (4.26) was attained at the lowest concentration (12% w/v) of a gelling agent. In contrast, at the highest concentration (21% w/v) of a gelling agent, the number of leaves per shoot was lowest (1.83) (Table 3).

Table 3: The effect of concentration of gelling agent on number of leaves and nodes, height and fresh weight of *in vitro* shoots at day 21 of culture initiation

Average Concentration	Leaves/shoot	Nodes/shoot	shoot height (cm)	Fresh weight (g)
12%	4.26±0.05d	4.42±0.05d	5.48±0.09d	0.47±0.00d
15%	3.64±0.05c	3.55±0.05c	4.62±0.09c	0.40±0.00c
18%	2.39±0.05b	2.40±0.06b	3.49±0.09b	0.29±0.00b
21%	1.83±0.05a	1.91±0.05a	2.45±0.09a	0.20±0.00a

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

It was also observed that, there were significant differences (P= 0.05) among the concentrations of gelling agent in influencing the number of nodes per shoot. At the minimum concentration (12% w/v), the number of nodes per shoot was the highest (4.42) while at the maximum concentration (21% w/v) had the lowest (1.91) (Table 3).

The effect of concentration of gelling agents on the height of shoots was significant (P = 0.05). From Table 3 it was noted that, increasing the concentration resulted in decreasing shoot height from 5.48 cm at the concentration of 12% (w/v) to 2.45 cm at the concentration of 21% (w/v) (Table 3). The results in Table 3 indicate that increasing the concentration of the gelling agent from 12% to 21% (w/v) resulted to a significant (P = 0.05) decrease in the fresh weight from 0.47g to 0.2 g respectively.

4.3.3 The effect of interaction between type and concentration of gelling agent on growth of sweet potato shoots *in vitro*

4.3.3.1 Number of leaves per shoot

The interaction between the type of gelling agent and the concentrations with which they were used to solidify TC media affected the number of leaves per shoot differently. The interaction between sweet potato starch and the concentration of 12% (w/v) produced shoots with number of leaves (6.87) which was higher than the control but also highest of all starch based media formulation (Figure 6). The number of leaves per shoot recorded on TC media gelled by cassava starch at the concentrations (w/v) of 12% (6.79) and 15% (6.71) outperformed the control (4.30).

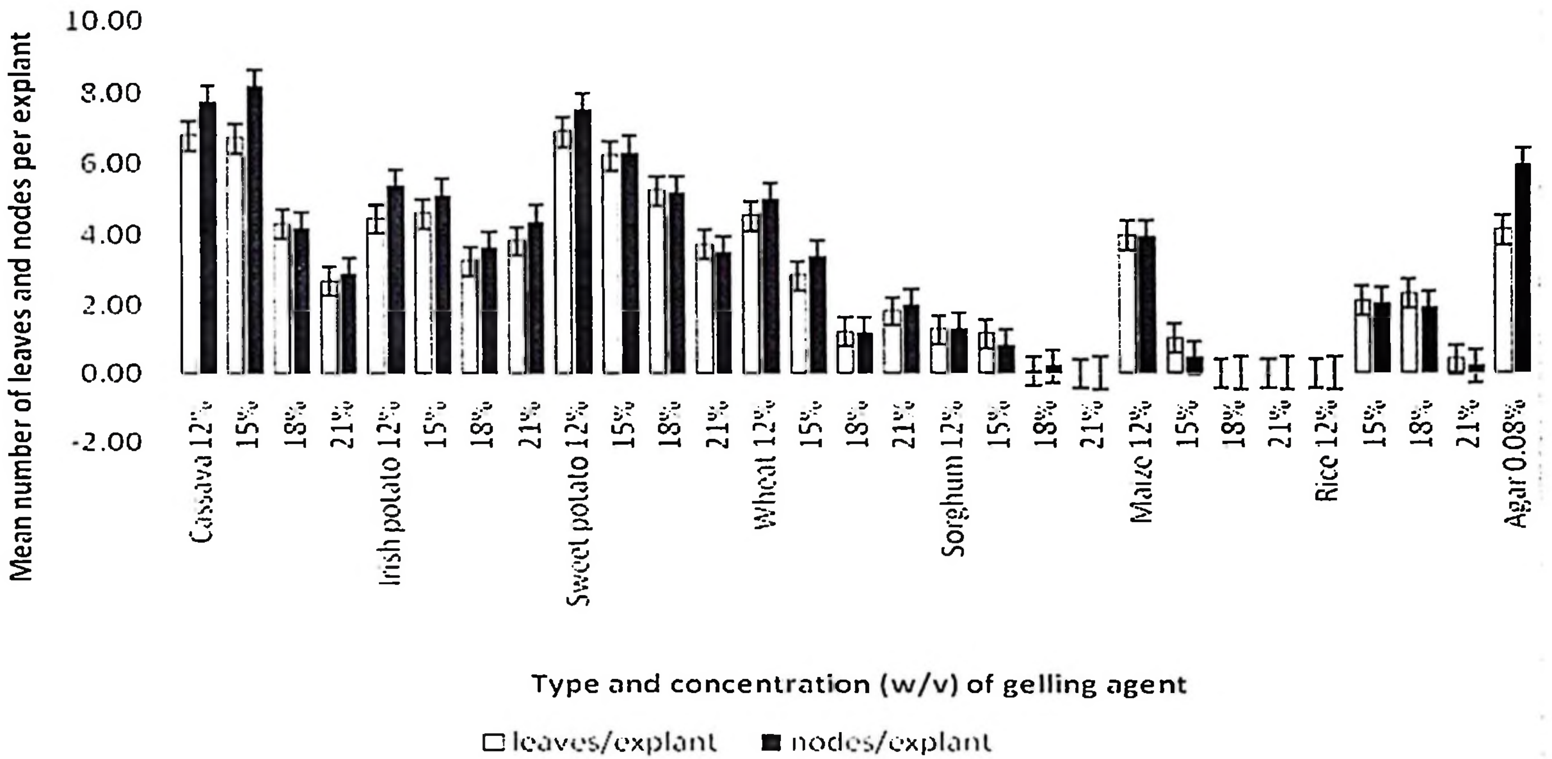


Figure 6: The interaction effect of type and concentration of gelling agent on number of green leaves and nodes of *in vitro* shoots at day 21 of culture initiation

Similarly, the number of leaves per shoot recorded in TC media gelled by 12% (w/v) irish potato starch (4.58) was higher than the performance of the control (4.30). Number of leaves per shoot in rice starch was maximum (2.27) at 18% followed by 2.07 leaves/explants for 15% (w/v) rice starch (Figure 6).

4.3.3.2 Number of nodes per shoot

The TC media gelled by cassava at the concentration of 15% produced shoots whose number of nodes was higher (8.14) than those of the control (5.87) but also was the highest of all starch based media (Figure 6). The number of nodes per shoot recorded in the control media was also lower than 6.27 recorded in TC media which were solidified by sweet potato starch at the concentration of 12% (w/v). The response observed in all TC media which were solidified by starch from cereal extracts ranged from 0 to 4.93 which were lower than the control.

4.3.3.3 Shoot height

The interactions between the type of gelling agents and the concentrations for which they were applied influenced the height of *in vitro* shoots differently. The interaction of irish potato starch gelled media with a concentration of 12% (w/v) produced shoots with height of 9.21 cm, which was significantly higher than the control (8.73 cm) (Figure 7). The response in the control media was also lower than 8.97cm of TC media, which was solidified by sweet potato starch at the concentration of 12% (w/v). In contrast, the responses recorded in all TC media, which were solidified by different concentration of cereal starch extracts were very far below (0 – 5.07 cm) the control (Figure 7).

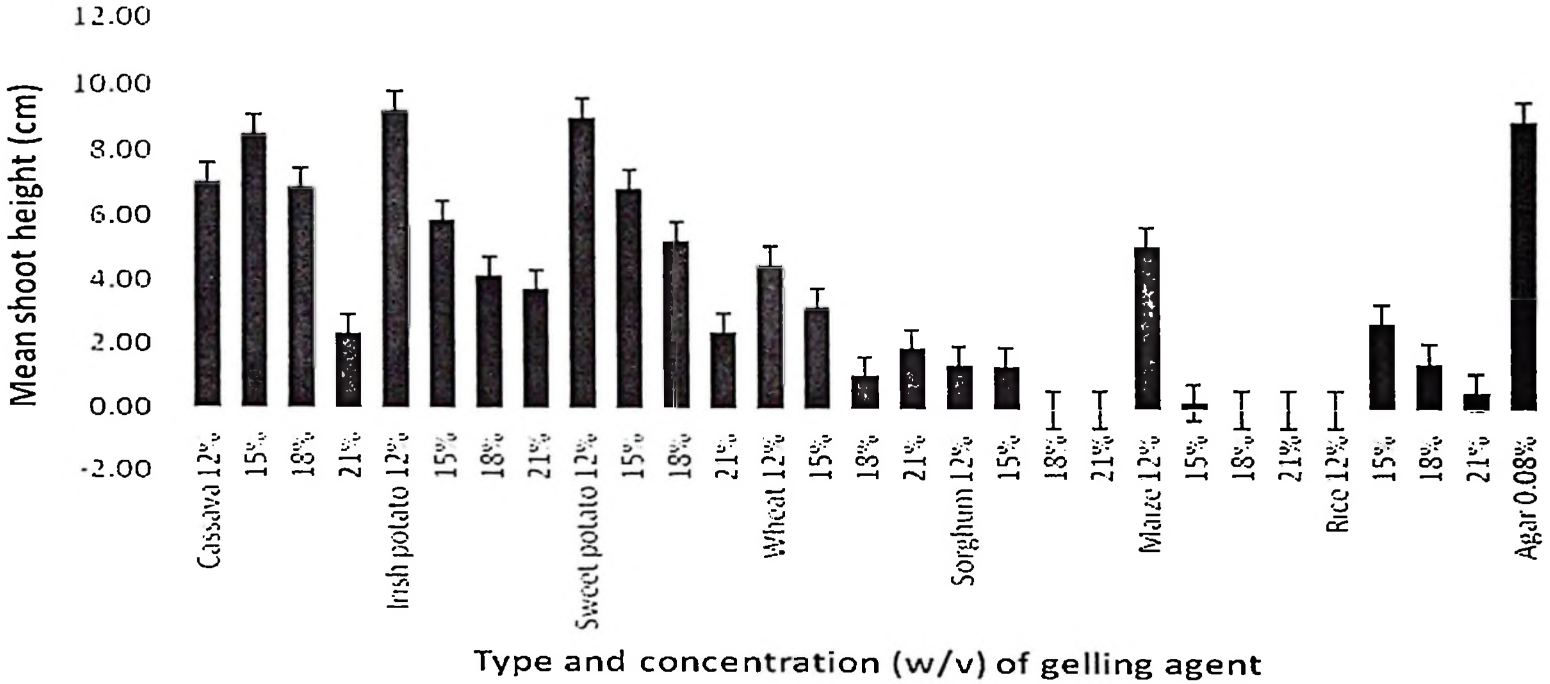


Figure 7: The effect of interaction of type of gelling agent with concentration on mean height (cm) of *in vitro* shoots at day 21 of culture initiation

