EFFECT OF DIFFERENT CONCENTRATIONS OF SUCROSE, GLUCOSE AND FRUCTOSE ON MICROTUBERIZATION OF SWEET POTATO (*IPOMOEA BATATAS*)

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ABSTRACT

The effect of different concentrations of sucrose, glucose and fructose on *in vitro* microtuberization of sweet potato (*Ipomoea batatas*) was studied. Plantlets were obtained from explants excised from mother tubers of sweet potato and cultured on Murashige and Stoog (MS) media without growth regulators. Growth characteristics after three weeks revealed that plantlets developed roots within 12 hours with lengths of 20-38cm; and developed shoots within 3-5 days with plantlet heights of 12-16. 5cm. Also the number of leaves per plantlet was 5-20 and the nodal number was 12-14 per plantlet. Microtubers were formed from plantlets cultured on 3%, 5% and 8% concentrations of the three sugars as basic carbon sources; with 5% and 8% concentrations yielding the biggest sizes of microtubers. This implies that concentrations of sucrose, glucose and fructose ranging between 3% and 8% are capable of forming sweet potato microtubers *in vitro*, hence facilitating mass production of microtubers which can be supplied to farmers at subsidized rates.

INTRODUCTION

In tackling the challenges of food security and meeting up with the millennium development goals (MDGs), the application of biotechnological tools in the agricultural sector cannot be over emphasized.

Tissue culture is a tool, in biotechnology, capable of creating genetic variability and producing plants with novel characteristics which can be more favourable than the existing crop varieties. The emergence of genetically modified crops (GMCs), and other products of tissue culture, is gaining popularity, though ethical and other problems do exist (Okereke *et. al*, 2009).

In tissue culturing of sweet potato for example, the stems, leaves, roots, flowers, seeds, etc, can be used as explant source, though younger and fresh explants are preferable (Ahmed *et al*, 1995). In micro propagation of sweet potato, production of virus-free plants is achieved. Here, the plantlets produced are induced under right concentrations of appropriate carbon sources to give rise to minitubers which can be sown as seeds.

Microtuberization of sweet potato (*Ipomoea batatas*) involves the production of tiny tubers of it *in vitro* under controlled environment. Reliable microtuber production has been obtained by culturing nodal explants of sweet potato on Murashige and Skoog (MS) media without the addition of growth regulating substances.

Generally, good microtuberization of sweet potato is influenced by factors such as the age of the explant, concentration of carbon source, temperature, light illumination and presence or absence of contaminants. Different sucrose levels have different effects on the growth parameters of potato plantlets. For instance, 6% sucrose concentration has been found to be better for good shoot regeneration and root length, 3%, for going shoot length and an average number of leaves. A concentration of 12%, is significantly better for shoot length, higher number of leaves, better root formation and root length (Fatima *et al*, 2005). Higher number of micro tubers can be induced using 8% sucrose in the MS medium, while a further increase in sucrose level reduces the number of microtubers (Wattimena *et al*, 1983). Garner and Jennet, 1989 and George and Hall, 2008, compared a sucrose range of 4%-12% and 5%-10% respectively, and obtained similar results, showing production of large microtubers after one month under 16 hours photo-period; with storage of food in form of sugar as tubers around the plant base.

The reported use of sweet potato in food industry, pharmaceutical industry, as well as in bioenergy (biofuel) production (e.g. in production of ethanol (Okereke *et al*, 2010), all serve as useful and interesting pointers for the future. Considering this trend, the present study aimed at assessing the best conditions (with respect to the concentrations of carbon sources) for the microtuberization of sweet potato, to enhance its quicker and larger production, as well as making it available to farmers at subsidized rates.

METHODOLOGY:

Media preparations for plantlet formations and microtuberization of sweet potato.

Following the manufacturer's guideline, the media for plantlets formation was mixed in 1000ml conical flask and made up to the 1000ml with distilled water. The pH of the mixture was adjusted to 5.8 with 1M KOH or NaOH, autoclaved at 121°C and 15psi for 15 minutes. After cooling, 5ml of the medium was removed while 5ml of filter-sterilized Vitamin x 200, was added to the 995ml of the medium. The mixture was shaken thoroughly and poured into sterile culture vessels (20-25ml) or test tubes, covered, labeled and kept to solidify for use.

In the preparation of medium for microtuberization, the vitamin used was Gamborg, *et al.*, Vitamin supplemented with 2mg/l glycine. Here 50ml of autoclaved and cooled potato medium was put into 5 conical flasks (labelled A-E.) and **250ml** of Gamborg *et al.* vitamin supplemented with glycine added, mixed and dispensed into sterilized test tubes containing different concentrations of sucrose, glucose and fructose respectively in the order of 3%, 5%, 8%, 10% and 12% concentrations.

Collection and Sterilization of Potato Explants:

A clean and sizeable sweet potato tuber was washed under running tap water. It was later fitted into a bottle containing some water in such a manner that the potato rested on the water beneath. The nodes which resulted after two weeks were harvested from the sprouted stems. The nodal cuttings were reduced to manageable sizes, washed about three times with distilled water and few drops of tween-20.

They were transferred into a sterilized conical flask using a pair of forceps and an ample volume of 70% ethanol was added and shaken for a minute.

After rinsing the nodal cuttings with distilled water they were transferred to a sterile conical flask, and further sterilization was carried out using 0.5% sodium hypochlorite, supplemented with three drops of tween-20 and shaken for 30 minutes. The steriliant was decanted and the explant rinsed 5 times with sterile water and poured out onto sterile cotton pads to blot.

