NIGERIAN AGRICULTURAL JOURNAL
ISSN: 0300-368X
Volume 51 Number 3, December 2020 Pg. 16-20
Available online at: http://www.ajol.info/index.php/naj

COMPARISON OF SUCROSE AND GLUCOSE AS CARBON SOURCES FOR *INVITRO* GROWTH OF *Moringa oleifera* Lam. EMBRYOS

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Abstract

The embryo of *Moringa oleifera* Lam. was cultured in vitro using sucrose and glucose as carbon sources at two different concentrations to check the effect of the different carbon sources and concentrations on the growth of the cultured explants. The concentrations used were 2% and 4% each of sucrose and glucose respectively, while 0% served as control. It was recorded that the explant in 2% and 4% sucrose attained 50% sprouting on the 4th day, and produced the highest root length and highest number of roots. Whereas, explants in 2% and 4% glucose attained 50% sprouting on the 6th day after inoculation, and produced the highest leaf length, shoot length, number of leaves and highest value of fresh weight in grams (g). Therefore, 2% and 4% sucrose supported root growth and elongation, while 2% and 4% glucose supported leaf growth and shoot growth.

Keywords: ***Moringa oleifera,*** **embryo culture, micropropagation, sucrose, and glucose**

Introduction

Moringa (*Moringa oleifera* Lam.) is a perennial softwood tree, known for its traditional, medicinal and industrial uses. Recently, this tree has become an outstanding indigenous source of highly digestible protein, calcium (Ca), Iron (Fe), Vitamin C, and carotenoids. These nutritional characteristics of the plant may be potentially beneficial to the developing regions of the world where undernourishment is a major concern. Also according to Fahey (2005), Moringa is one of underutilized tree species. The large numbers of underutilized species of this plant represent an enormous food resource which can narrow the gap to the increasing demand for food and nutrition, energy, medicines and industrial needs.

Micropropagation or clonal propagation through tissue culture; an *invitro* technique has been introduced and being used over time in the production of Moringa. This technique has been proved to be efficient and good in the propagation of Moringa and other plants. Plant tissue culture offers the possibility of rapid clonal propagation, which provides potential for large scale production of genetically identical superior strains for crop improvement, and also for human consumption (Sharp *et al*., 1980; Al-Sulaiman, 2010). Tissue culture has helped in the multiplication of plants throughout the year despite seasonal variation (Rahman *et al*., 2004). Various parts of this plant has been used as explant including the embryo.

Carbon source (sucrose and glucose) has been reported by researchers as the major energy source in plant tissue culture as explants cannot grow on a culture medium without a carbon source. Over time it has been identified that there is need for specie-specific carbon sources and concentrations for optimal growth in *invitro* propagation (Al-khateeb, 2008). Sucrose as a carbon source is by far the most widely used in micropropagation, but studies have also reported that some plants grow better with glucose as a carbon source than sucrose (Noggle and Fritz, 2006; George, 2008). Glucose has been reported to be required for the culture of isolated roots of wheat, and some other monocotyledons (Bhojwani and Razdan, 1996).
Irrespective of the knowledge on specie-specific carbon source, there is limited information on the proper carbon source and concentration for *Moringa oleifera* explants, especially the embryo culture. Therefore there is need to determine the optimal carbon source for better growth of the plant *invitro.* The aim of this experiment is to determine the optimal carbon source and concentration for Moringa embryo tissue culture. The aim of this research study was to compare extensively, the strengths of sucrose and glucose at three levels (0%, 2% & 4%) as carbon sources for the propagation of *Moringa oleifera* embryos in *vitro,* using Murashige and Skoog's (1962) basal medium.

Materials and Methods

Source of material and experimental location

This study was conducted at the Plant Tissue Culture Laboratory located at the University of Nigeria, Nsukka. Seeds of *Moringa oleifera* used for this work was purchased from Ogige Market, university road, Nsukka Local Government Area of Enugu State, Nigeria.

Seed viability test

The viability of the purchased seeds was tested by soaking them in sterile distilled water, in a beaker. The seeds that floated were carefully removed from the water and those that sank were collected and used for the study.