4.3.3.4 Weight of fresh *in vitro* shoot

The results in Figure 8 show that the interaction between types of gelling agents and the concentration with which they were applied affected the fresh weight of the *in vitro* shoots differently. The fresh weight of shoots grown on TC media solidified by root/tuber starches ranged from 0.16 to 0.8 g which was higher than the range of 0 – 0.35 for shoots produced on cereal starch (Figure 8). Among the root/tuber starches, both irish and sweet potato produced shoots with highest (0.8 g) fresh weight at 12% (w/v) concentrations as compared to the 15% (w/v) cassava starch gelled medium and the control which produced shoots which weighed 0.77 g and 0.76 g respecively (Figure 8). Generally, the biomass accumulation in 12% (w/v) sweet and irish potato and 15% (w/v) cassava starch gelled media were higher than the control.

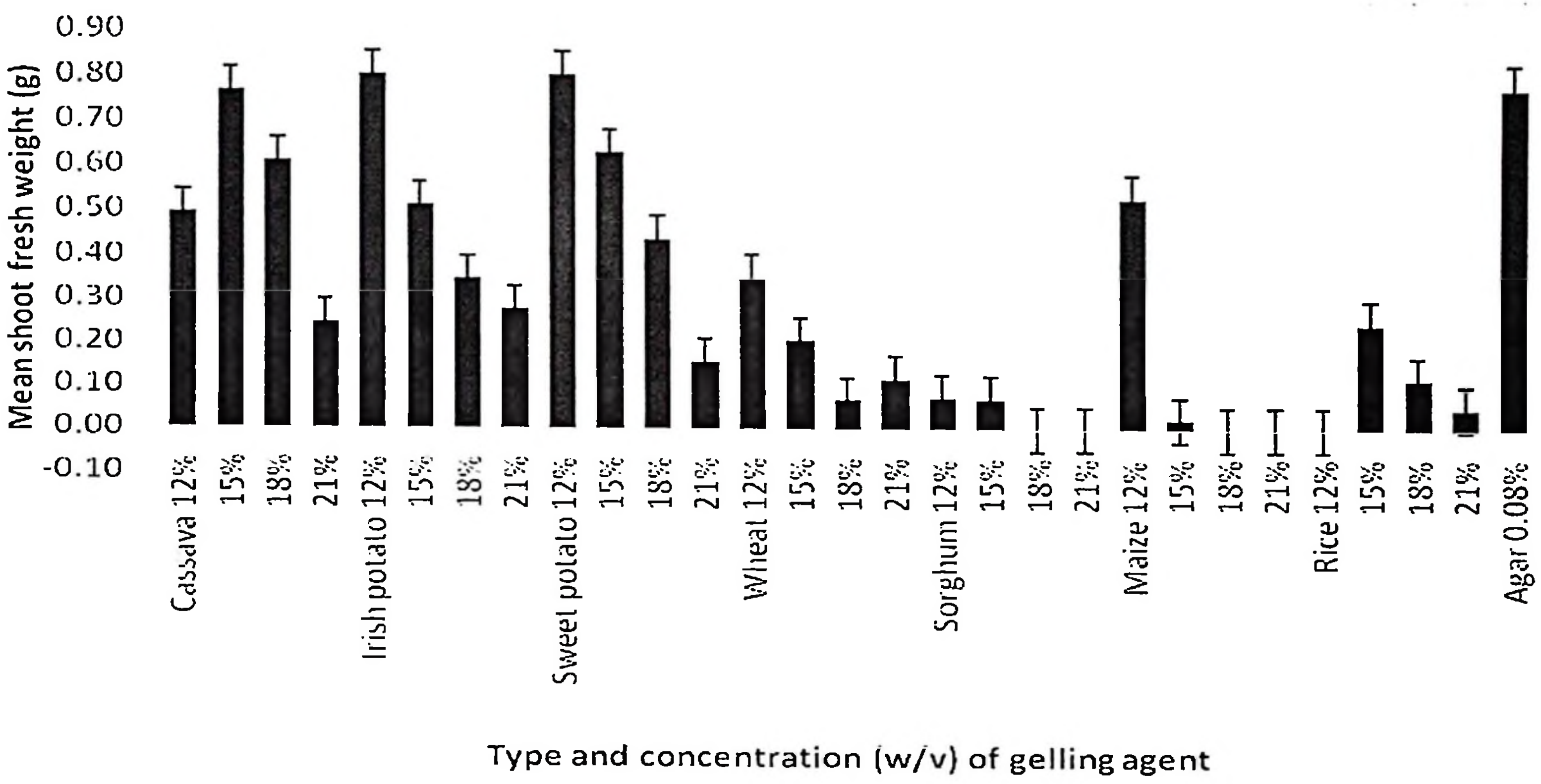


Figure 8: The effect of interaction of type of gelling agent with concentration on mean fresh weight (g) of *in vitro* shoots at day 21 of culture initiation

Based on the preliminary observation from the experiment in section 3.3.5 whose results are presented in sections 4.3, it was established that the performance recorded on TC media which were solidified by cereal starch extracts were too inferior and uneconomical to continue investigating their effects on other micropropagation variables. Therefore, only starch extracts from cassava, sweet potato and irish potato were used for the experiment indicated in sections 3.3.6 and 3.3.7 whose results are presented in sections 4.4 and 4.5.

4.4 The effect of type and concentration of gelling agent on the survival of nodal explants in multiplication media

During the first subculture, the survival of nodal explants on TC media, which were gelled by starches from cassava, sweet potato and irish potato varied from 73% to 93% as opposed to 100% on control medium (Figure 9). On the cassava starch gelled medium, the highest (93%) survival was recorded at 15% (w/v) and the lowest (80%) at 13% (w/v) (Figure 9). The TC media, which were solidified by 11% of both sweet and irish potatoes had the highest survival of 93% and 87% respectively.

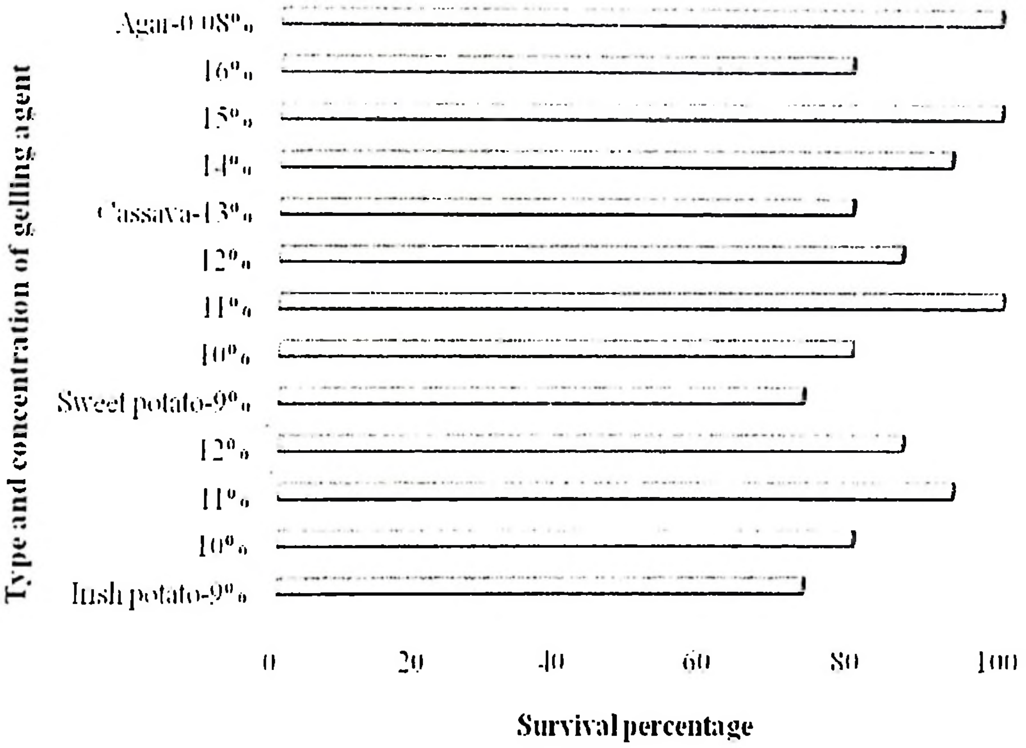


Figure 9: The influence of type of gelling agent on the survival of the nodal explants on first subculture

4.5 Effect of types of gelling agents on trend of in vitro growth of sweet potato

4.5.1 Trend of leaf development

During the three weeks of culture initiation, each shoot grown on TC media that was solidified by 15% cassava and 12% (w/v) sweet potato starches developed an average of one leaf per shoot per week while those grown on TC media solidified by agar and irish potato produced an average of 0.5 of a leaf per week (Figure 10).

It was observed that shoots that grew on 15% cassava starch gelled media developed leaves faster than the control and maintained high growth rate throughout the culture period (Figure 10). On the other hand, the shoots that grew on 12%

(w/v) sweet potato starch gelled medium began to develop leaves at a rate that was lower than those of 15% (w/v) cassava and 12% (w/v) irish potato starch media though a bit higher than that of the control. This growth rate increased uniformly such that before the end of second week it was higher than that on 12% (w/v) irish potato starch media and at the end of the third week the response was greater than that on 15% (w/v) cassava starch media (Figure 10).