Explant Culturing and Microtuberization:

The blotted explants were reduced to the sizes of 2-3cm using sterile scapel and were transferred and cultured in the test-tube containing sweet potato media. The test tubes were sealed with paraffin, labeled and transferred to *in vitro* plants growth room where they were subjected to a temperature of about 27°C for 16 hours photoperiod.

After about 3-4 weeks during which the nodes had developed into plantlets with roots, leaves and shoots, they were taken to the laminar flow hood, the test tubes opened, plantlets brought out and roots sizes reduced with the aid of a sterile scapel on sterile cutting pad. The plantlets were transferred into labeled test-tubes, with each plantlet/test tube containing different concentrations (3%, 5%, 8%, 10% and 12%) of sucrose, glucose and fructose respectively. The test tubes, with each were later sealed with paraffin and kept in the *in vitro* plants growth room or growth chamber at a temperature of 26°C and 10 hour photoperiod for one to three weeks under close observation for possible changes.

RESULTS

As shown in Table 1, rooting of sweet potato explants was observed within 12 hours of culturing on MS Medi without growth regulators while shoots developed within 3-5 days.

The observed root lengths were between 20-38cm; plantlet heights, 12-16.5cm; and number of leaves per plantlet, 5-10 and number of nodes per plantlet, 12-14.

The potato plantlets cultured in the microtuberization medium with different concentrations of the three carbon sources (sucrose, glucose and fructose) after one week, showed microtuber formation in the 8% concentration only, of all the 3 sugar sources (Table 2).

However, after two weeks, 5% and 8% concentrations only (of all the sugar sources), supported formation of microtubers, while it was only 3%, 5% and 8% concentrations of sucrose, glucose and fructose that supported the microtuberizing of the sweet potato plantlets after three weeks.

Inasmuch as the 3%, 5% and 8% concentrations of sucrose, glucose and fructose supported microtuber formation by the sweet potato plantlets, the sizes of microtubers formed differed. The 5% and 8% concentrations of the respective sugars, recorded highest sizes (+++) of microtubers formed, while 3% concentrations gave the least sizes (+) of microtubers (Table 3).

Table 1: Growth performance of sweet potato plantlets after 3 weeks of culture

GROWTH CHARACTERISTICS	TIME, SIZE OR NUMBER
Rooting	12 hours
Shooting	3-5 days
Root length	20-38cm
Plantlet Height	12-16.5cm
No. of leaves/Plantlet	5-10
No. of Nodes/Plantlet	12-14

Table 2: Microtubers formation in different concentrations of sucrose, glucose and fructose within 3 weeks

CULTURE PERIOD		CONCS. OF SUGAR SOURCES				
		3%	5%	8%	10%	12%
Sucrose	1 week	-	+	-	-	
	2 weeks	-	+	+	-	-
	3 weeks	+	+	+	-	-
Glucose	1 week	-	+	-	-	
	2 weeks	-	+	+	-	-
	3 weeks	+	+	+	-	-
Fructose	1 week	-	+	-	-	
	2 weeks	-	+	+	-	-
	3 weeks	+	+	+	-	-

KEY: + = Microtuber formation
- = No microtuber formation

Table 2: Microtubers formation in different concentrations of sucrose, glucose and fructose within 3 weeks

CONCENTRATION (%)	SUGAR TYPE		
	Sucrose	**Glucose**	**Fructose**
3	+	+	+
5	+++	+++	+++
8	+++	+++	+++

KEY: **+: Large sizes of microtubers**
++: Larger sizes of microtubers
+++: Largest sizes of microtubers

DISCUSSION AND CONCLUSION

In microtuberizations of sweet potato using Mirashige and Skoog (MS) Media without growth regulators, the development of roots within 12 hours (with length of 20-28 cm within 3 weeks), shooting within 3-5 days as well as a good number of nodes per plantlet of cultured explants, indicate possible multiplication, propagation and mass production for commercialization of this food crop.
Ahmed *et al* (1995), asserted that stems, leaves roots, flowers, and seeds, etc can be used as sources of explants in sweet potato tissue culture through leaf fragmentation and protoplast fusion; and better hybrids of sweet potato can be micropropagated using appropriate biotechnological processes (William and Michael, 2009).

Sweet potato plantlets from this study when grown in culture media with 3%, 5% or 8% concentrations of sucrose, glucose, or fructose form microtubers *in vitro* within 3 weeks under 10 hours photoperiod. According to Onovo *et al*., (2009), about 100% of sweet potato plantlets formed microtubers in the presence of 3% sucrose in contrast with 29% in the presence of 1% sucrose. He also obtained similar results with 1% and 3% concentrations of fructose and glucose respectively. George *et al*; (2008) had observed that sucrose at 8% concentration as compared with 4% or 12%, advanced the initiation of tuberization of sweet potato and gave large microtubers after one month, under 16 hours photoperiod, followed by transfer to 8 hours photoperiod for rapid microtuber development. Nature of growth media with emphasis on carbon source, presence or absence of growth regulators, etc, play very important roles in microtuberization of sweet potato and other tubers (Tarique *et al*; 2008).

Other factors such as explant source, sub-culture frequency, contaminants, environmental factors (e.g. photoperiod, humidity, temperature), etc, also contubute to results obtained in any plant tissue culture.

Though tuberization was supported by 3%, 5% and 8% of sucrose, glucose and fructose, the sizes of the microtubers differed, with only 5% and 8% concentrations yielding more sizeable microtubers than 3% concentration. This invariably affects the quality of seed potato cultivar (Sewan *et al*, 2009).

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