Sterilization, preparation of media and culturing

The seeds were surface sterilized in 70% alcohol to which 0.1% wetting agent was added for 10min after which it was transferred into 10% sodium hypochlorite for 30min and rinsed three times in sterile distilled water. The explant used is the embryo and was obtained by excising the seeds (separating the cotyledons since the plant is dicotyledonous in nature). The stock solutions were prepared according to the modified method of Murashige and Skoog (1962). The stock solutions made comprised of macro salts (NH4NO3, KNO3, CaCl2.2H2O, MgSO4, 7H2O, KH2PO3), micro salts, iron compounds and organics (myo-inositol, thiamine-HCl, nicotinic acid, pyridoxine and glycine). Appropriate quantities of the stock solutions were measured into a flat conical flask. The pH of the medium was adjusted to 5.8 with 1M NaOH. 8 grams of Fluka agar added to conical flask and the volume was made up to 1 litre by adding sterile distilled water. The media was distributed into 5 beakers.

In beaker A, 20g of sucrose was added to make up 2% level, beaker B contains 2% level of glucose, beaker C had 4% level of sucrose and beaker D contained 4% of glucose, while Beaker E was left at 0% sugar concentration and served as control. These were further distributed into labeled test tubes for culture. All operations starting from the preparation of explants to establishment of cultures were carried out in a laminar air flow hood chamber previously kept sterile by swabbing with alcohol and exposure to ultraviolet light for 3mins. The embryos were transferred into the cultured tubes and left to grow.

Experimental design

The treatments used were two carbon sources: sucrose and glucose at two different concentrations (2% &4%) respectively, while 0% serves as control. The experiment was carried out in a completely randomized design with 10 replicates. The growth was allowed for three weeks after which they were scored for the requisite growth parameters.

Sprouting studies and growth parameters determination

After inoculation in the laminar air flow chamber, the embryo culture was keenly monitored as radicle surfaced after 4days, leaf primordial became clearer after 8-9days. The percent sprouting was recorded for each treatment until about 50% sprouting was obtained. The total number of embryos that sprouted per day in various treatments were counted and expressed as a percentage of the total number of embryo explants cultured.

$$\text{Percentage sprouting} = \frac{\text{Number of sprouted embryo}}{\text{Total number of cultured embryos}} \text{ X } 100$$

The rate of sprouting under each treatment was expressed as the reciprocal of the time to attain 50% sprouting. Sprouting rate or Germination rate = 1/T. The following parameters were determined for each sample from each treatment: number of leaves, number of roots, length of leaves, length of shoot, length of roots and fresh weight of plantlets.

Analysis

SPSS software was used to carry out data analysis. One-way analysis of variance (ANOVA) followed by Duncan's New Multiple Range Test (DNMRT) was used to test for significance difference ($P \leq 0.05$) and comparison of means.

Results and Discussion

Although sucrose is the most commonly used carbon source in the micro-propagation of plants due to its efficient uptake across the plasma membrane (Noggle and Fritz, 2006), a number of studies have shown positive effects of other sugars on plant regeneration. Specie-specific carbon sources and carbon concentrations for optimal growth rates have been identified for various culture systems (Bhojwani and Razdan, 1996; Noggle and Fritz, 2006; George, 2008). Romano *et al*. (1995) revealed that different patterns of morphogenesis were attributed to the type of carbohydrate and its concentration which was supported by this study. The sprouting rate and sprouting percentage differed significantly ($P<0.05$) between the two carbon sources likewise with the control (Table 1). Table 2 showed that glucose as a carbon source enhanced leaf length of the plantlet with 4% glucose having the highest mean, while sucrose concentrations had no effect on the plantlet as there was a significant difference ($P<0.05$) between the two carbon sources, but 4% sucrose produced the highest root length and significantly different ($P<0.05$) from glucose, while decrease in concentration of glucose from 4% to 2% decreased the length of the root. This is in accordance with the study of Thwe *et al*. (2013), who reported that root development increased with increasing sucrose concentrations in the media. However, he recorded a gradual decline in root growth below a concentration of 3% sucrose. Also Cuenca and Vitez (2000) reported positive effect of glucose on leaf production, while the control had no effect on the leaf length and root length.