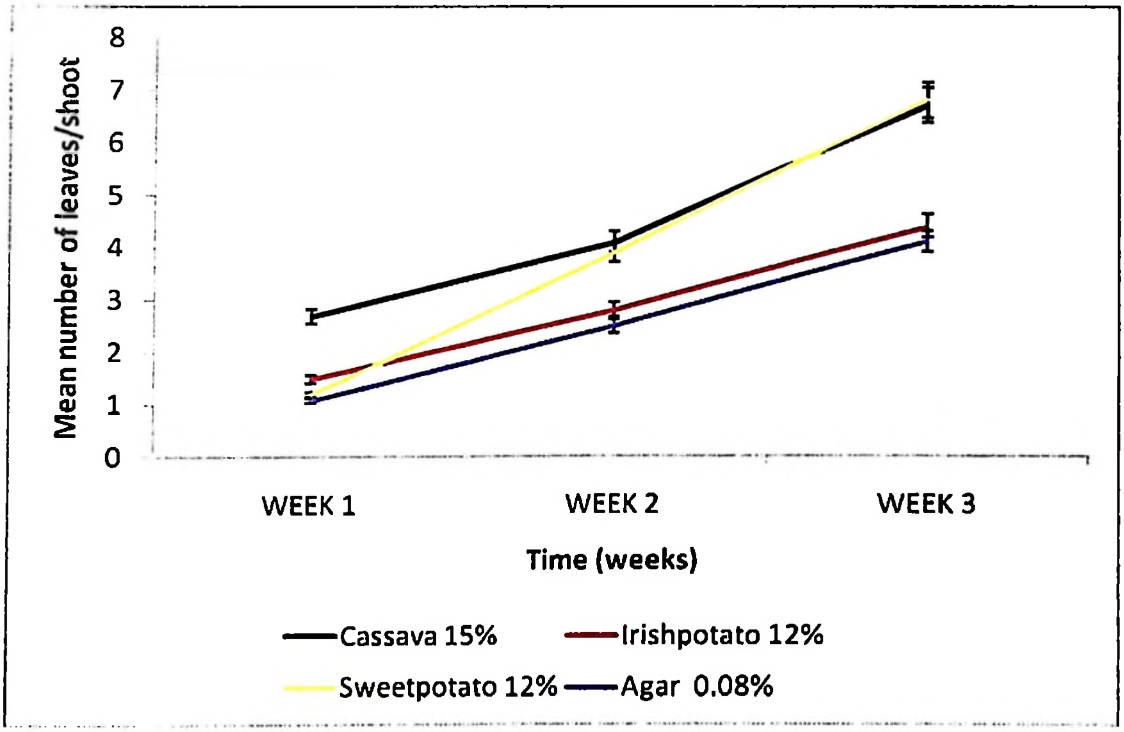


Figure 10: The effect of type of gelling agent on rate of development of in vitro leaves during culture initiation

It was observed from figure 10 that, the *in vitro* shoots which were grown on starch gelled medium developed leaves faster (1 leaf/week) than the same on agar gelled medium.

4.5.2 Trend of node development

In vitro shoots grown on starch gelled media grew faster than the same on agar gelled medium resulting in the increase of nodes at 2 nodes/week than those which grew on agar gelled medium which was 1.5 nodes/week (Figure 11).

The shoots which grew on 12% (w/v) sweet potato starch gelled medium increased the number of nodes steadily at the rate of at least 2 nodes per week (Figure 11). The rates observed in both cassava and sweet potato gelled media were higher than that of agar, which maintained and increment of one node per week.

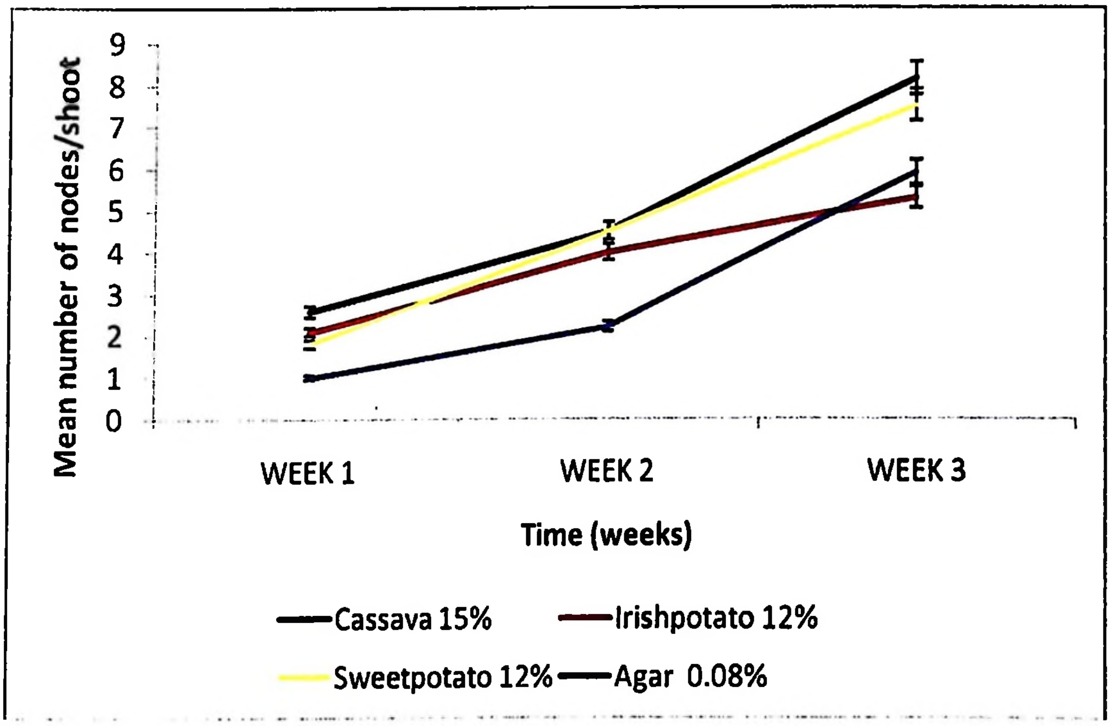


Figure 11: The effect of type of gelling agent on rate of development of in vitro nodes during culture initiation

4.5.3 Trend of increase in shoot height

The results showed that shoots regenerated on 15% (w/v) cassava starch gelled media gained 3.1 cm by the end of the first week of culture initiation as opposed to the control whose shoots grew up to 1cm (Figure 12). The superiority of cassava starch over the control was maintained during the second week, whereby the shoots increased height by 1.6 cm and this rate tripled in the third week to increase height by 3.8cm (Figure 12).

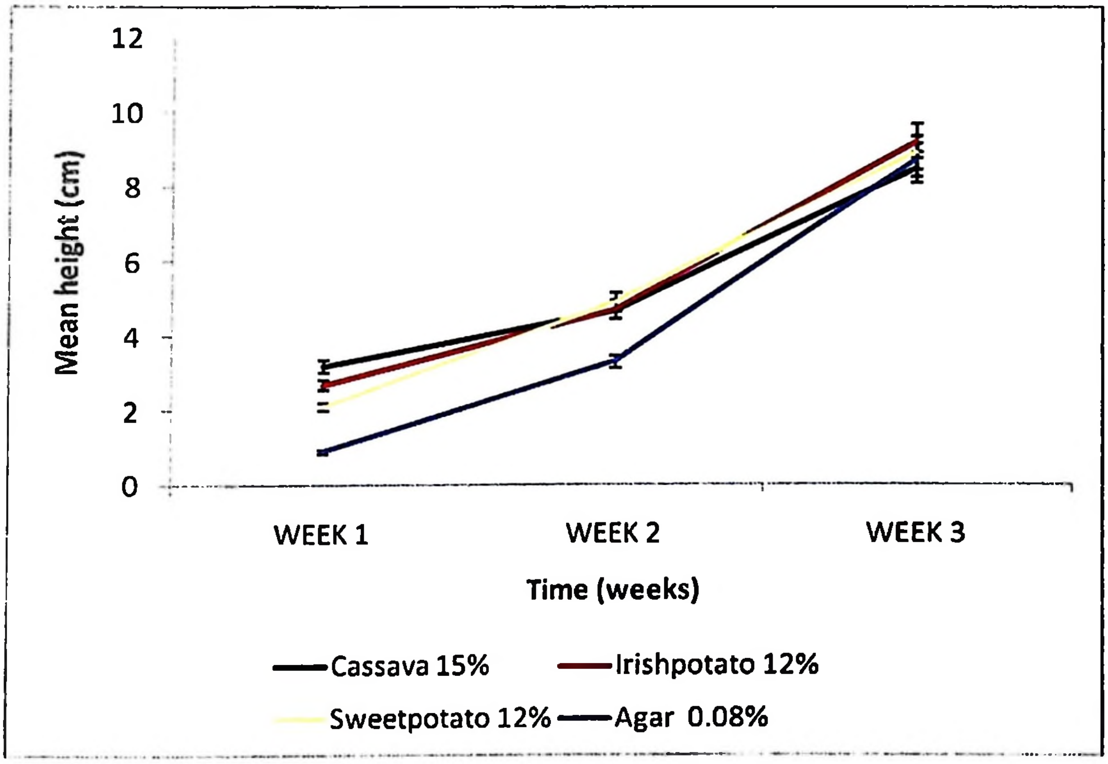
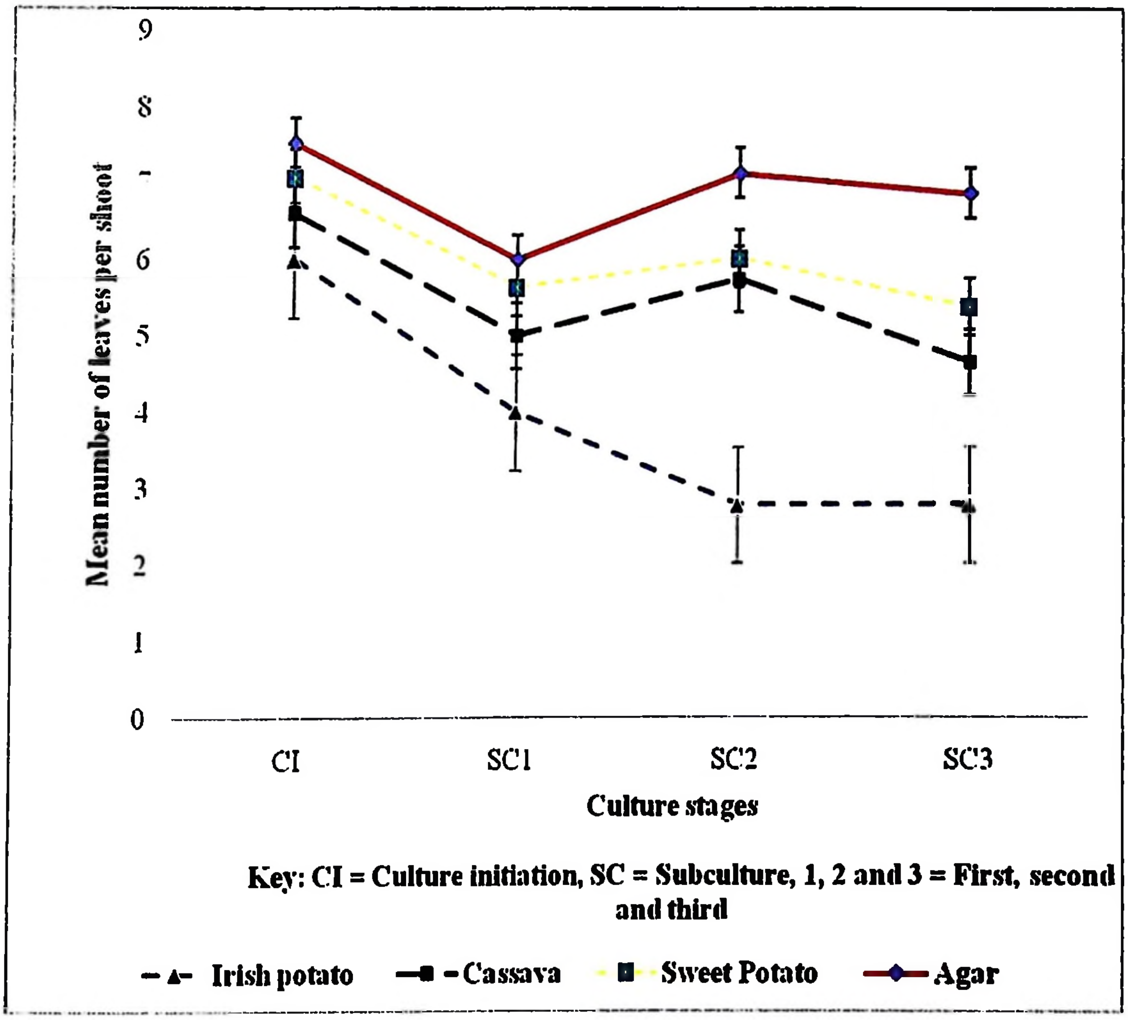


Figure 12: Effect of type of gelling agent on rate at which the height of *in vitro* shoots increase during culture initiation

4.6 Effect of types of gelling agents on components of micropropagation quality at different culture stages

4.6.1 The number of photosynthetic leaves per shoot

Variations were observed among types of gelling agent in the ability to influence the number of photosynthetic leaves of *in vitro* shoots at different stages of culture. At the culture initiation stage, the highest number of photosynthetic leaves (7.5) was recorded on the control medium while the lowest (6.00) was observed on 12% (w/v) irish potato starch gelled medium (Figure 13).



Key: CI = Culture initiation, SC = Subculture, 1, 2 and 3 = First, second and third

Figure 13: Effect of the type of gelling agent on number of photosynthetic leaves per shoot in different stages of *in vitro* culture

The results indicate that, the numbers of leaves per shoot in all treatments declined as the *in vitro* shoots were transferred from culture initiation medium to multiplication medium at the first subculture (Figure 13). However at the second and third subcultures, the performance of the control increased rapidly while that of irish potato starch continued to decline. It was also observed that, during the multiplication phase the mean number of photosynthetic leaves per shoot obtained from agar gelled medium produced was the highest (i.e. 6.00, 7.13, and 6.87 at the first, second and third subculture respectively) (Figure 13). Of the three starches, the number of photosynthetic leaves recorded in 11% sweet potato starch was close to that of agar in all culture stages.

4.6.2 Fresh weight of *in vitro* shoots

Variations were observed on the effect of the treatments on the fresh weight of the *in vitro* shoots. After 21 days of culture initiation, agar gelled media produced the highest fresh weight (1.24 g) compared to all other starch gelled media. Of all the starch gelled media, 12% sweet potato starch gelled media produced the highest (0.82 g) (Figure 14). The irish potato starch gelled media produced the lowest (0.49 g) fresh weight.

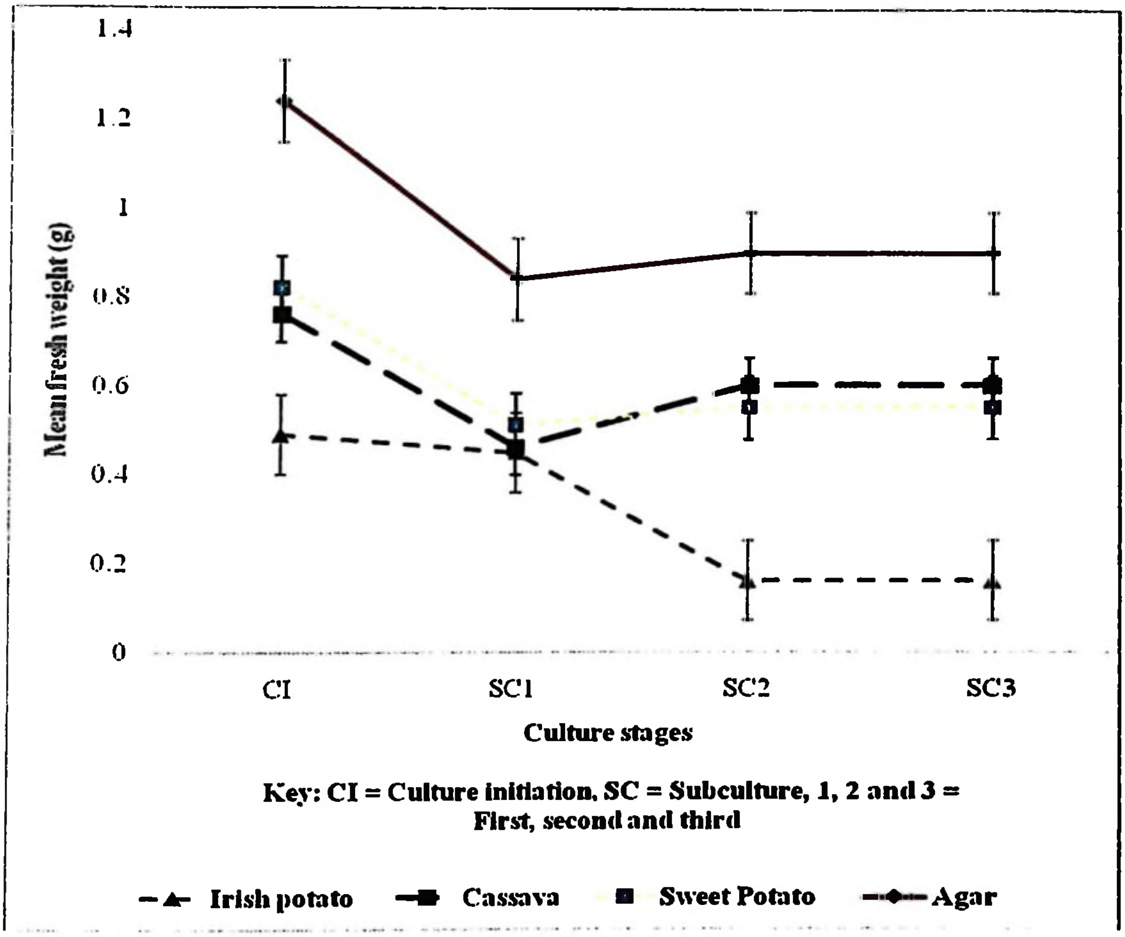


Figure 14: Effect of the type of gelling agent on fresh weight of *in vitro* shoots in different stages of culture

Similarly, in all the three subcultures, agar gelled media promoted the highest fresh weight of 0.84 g for the first and 0.90 g for both second and third subculture while the media gelled by 11% irish potato produced shoots with the lowest mean fresh weight (Figure 14). With exception of irish potato starch, the mean fresh weight of all other treatments were highest at the culture initiation and declined at the first subculture. At the second and third subculture, the mean fresh weight of *in vitro* shoot that was observed in TC media, which were solidified by agar, sweet potato and cassava starch increased while that of irish potato starch declined from 0.45 g at the first subculture to 0.16 at the second and third subculture (Figure 14).

4.6.3 Dry matter content of *in vitro* shoots

During the four culture stages, agar solidified media produced shoots with the highest DMC (10%) except at the second subculture where the cassava starch solidified media slightly outperformed (9.23%) the control (8.99%). The performance of sweet potato starch increased from 8.22% at the culture initiation to 8.71% at the third subculture while the performance of the control decreased from 10% at culture initiation to 8.95% at the third subculture (Figure 15). During the three subcultures, lowest performance varying between 7.66% and 7.54 % was observed in irish potato starch gelled media.