Table 1: Mean effect of carbon sources and concentration on the sprout rate and percentage of *M. oleifera*

Treatments	Sprout rate	Sprouting %
2% Sucrose	0.25±0.00a	100.00±3.04a
4% Sucrose	0.25±0.00a	70.00±2.40b
2% Glucose	0.17±0.00b	60.00±1.91bc
4% Glucose	0.17±0.00b	50.00±1.02c
Control	0.00±0.00c	0.00±0.00^{d}

Mean values in the same row with different superscripts are significantly different (P<0.05)

Table 2: Mean effect of carbon sources and concentration on the leaf length and root length of *Moringa oleifera* embryo explants

Treatments	Leaf length	Root length
2% Sucrose	0.00±0.00^{a}	4.50±1.32^{c}
4% Sucrose	0.00±0.00^{a}	12.00±5.06^{d}
2% Glucose	1.35±0.45^{b}	1.00±1.00^{b}
4% Glucose	1.75±0.75^{b}	4.50±4.50^{c}
Control	0.00±0.00^{a}	0.00±0.00^{a}

Mean values in the same row with different superscripts are significantly different (P<0.05)

At 4%, sucrose had the highest number of roots and was significantly different from 2% sucrose, glucose and control (Table 3). Also, between 4% and 2% glucose, there was no significant difference on the number of roots. The control had no effect on the shoot length and number of roots of the plantlet. Four percent glucose recorded the highest mean on shoot length, though not significantly different from 2% glucose, while there was a significant difference between sucrose and glucose on the shoot length. It was also recorded in Table 3 that 2% sucrose had the least mean effect. There was a significant difference between sucrose, glucose and control on number of leaves (Table 4). Though sucrose had no effect on the number of leaves, increase in glucose concentration had a significant (P<0.05) decrease on the number of leaves produced. A significant difference was observes among sucrose, glucose and control on the fresh weight of the plantlet; but within each carbon source, the variation in concentration had no significant difference on the fresh weight of the plantlet. This study supported the findings of Cuenca and Vitez (2000) who reported glucose as the best carbon source for leaf proliferation. From this study, 4% glucose induced the highest number of leaves, highest fresh weight and highest shoot length of *Moringa oleifera* plantlets, but the least for root length. This is in conformity with the study of Harda and Murai (1996), who reported that regeneration of *Prunusmume* was more effective on medium with glucose than that with sucrose. Also Atawodi *et al*. (2010) reported similar effect of glucose on shoot regeneration.

Table 3: Mean effect of carbon sources and concentration on shoot length and number of roots of *Moringa oleifera* embryo explants

Treatments	Shoot length	Number of roots
2% Sucrose	5.00±1.08^{b}	12.50±2.50^{c}
4% Sucrose	5.80±1.28^{b,C}	16.00±16.31^{d}
2% Glucose	9.50±0.50^{c}	5.00±5.00^{b}
4% Glucose	10.00±2.00^{c}	5.00±5.00^{b}
Control	0.00±0.00^{a}	0.00±0.00^{a}

Mean values in the same row with different superscripts are significantly different (P<0.05)

Table 4: Mean effect of carbon sources and concentration on number of leaves and fresh weight of plantlets of *Moringa oleifera* embryo explants

Treatments	Number of leaves	Fresh weight
2% Sucrose	0.00±0.00^{a}	0.02±0.00^{a,b}
4% Sucrose	0.00±0.00^{a}	0.03±0.01^{b}
2% Glucose	5.00±3.00^{c}	0.05±0.00^{c}
4% Glucose	1.50±0.50^{b}	0.05±0.00^{c}
Control	0.00±0.00^{a}	0.01±0.00^{a}

Mean values in the same row with different superscripts are significantly different (P<0.05)

Plate A

Plate B

Plate C

Plate D

Plate E

Dmag. x10

A-Fourteen-day old plantlets in control; B- Fourteen-day old plantlets on 2% sucrose in MS media; C- Fourteen-day old plantlets on 2% glucose in MS media; D- Fourteen-day old plantlets on 4% sucrose in MS media; E- Fourteen-day old plantlets on 4% glucose in MS media

Conclusion

Although plantlets produced from these concentrations were small in size and unit, they still sprouted faster than sown *Moringa* seeds, considering the fact that the explants used is the embryo. Viable seeds of *Moringa* start germinating within two weeks of planting (Gantra *et al.,* 2012). Glucose as a carbon source had a positive effect on number of leaves, leaf length, fresh weight and shoot length of *Moringa oleifera* plantlets, while sucrose as a carbon source enhanced root length and number of roots. In conclusion, 4% glucose may be used for leaf proliferation and regeneration of embryonic explants of *Moringa oleifera* and 4% sucrose for root development. Also in addition, the use of growth hormones will likely enhance initiation, proliferation and growth of cultured embryo of *Moringa oleifera*.

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