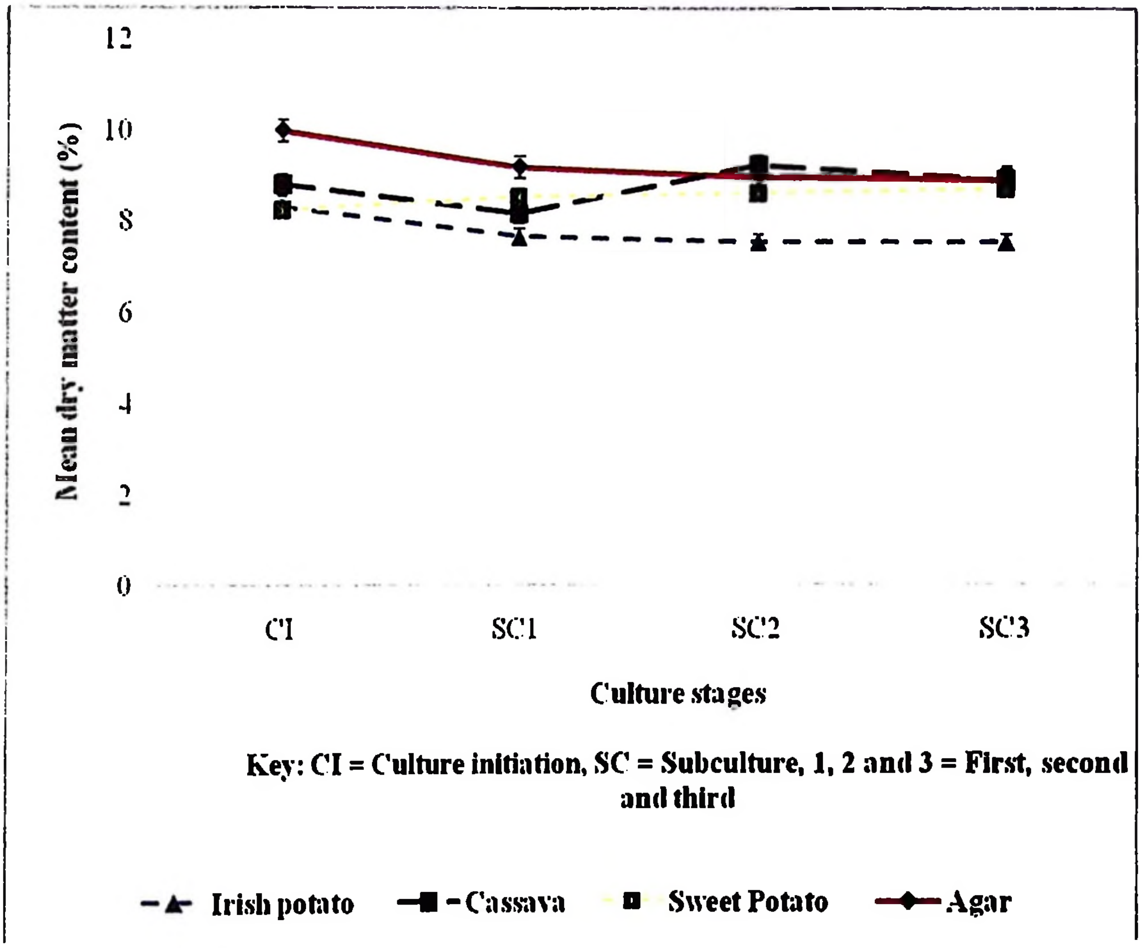


Figure 15: Effect of the type of gelling agents on dry matter content of in vitro shoots in different stages of culture

4.7 Effect of type of gelling agent on components of micropropagation rate at the culture initiation phase

Significant differences were observed among the treatments in their influence on the shoot height during culture initiation (P = 0.05). The TC medium which was solidified by 0.08% agar produced the highest mean height (10.98 cm) while the one which was solidified by 12% irish potato starch produced the lowest (6.55 cm) (Table 4).

Table 4: The effect of type of gelling agent on number of nodes and the nodal propagules per shoot, height and internode length at the 21st day of culture initiation

Gelling agent (w/v)	Plant height (cm)	Nodes/shoot	Internode length (cm)	Propagules per shoot
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

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

Among the starch based TC media, the one solidified by 12% sweet potato starch performed not very far (8.42 cm) from the control. Significantly different ($P = 0.05$) number of nodes per shoot were observed among TC media, which were solidified by different types of gelling agents for both culture initiation and multiplication media. During culture initiation, the highest mean number (8.73) of nodes per shoot was observed on TC media gelled with agar while the irish potato starch gelled media promoted the lowest (5.40) (Table 4). Among the TC media, which were solidified by starch, those solidified by 12% sweet potato and 15% cassava starches gave better responses of 7.73 and 6.13 nodes per shoot respectively than the rest.

No significant differences (P = 0.05) in internode length were observed as a result of different types of gelling agents during culture initiation (Table 4). The mean length of internode of shoots produced from both starch and agar gelled medium varied from 1.08cm to 1.26cm (Table 4). The effect of the type of gelling agent on the number of nodal propagules per shoot at multiplication phases was significantly different (P = 0.05). The mean number of nodal propagules per shoot, which were produced from initiation medium which was solidified by agar was the highest (7.9). Sweet potato starch gelled media outperformed the other starches by producing 7.33 nodal propagules per shoot.

4.8 The effect of type of gelling agent on components of micropropagation rate at the multiplication phase

The results in Table 5 show that, in all the three subcultures, the effect of treatments on shoot height was significantly different between them (P = 0.05). The control medium produced shoots with the highest shoot height in the first (9.3 cm), second (10.29 cm) and third (9.56 cm) subcultures (Table 5).

Table 5: The effect of type of gelling agent on number of nodes and the nodal propagules per shoot, height and internode length at the 21st day of first (SC1), second (SC2) and third (SC3) subcultures

Subculture	Gelling agent (w/v)	Shoot height (cm)	Nodes/shoot	Internode length (cm)	Propagule/shoot
SC1	Round 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
SC2	Round 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
SC3	Round 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 $P \leq 0.05$.

The lowest responses were observed in TC media which were solidified by 11% irish potato starch which were 5.22 cm, 3.02 cm and 3.03 cm in first, second and third subcultures respectively (Table 5). Among the starch gelled media, 11% sweet potato starch had significantly higher responses of 7.26 cm, 7.33 cm and 7.43 cm during the 1st, 2nd and 3rd subculture than 15% cassava starch (Table 5).

The effect of the different types of gelling agent on the number of nodes per shoot was significantly different in all the three subcultures (P = 0.05). The agar gelled media produced shoots with highest numbers of nodes of 6.93, 8.20 and 8.00 during the first, second and third subcultures respectively (Table 5). The TC media gelled by 11% sweet potato starch was better than the other two starches by producing shoots with the mean number of nodes of 6.00, 6.91 and 5.73 at the first, second and third subculture respectively. The lowest mean number of nodes per shoot were observed in TC media gelled by 11% irish potato starch where by the numbers were 4.00 at the first subculture, 2.56 at the second subculture and 2.22 at the third subculture.

No significant differences (P = 0.05) in internode length were observed as a result of different types of gelling agents during multiplication phase (Table 5). The mean length of internode of shoots produced from both starch and agar gelled medium varied from 1.08 cm to 1.26 cm at the stage of culture initiation (Table 5). The length of internodes also varied from 1.3 cm to 1.35 cm at the first subculture, 1.14 cm to 1.3 cm at the second subculture and 1.2 cm to 1.5 cm at the third subculture.

Similarly, the agar gelled multiplication media produced the highest number of propagules per shoot which were 7.6 for the first subculture, 7.47 for the second subculture and 8.20 for the third subculture (Table 5). The response on irish potato starch solidified media was the lowest. This treatment produced only 4.01, 2.25 and 2.33 propagules per shoot during the first, second and third subcultures respectively (Table 5). However, the number of nodal propagules produced per shoot was higher in sweet potato starch gelled media than the rest of the starch treatments. This treatment outperformed all other treatments by producing 6.63 nodal propagules per shoot at the first subculture (Table 5). The same media formulation produced up to 6.90 nodal propagules per shoot at the second subculture and 6.09 at the third subculture. These results had a direct implication on the final micropropagation rate as was observed in the results presented in Table 4.

From the number of nodal propagules per shoot produced in each treatment as presented in Tables 4 and 5, and the corresponding multiplication factors, it was established that the control treatment had the highest micropropagation rate (3 584 propagules/84 days) (Figure 16)

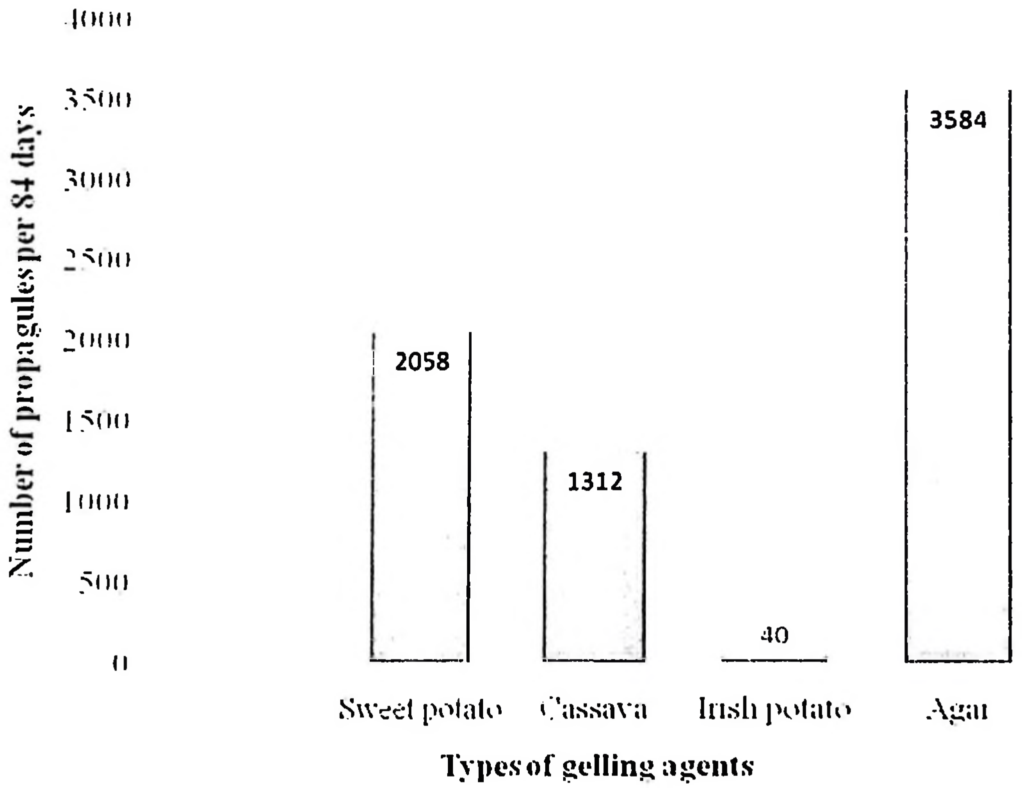


Figure 16: Micropropagation rate for sweet potato, cassava, irish potato and agar gelled TC media.

4.9 The implication of using starch as agar substitute on micropropagation cost

The cost of making a litre of the control medium (agar gelled) was the highest (Tsh 1357/=), 66% of which was the cost of agar as gelling agent (Table 6). Of the three starchy gelling agents, irish potato starch was the most expensive (Tsh 1135/=) while sweet potato starch was the cheapest (Tsh 763/=) (Table 6). From these results, the highest cost reduction of 44% was achieved in making one liter of TC media by using sweet potato starch at 11% (w/v) concentration while lowest cost reduction of 14% was achieved by using irish potato starch at a concentration of 12% (w/v).

Table 6: The effect of type of gelling agent on media cost

Gelling agent (GA)	Concentration (%w/v)	GA cost/l (TSh)	Media cost / l (TSh)	Cost reduction (%)
Cassava	15	420	883	35
Sweet potato	12	300	763	43
	11	275	778	44
Irish potato	12	672	1165	14
	11	616	1135	16
Agar	0.08	894	1357	

The reduction in total cost of media due to cheaper gelling agents was implied on the cost of the production of propagules in 84 days through three subcultures. The production cost per propagule due to gelling agent on 11% sweet potato starch gelled medium was reduced by 67% while the use of 11% irish potato starch increased the cost per propagule by 17% (Table 7).

Table 7: The effect of type of gelling agent on cost of *in vitro* propagules

Gelling agent	Concentration (% w/v)	Propagule cost (Tsh)	Propagule cost reduction (%)
Cassava	15	10	44
Sweet potato	12	7	61
	11	6	67
Irish potato	12	21	-17
	11	19	-5
Agar	0.08	18	

CHAPTER FIVE

5.0 DISCUSSION

5.1 Physicochemical properties of the starch extracts

5.1.1 Starch purity

The results indicate that, starch extracts from cassava and sweet potato contained the highest amount of pure starch. Such high amount of starch in cassava and sweet potato extracts can be attributed to the characteristic behavior of root crops of accumulating large amount of starch in roots and small amounts of other components as was also observed by Onuwene (1982). These results were comparable to those reported by Chen *et al.* (2006a) for sweet potato and Niba *et al.* (2001) for cassava.

Although the amount of pure starch in extracts from cereals was lower than those in root crops, these results are within ranges reported for the same cereals but different genotypes by Kouakou *et al.* (2008) and ISI (2007). These observations in cereal extracts are attributable to the genetic tendency of many cereals to accumulate larger amounts of non starch biopolymers (Protein, fats, organic acids etc). Unlike root crops, the non starch biopolymers in cereals are tightly entangled in grain endosperms as observed by Kouakou *et al.* (2008) in maize, rice, millet and wheat. Large amount of protein and fat of cereal grains is accumulated in the germ parts as food for the growing embryo during seed germination. For the same reason, this study showed that protein and fat contents of starch extracts from cereal crops were higher than those of root crop.

According to Chen *et al.* (2006b), the molecules of non starch biopolymers like fat seem to be tightly bonded to the other biopolymers (e.g. starch) contained in the endosperm. As a result, the extraction process to isolate pure starch from cassava and sweet potato was simpler and more efficient than it was with cereal crops used in this study. Hence, these results suggest that it could be more economically efficient to extract starch from cassava and sweet potato than from cereals. Since for the purpose of this study, the compound of interest is the pure starch, an extract with higher pure starch content may have greater potential to the intended function as gelling agent than those with low.

The low amount of pure starch in extract from irish potato coupled with high browning of the starch paste during air drying suggest that this extract might have higher levels of polyphenolic compounds. This implies that removal of non-starch biopolymers in potato (*S. tuberosum* L) was less complete as compared to cassava and sweet potato starches. Therefore adding antioxidants in the extraction mixture for irish potato may be important although will increase the cost of production.

5.1.2 Amylose content

The observed differences in amylose contents between starch extracts may be due to genetic differences between plant species from which they were extracted. This is because according to Mohammadkhan (2005) and Rai (2005), the variation between and within species (among cultivars) in terms of amylose content is genetically controlled and is a trait with high heritability.

This study showed that the amylose contents of starch extracts from cereals was higher than those of root and tuber crops and these results are similar to those reported by Satin (2005), Spigno and De Faveri (2004) and Herero-Martinez *et al.* (2004) for the same species but different cultivars. Due to the direct influence of amylose content on gelation properties of starch and that it has a negative correlation with gel firmness (Sasaki, 2005), it can be predicted that, starch extracts with low amylose content may form semisolid TC media at a lower concentration than those with high amylose content. In this case therefore high amylose starch extracts like those from rice and sorghum will require large amount of starch powder to form a standard semisolid TC media.

5.1.3 Swelling power

The observed differences in swelling power among starch extracts may be attributed to amylose content, granular structure and amounts of non starch biopolymers of each starch extract. The low swelling power observed on some of the cereal starches may be a result of the extensive and strongly bonded micellular structure which makes them relatively resistant to swelling as observed by Sandhu and Singh (2006). This study indicated high swelling power in starch extracts with low amylose content such as cassava, sweet potato and wheat (Figure1). This is expected because, as the starch granules absorb water the amylose molecules reinforces the internal network within the granules thus restricting swelling of starch with high amylose content compared to those with small amounts of amylose (Riley *et al.*, 2006).

Furthermore, the low swelling power of starch extracted from irish potato, rice, maize and sorghum can be associated with the low starch purity as was observed in Table1. This is because according to Riley *et al.* (2006) starch extracts with large amounts of non starch biopolymers like lipids seem to inhibit swelling of starch granules. This observation is strongly supported by the report by Tester and Karkalas (1996) who established that swelling power of many starches are negatively correlated to amylose and lipid contents.

Also lower value of swelling power of starch extracts from maize, rice and irish potato might be due to the high protein content as observed in Table 1. The protein molecules have a tendency to interact with amylose molecules due to opposite charges hence they form protein-amylose complex which according to Shimelis *et al.* (2006) restricts swelling.

Swelling power of starches is of great significance in TC media formulations as it enhances movement and availability of water, growth regulators and nutrient solutes to the *in vitro* plants (Mohammadkhan, 2005). The results therefore suggest that the TC media gelled by starches with higher swelling power would be expected to release the nutritional ingredient from its compacts at a faster rate than those with low swelling power. Therefore, these results further imply that the TC media gelled with wheat, cassava and sweet potato starches would support *in vitro* plant growth better than those solidified by rice, maize, sorghum and irish potato.

Increasing swelling power with temperature could be due to the fact that, as the insoluble starch granules starts to swell due to hydration they begin to integrate with water molecules. This integration, hence the rate of swelling, is probably enhanced by increment in incubation temperatures. However within the same change in incubation temperature, wheat, sweet potato and cassava showed higher rate of increasing swelling power than the rest of starch extracts. For cassava and sweet potato starch extracts which had low amylose content, this is probably due to the longer chains in amylopectin structure which is water loving (Riley *et al.*, 2006)

These findings agree with those reported by Chen (2003) for sweet potato starch, Riley *et al.* (2006) for Yam (*Diascorea alata* L) starch, Shimelis *et al.* (2006) for common bean (*Phaseolus vulgaris* L) starch and Yuan *et al.* (2007) for cassava and irish potato starches. However the decrease in swelling power of maize starch disagrees with the observation reported by Fasasi *et al.* (2007) who reported that the swelling power of both starch and flour from maize increased with increasing temperature. These results imply that, since high swelling power is preferred for a suitable starch gelled TC media, increase in temperature during media preparation as well as in incubation rooms would have no detrimental effects on the ability of the media to nourish the explants except TC media solidified by maize starch.

5.1.4 Acidity of starch extracts

Compared to cassava and sweet potato starches, the low pH of cereal starches might be due to the presence of high amounts of non starch impurities which are acidic in nature like amino and fatty acids from protein and fat biopolymers as was noted in

Table 1. The normal pH of TC media for many plant species including sweet potato is usually around 5.7. Therefore the TC media which is solidified by gelling agents like starch from maize, rice, wheat and sorghum with lower pH than the normal values would need a lot of base/alkali to adjust the pH to normal.

Similarly, gelling agents like cassava, sweet potato and irish potato which have pH that is higher than the normal values would need a lot of acid to neutralize it to normal. Excessive application of acid or base to bring the pH of TC media to normal has implication on the production cost. Therefore, gelling agents with extremely low or high pH values may be expensive in terms of acid-base requirements.

5.2 Properties of starch gels for making solid TC media

5.2.1 Gelation concentration and gel firmness of TC medium

The observed differences between treatments in terms of gelation concentration and gel firmness are probably due to differences in the physicochemical properties of the respective starch extracts as discussed in section 5.1. Due to these differences, rice starch required the highest (24%) concentration to achieve semisolid level of gel firmness, while sweet potato, irish potato and maize required the lowest (12%). Therefore, the amount of starch extracts from sweet potato, irish potato and maize which was required to prepare a s emisolid TC media whose gel firmness and stability were similar to the control was lower than the rest of the starch extracts. The stable TC medium of the control treatment was characterized of being semisolid, which adheres well to the bottom of the container (e.g. culture bottle) in a manner that it does not become sloppy and flow when the container is tilted (Napan, 2008). Hence, the use of starch extracted from rice, cassava, wheat and sorghum as alternative gelling agents are likely to be expensive because the amounts that would be required to prepare a given volume of TC media would be higher than the amount of sweet potato, irish potato and maize starch required to prepare the same volume of TC media.

5.2.2 Colour and clarity of TC media

The results indicate that, while the control media was white and semi transparent, all starch gelled media were opaque and had colours varying from light medium gray to dark gray. Since starch granules are usually white, occurrence of different colours of the starch gelled TC media may be a result of presence of non starch impurities such as polyphenols, ascorbic acid and carotene. According to Shimelis *et al.* (2006) these impurities may have negative effects on the starch quality and the final functionality of the product.

It was also established that, the colours of the starch gelled media changed with heat treatment from cooking temperature of about 100°C to autoclaving temperature of 121°C. The observed changes in colour during preparation of TC media can be explained as due to irreversible structural change of the molecular order of the starch granules that occurs after a gelatinization temperature is reached during heat treatment as described by Sasaki (2005). Similar observations are reported by Spigno and Favery (2003) who also pointed out that it could be due to structural change which results to changes of shape and size of granules, absorption of water and swelling, crystallite melting, and leaching of amylose from the granules.

Lack of transparency or clarity of starch gels is attributable to presence of non starch components in the extract such as protein, lipids and phosphorous which according to Shimelis *et al.* (2006) usually influences the transparency and clarity of starch gels. Not only that but also the observed lack of transparency in starch gels may be associated with the irreversible destruction of the structural molecular order of the starch granules which changes their physical appearance as reported by Spigno and Faveri (2003).

Both gel colour and clarity of TC media were important properties in management of *in vitro* culture especially the identification of contaminated cultures as well as the type of contaminant. This is because it was observed that, some bacterial and fungal contaminants do develop colonies with colours similar to the colour of the starch based TC media. In such a situation it becomes difficult to note the growth and identify the type of contaminant as early as possible. The colours and lack of clarity of the starch gelled media make the use of starch as alternative gelling agent, unattractive option especially in research laboratories where close and frequent observations is required.

5.2.3 Handling of starch gelled media

Due to irreversible formation of semisolid starch gels, once prepared, the starch gelled TC media had to be dispensed into culture vessels while in their semisolid state before autoclaving to avoid likely contamination due to handling. Hence it was noted that dispensing semisolid TC media was more tedious and time consuming than it was for the hot liquid agar gelled media. it was also noted that, all starch gelled TC media were forming either, a semi opaque or opaque thin film sticking on the walls of the container as the starch gelled media cooled after autoclaving. This observation suggests that, the amount of light entering into the culture vessel would be low and might have detrimental effect on the growth of the plants *in vitro*.

5.3 The potential of starch from selected crops as gelling agents to support in vitro growth of sweet potato (*I. batatas* L,)

5.3.1 The effect of source of starch on *in vitro* growth characteristics under culture initiation

The growth of shoots *in vitro* on agar gelled media outperformed those of starch gelled media except for the number of leaves per shoot for which cassava starch media recorded significantly the highest response (P = 0.05). Of all the starch gelled media, the best growth response was observed with cassava. In comparison with other starch based media, high numbers of nodes and leaves per shoot, shoot height and fresh weight on cassava starch media may be due to its characteristic low gel firmness (viscosity) which probably enhanced the availability of water and nutrients due to low resistance to diffusion and closer contact between the explant and the medium.

Alternatively Kuria *et al.* (2008), proposed that the better response on cassava starch gelled media could be due to the absence of inhibitors which have been reported to be present in agar. These results are in agreement with those reported by

Maliro and Lameck (2004) for cassava flour, Puchooa *et al.* (1999) for corn starch and Ibrahim *et al.* (2005) for irish potato starch

Furthermore, it can be speculated that, the good growth responses on cassava and sweet potato starches based media may be due to their tendencies to act as an additional source of carbon which was also reported by Onuweme (1982). Also, they could be source of beneficial nutrients present in starch extracts which act as ionic supplements. This is because these crops are reported to have about 35% carbohydrates and 1% mineral matter some of which may be taken into the medium (Zimmerman *et al.*, 1995). Consequently, they may have resulted into improved cell growth and morphogenesis. Good growth of plantlets on cassava and sweet potato starch gelled media as compared to agar is an indication of the potential of cassava and sweet potato starches as agar substitute in plant tissue culture. On the other hand, these results show that the root crop starches have higher potential to support *in vitro* growth of sweet potato than starch extracts from cereal crops (Wheat, sorghum, rice and maize).

5.3.2 The effect of concentration of gelling agent on growth of *in vitro* shoots

The results indicate that increasing the concentration of a gelling agent resulted into retardation of *in vitro* growth of shoots. This relationship is due to the fact that the concentration of gelling agents beyond certain optimal level has a close relation with water stress. This observation was also reported by Scholten and Pierik (1998) that the concentration of gelling agent which is beyond established optimum level causes high water stress due to difficult uptake of water and nutritional elements from culture medium. The vigorous growth of the shoots at 12% concentration was an indication that, availability, absorption and assimilation of nutrient by the shoot were optimally favored.

The relationship between the concentration of a gelling agent and the absorption of water and mineral nutrients by explants is based on the fact that increasing the concentration increases the gel firmness of the TC media as was observed in Figure 5. The increase in gel firmness (viscosity) from liquid to semi liquid then to semisolid or solid may have a corresponding decrease in water potential (more negative) resulting from the matrix potential of a gelling agent that limits mobility of solution in the TC media through diffusion. As a result the rate of absorption of water and mineral nutrient from TC medium into tissues of the explant is reduced. The lower uptake of nutrients could explain the lower growth and development of *in vitro* shoots as the concentration was being increased. Similar observations were documented by Kuria *et al.* (2008) and Napan (2008) who worked on cassava starch as agar substitute for TC media.

5.3.3 The effect of interaction between type and concentration of gelling agent on growth of sweet potato shoots *in vitro*

Although results show that, growth was negatively affected by increasing the concentration of gelling agents, it has been established that, the number of nodes and leaves, shoot height and fresh weight were higher at 15% concentration than at 12% (w/v) (Figure 6, 7 and 8). This can be explained as probably due to synergistic effect resulting from the interaction of the gelling agent with the concentration for which they were applied into the TC media. Different gelling agents require different optimal concentration with which they are applied into TC medium to provide the necessary physical and nutritional support to shoots growing *in vitro*. This explains why shoot growth in TC media which were solidified by sweet potato, irish potato, and wheat and maize starches was highest at 12% concentration while rice starch gave best performance at a concentration of 18% (w/v).

The low performance on TC medium solidified by cassava starch at 12% (w/v) may be explained to be due to failure of the medium to provide adequate anchorage which instead of keeping the explant upright, it was submerged. The explants which were submerged into semi opaque medium had no sufficient exposure to light which may have caused poor photoperiodic responses. The low performance on TC medium solidified by cassava starch at 12% (w/v) and rice starch at 12% and 15% may be explained as due to failure of the medium to support the explants upright, instead they were submerged giving the explants insufficient exposure to light.

While the gelling agent facilitates good contact and anchorage of the explants on the TC medium, the concentration is responsible for the optimal availability and uptake of water, growth regulators and nutrients from the culture medium into the explant's meristematic tissues for growth (Selby *et al.*, 1989). However the synergistic effect was significantly higher in cassava and sweet potato starch based media than in irish potato and cereal starches. This could probably be caused by the presence of a variety of non starch impurities as observed in Table 1. These impurities may have inhibited the growth of *in vitro* shoots.

Therefore the good responses in terms of the number of leaves and nodes on the 12% (w/v) observed on both sweet potato and irish potato starches and 15% (w/v) cassava starch gelled media suggest the formulation which make good TC media for production of nutritionally healthy shootlets. It is important to note that production of large number of photosynthetic leaves is an important parameter for growth *in vitro* as well as for the quality of micropropagation. Good quality plantlets from micropropagation should have sufficient number of photosynthetic leaves to ensure that the plants have high photosynthetic ability in *ex vitro* environment for high survival and growth. The shoot height and fresh weight in 15% (w/v) cassava, 12% (w/v) sweet potato and irish potato starch based TC media formulations, suggests that, the nutrient availability, absorption and assimilation was almost equally favorable as in the control (Figure 7 and 8).

5.4 The effect of type and concentration of gelling agent on the survival of nodal explants in multiplication media

Comparative studies of sweet potato micro-propagation in cassava, sweet potato and irish potato starch gelled media revealed that, the regeneration capacity of nodal sections varied between types of gelling agents and concentrations within those types as indicated by survival percentage in Figure 9. The low survival on 13% cassava starch gelled medium could be due to very week semisolid condition of the medium which kept the explant totally submerged into the opaque medium. Similar reasons may account for the low survival in TC media gelled by less than 11% (w/v) sweet and irish potato starch concentration.

In contrast, the low survival in TC media which was solidified by more than 11% (w/v) sweet and irish potato starch concentration, could be due to very hard media. Such a condition inhibits movement of nutritional ingredients contained in the media matrix towards the explants. These results therefore suggest that, for the purpose of subcultures the multiplication media which was solidified by cassava starch, the optimal concentration was 15% (w/v). On the other hand, the multiplication media which was solidified by both sweet and irish potato starches, the optimal concentration was 11% (w/v)

5.6 The growth rate of in vitro shoots as effected by types of gelling agents

It was noted from figure 10, 11 and 12 that, the rate (number or cm/week) at which the leaves, nodes and height increased was higher in starch gelled media than in the control (agar). Cassava starch based medium had the highest shoot multiplication rate probably because apart from being a gelling agent it can act as additional source of carbon and some mineral matter as reported by Onuwene (1982). Also the results suggest that the quality, hence the ability of TC media which were solidified by cassava and sweet potato to supply nutritional ingredients to the growing plant did not adversely deteriorate during that time of culture. This finding is contrary to the deterioration over storage time of cassava starch based media in the incubation room that has been reported by Kuria *et al.* (2008).

According to Napan (2008) the number of nodes per shoot and the rate at which they were formed was an important component of rapid multiplication for sweet potato that is based on nodal cuttings. This is the case for many other plants whose multiplication of propagules *in vitro* is based on their vines like vanilla (George and Ravishankry, 1997). Hence the cassava and sweet potato starch gelled media which enhance plant growth to form large number of nodes within short time is preferred for rapid multiplication of propagules *in vitro*. Therefore according to Figure 11, the potential of 15% (w/v) cassava starch and 12% (w/v) sweet potato starch gelled media for the rapid multiplication of sweet potato is higher than that of the control (0.08 agar).

It can therefore be envisaged that, the potential of starch gelled media to produce *in vitro* plantlets with good survival during acclimatisation is high. These observations suggest that the quality and ability of TC media which were solidified by cassava and sweet potato to supply nutritional ingredients to the growing plant did not adversely deteriorate during that time of culture. This finding is contrary to the deterioration over storage time of cassava starch based media in the incubation room that has been reported by Kuria *et al.* (2008).

As noted earlier the number and quality (presence chlorophyll) of leaves developed by *in vitro* shoots is an important quality parameter to predict the *ex vitro* survival of the plantlet during acclimatisation. According to Dewir *et al.* (2005) the higher the number of photosynthetic leaves the greater the chance of *ex vitro* survival of plantlets during acclimatisation.

Since under normal growth conditions, increment in shoot height is positively correlated with the number of nodes (Geetha and Shetty, 2000) which are the basic component of plant micropropagation through nodal cuttings, the starch based media which promote vigorous growth is a suitable alternative to agar. Therefore according to Figure 11, the potential of 15% (w/v) cassava starch and 12% (w/v) sweet potato starch gelled media for the rapid multiplication of sweet potato are preferred as they were better than that of the control (0.08 agar).

5.7 The einfluence of types of gelling agents on quality of micropropagation at different culture stages

The results indicate that number of leaves (Figures 13), fresh weight (Figure 14) and the dry matter content (Figure 15) at all culture stages were highest in the control medium and lowest in round potato starch gelled media. Since these are important quality parameters of micropropagation, the quality of produced shoots at different stages of cultures was highest in the control and lowest in the irish potato starch gelled media. This may be due to the higher diffusion rate of water and nutrients in the agar based media than the starch based media as observed by Selby *et al.* (1989). Therefore, the movement of water and mineral nutrients towards the explants could be higher in agar based media than it is in the alternative gelling agents. Not only that, but also unlike the starch based media, there is high transparency of agar based media which may have facilitated optimal photosynthetic and photoperiodic responses of the *in vitro* shoots.

The results also show that number of leaves per shoot, shoot fresh weight and DMC in all treatments declined as the number of culture cycles increased from culture initiation through the three subcultures. This means that the quality of propagules produced deteriorated as the number of culture cycles increased. Such deterioration is probably caused by the diminishing regeneration potential of the *in vitro* derived explants due to repeated subculturing. A similar observation was reported by Selby (1989) on needle cultures of Sitka spruce (*Picea sitchensis* (Bong.) Carr.).

The results imply that if starch is used as agar substitute, propagules with relatively high photosynthetic ability can be obtained in 12% and 11% (w/v) sweet potato starch gelled media for culture initiation and multiplication respectively. Also the nodal explants subcultured on agar gelled media had greater access to nutritional resources for accumulation of biomass than it was for those cultured in starch gelled media. However, among the starch gelled media, the nodal explants subcultured on 11% sweet potato starch gelled media were more nourished than the rest of starch gelled media. These differences could be due to their differences in gel firmness and hence the availability of water and mineral nutrients for absorption and assimilation by the nodal explants.

The amount of biomass which the plantlets accumulate during growth *in vitro* is an important quality parameter for survival in continuing multiplication subcultures and finally in the *ex vitro* environment. These results suggest that, although the response is inferior to that of agar, sweet potato and cassava starches as gelling agents for micropropagation of sweet potato propagules have high *ex vitro* survival.

The results of DMC indicated how efficient the nodal explants were able to absorb and metabolically assimilate the organic and inorganic nutrients contained in the media to promote cell division and morphogenesis. According to Te Chato *et al.* (2005) the amount of dry matter accumulated by a shoot at one stage of culture determined the survival and growth of that plant on the next stage of culture. Based on these results the TC media gelled by sweet potato, cassava and irish potato starches have high potential to produce shoots with good survival in both *in vitro* and *ex vitro* conditions.

5.8 The influence of types of gelling agents on rate of micropropagation at different culture stages

Comparison between treatments on their influence on components of micropropagation rate (number of propagules produced per unit of time) show that, there was persistent superiority of the control (agar) over the three starch based treatments in both culture initiation and subculture cycles of the multiplication phases. These components include shoot height, number of nodes per shoot, internode length (cm) and propagules/shoot (Tables 4 and 5). 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 through out the culture period.

Furthermore, agar solidifies the TC media in a manner that, there is no restriction of movement of water nutrients and growth regulators within the matrix and from the matrix to the explants as observed by Scholten and Pierik (1998). The fact that naturally agar produces transparent gels of TC media may be another reason contributing to its superiority. This is because it facilitates good exposure of the growing shoot to light for maximum photosynthetic and photoperiodic functions as observed in Plates 2 and 3.

During culture initiation and multiplication subcultures, the poorest responses in terms of shoot height, number of nodes per shoot, internode length and number of propagules per shoot were observed in TC media which was solidified by irish potato starch extract. This may be due to the observation that, the gel firmness of TC media which was solidified by irish potato starch was higher than that of agar based media. This might have caused restricted movement of water and mineral solutes within the media matrix and across cell walls of the explants cells.

Apart from the restricted movement of the nutritional materials, the opaque thin films forming along the walls of culture vessels observed in Plate 3 might have reduced the exposure of the growing shoot to light. Also it was established in Table 1 that, irish potato starch extract had the lowest (29.32%) amount of pure starch (purity). Therefore, other compounds included in the extract may have played an inhibitory role to somatic cells division (mitosis) of the explants and the general growth and development of the shoot *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 under optimal growth conditions the height of a shoot is positively correlated to its number of nodes which determines the number of nodal propagules produced for further subculture and rooting (Geetha and Shetty, 2000). Moreover the height of shoot determines the size of nodal propagule and the *in vitro* plantlet for adaptation in *ex vitro* environment as reported by Smith *et al.* (1986).

Since the nodal propagules which were used in subcultures needed to be at least 1cm long, the length of internode was important in determining the number of nodes for each nodal propagule. As long as the internode length was not shorter than 1cm each nodal propagule had a single node. Therefore from these results, internode length shorter than 1cm would lead to production of propagules with multiple nodes to produce the required 1cm size of propagule for a better survival *in vitro*. Hence, using nodal propagules with multiple nodes, the number of nodal propagules per shoot would be fewer than its corresponding number of nodes. Hence the internode length has direct effect on the final micropropagation rate.

The superiority of agar in commanding rapid and vigorous growth in terms of the components of micropropagation rate was implied in the amount of propagules produced in 84 days (Figure 16) from culture initiation and the three multiplication subcultures. Although cassava starch has been reported by many researchers as potential agar substitute (Maliro and Lameck, 2004; Ibrahim *et al.*, 2005; Kuria *et al.*, 2008) the results of this study show that sweet potato starch is a substitute which may be superior to cassava starch.

The high performance of sweet potato over agar may be caused by differences on the gel firmness which is usually implied on the ability of the TC media to provide anchorage, nutrients and growth hormones to the explants and the growing shoots for rapid morphogenesis. Also it can be speculated that, unlike cassava starch, sweet potato starch could be acting as source of additional organic and inorganic nutrients for growth of the shoots *in vitro*.

5.9 The implication of using starchy gelling agents on micropropagation costs

The results in Tables 6 and 7, suggest that sweet potato starch is a better candidate to replace agar because it commanded the highest reduction in both total media and propagule costs. Such cost reduction can be attributed to high availability (supply) of the sweet potatoes especially during the harvest season which commanded low purchasing prices. The low prices my also be due to low demand of the material because so far it has end uses in the food market where there is stiff competition with cereal products. Apart from low prices, the ability to promote higher micropropagation rate (number of propagules per unit of time) may have led it enjoy the economies of scale due to increased number of marketable units (propagules).

Generally upon improvement in quality and rate of micropropagation, the application of starch as substitute to agar has high potential of cost reduction. Apart from this study, many other workers (Ibrahim *et al.*, 2005; Kuria *et al.*, 2008; Napan, 2008) have reported significant cost reduction due to replacement and/or combination of cassava, irish potato, sago and maize starches with agar because of low prices

CHAPTER SIX

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

1. The physicochemical properties of the starch extracts varied with the type of the crop from which they were extracted. The results suggest that sweet potato, and cassava starches could be potential candidates to replace agar for tissue culture due to large amounts of extractable pure starch, high amylose content and swelling power. Also they have demonstrated high ability to support and nourish the explants for rapid regeneration and vigorous growth.

2. The irreversible semisolid starch gels and their behavior of sticking on the walls of containers made the work of dispensing a given amount of starch gelled TC media into culture vessels more difficult and labour intensive than dispensing the same amount of agar gelled media which are usually in liquid condition when hot above 75°C .

3. Sweet potato and cassava starches outperformed the control in some aspects of growth of plants while irish potato starch was insignificantly lower than the control at the initiation phase. Starch extracts from cereal crops were very inferior to the control in all assessed growth parameters hence were eliminated from further studies.

4. On multiplication media, best survival of up to 100% of the explants was observed in 15% cassava and 11% sweet potato and irish potato starches. During the

multiplication phase, the control commanded the highest quality and rate of micropropagation.

5. The quality of propagules produced on sweet potato starch media was comparable to those of agar solidified medium and the micropropagation costs due to sweet potato starch are also very low. Therefore the results suggest that sweet potato starch has high potential to replace agar in commercial micropropagation due to high amount of extractable pure starch, good gelling properties, good ability to support growth of *in vitro* plants and low cost.

5.2 Recommendations

1. Genotype plays a big role in determining the functional properties of starch. Therefore genotype whose starch is used as alternative gelling agent is extracted has to be considered.

2. Sweet potato and cassava starches are recommendable as gelling agents, due to their high starch yields, low amylose content and high swelling power. Research to establish genetic variability of starch properties among varieties of sweet potato and cassava may help to identify materials with better gelling properties than those used in this study.

3. The colour and lack of clarity of starch gels due to presence of non starch biopolymers make them unattractive options because they create darkness in the culture vessel which is unfavorable for plant growth. Also such colours and lack of

clarity make uneasy culture management practices like observation of growth and identification of contaminants. Highly efficient starch extraction technologies are therefore required to isolate pure starch and completely eliminate non starch biopolymers like protein, fats, organic acids, inorganic minerals.

4. Also the irreversible formation of starch gels which stick on the walls of containers, make the process of dispensing the media into culture vessels difficult, tedious and labour intensive. Therefore, an innovative research is needed to find out simple, user friendly and less labour intensive mechanisms of dispensing starch gelled media.

5. The use of sweet potato starch as alternative gelling agent at 12% and 11% (w/v) concentration may be advantageous due to its high potential to support growth of *in vitro* plants and low cost.

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APPENDICES

Appendix 1: Composition of Murashige and Skoog (1962) basic salt solution and organic additives

Name	Required concentration (mg/l)
Major Salts	
NH_4NO_3	1.650
KNO_3	1.900
$CaCl_2$-$2H_2O$	440
$MgSO_4.7H_2O$	370
KH_2PO_4	170
Na_2EDTA	37.5
$FeSO_4.7H_2O$	27.8
Minor Salts	
H_3BO_3	6.2
$MnSO_4.4H_2O$	22.3
$ZnSO_4.4H_2O$	8.6
KI	0.83
$Na_2MoO_4.2H_2O$	0.25
$CuSO_4.5H_2O$	0.025
$CoCl_2.6H_2O$	0.025
Organic additives	
Myoinositol	100
Glycine	2
Nicotinic acid	0.5
Pyridoxine	0.5
Thiamine	1