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EFFECT OF VARYING CONCENTRATIONS OF GLUCOSE AND MALTOSE ON THE ZYGOTIC EMBRYO PROPAGATION OF *GLYCINE MAX* L. (*INVITRO*)

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Abstract
The effect of varying concentrations of glucose and maltose was studied on the growth of *Glycine max* L. *in vitro* using Murashige and Skoog media. Glucose and maltose concentrations were 2% and 4% each, while 0% (no carbon source) served as control. Embryo explant of *Glycine max* L. in 4% glucose attained 80% growth from the second day of inoculation followed by glucose at 2% level with 70% induction, whereas maltose at 4% and 2% started also from the second day but with percentage induction of 60% each. Glucose based medium generally exhibited the highest mean value of root and shoot length when compared to maltose based medium. Four percent glucose has the highest mean, though not significantly different ($P \leq 0.05$) from the control medium. The glucose based medium shows greater efficiency both in shoot length and embryo growth induction than the maltose based medium. However, glucose at 4% level is preferable to that of 2% level as observed from the growth parameter analysis.

Keywords: *Glycine max,* Carbon sources, Embryo explant, *In vitro* propagation

Introduction

Glycine max (L) Merr. popularly known as soybean, belongs to the family *Fabaceae.* It is a naturally diploid (2n=40) legume used both as feed crop and industrial crop (Isler and Vural, 2010). Its importance as one of the world's oil and protein crop has increased the need for its mass propagation and crop improvement. Effective and efficient techniques have been developed over time to meet these demands. These techniques include *in vitro* cultures and genetic engineering. These cultures have been found convenient for genetic transformation of soya bean via particle bombardment and recovery of transgenic plants (Droste *et al.,* 2001). Increase in temperature above 30 °C greatly affects the yield, while exposure of the plant for a long period at its flowering stage to temperature below 13°C inhibits flower and seed formation. The temperature requirement of soybean at various growth stages differs. To stimulate its germination, soil temperature of about 15°C will be preferable. Generally for all growth stages, 25°C can be taken as the optimum temperature (Romano, 1995). Soybean is a short day plant and with the rapid changing climate conditions, such as erratic rainfall, global warming and its attendant effect on agriculture, every research effort to improve on its yield and acceptability will be a major boost towards solving the problem of hunger and malnutrition in most parts of Africa and beyond. However, through *in vitro* micro propagation, it can be mass propagated at a faster rate when compared with conventional propagation (Ahmed *et al.,* 2003). Also plantlets produced through *in vitro* are disease free. The adoption of *in vitro* techniques in plant propagation helps solve problems like: regenerating new cultivars, rare species and difficult-to-propagate plants (Sadhu, 2007). Plant cultures have poor photosynthetic ability due to poorly developed cells and tissues and as a result, needs a continuous supply of carbohydrate from the medium in order to survive *in vitro*. Therefore, sugars such as sucrose, glucose, fructose and sorbitol are generally added as energy sources, signal molecules in plants, stress protectants, osmotic agents and carbon sources (Lipavska and Konradova, 2004). The type, concentrations of carbon source and its combinations are important things to consider in the growth of a plant *in vitro* (Younas *et al.,* 2008). Over time it has been identified that there is need for species-specific carbon sources and concentrations for optimal growth in *in vitro* propagation (Al-khateeb, 2008). Therefore, the aim of this study is to investigate the effects of varying different carbon sources (glucose and maltose) on the *in vitro* micro propagation of *Glycine max*.

Materials and Methods

Source of explant and experimental location

This experimental study was conducted at the Plant Tissue Culture Laboratory of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. The explants (matured zygotic embryo) employed in this study were excised from matured seeds of *Glycine max* obtained from Ogige Main Market Nsukka, Enugu State, Nigeria.

Sterilization, preparation of media and culturing of explant

The seeds of *Glycine max* were washed in running tap water for 30mins to remove visible dirt followed by surface sterilization in 70% ethanol (v/v) for 5mins, Sodium hypochlorite (NaOCl) for 20 minutes after which it was rinsed 4 times with sterile distilled water. Thereafter, the seeds were soaked in sterile water for three hours to make them soft for easy removal of the seed coat and excision of the embryo. The stock solutions were prepared following a modified method of Murashige and Skoog (1962). Two carbon sources (glucose and maltose) at two different concentrations (2% and 4%) respectively were introduced which was not adopted by Murashige and Skoog, while 0% (no carbon source) served as the control. The pH of the media was raised to 5.8. Embryo explants were transferred into culture vessels containing the different treatment levels under a sterile condition and allowed to grow.

Experimental design and parameters measured

The experiment was completely randomized in 10 replicates each. The growth parameters measured include; shoot lengths per culture, root lengths per culture, shoot dry weight and root dry weight.

Analysis

The data analysis was done using analysis of variance. The significant differences between means were estimated by Duncan tests using the Statistical Package for Social Science (SPSS) version 17.0.

$$\% \text{ Regeneration} = \frac{\text{Number of regenerated embryo}}{\text{Total number of calculated embryo}} \times 100$$

Results and Discussion

The results in Table 1 show the mean effect of different concentrations of carbon sources on the growth parameters of *Glycine max*.

Table 1: **Mean effect of different concentrations of carbon sources on the growth parameters of Glycine max**.

Treatments	Shoot length (cm)	Root length (cm)	Shoot dry weight(g)	Root dry weight (g)
Control	3.54 ± 0.29^{c}	1.13 ± 0.16^{bc}	0.030 ± 0.40^{c}	0.011 ± 0.008^{b}
Glucose (4%)	3.53 ± 0.28^{c}	1.22 ± 0.14^{c}	0.017 ± 0.010^{b}	0.008 ± 0.008^{ab}
Glucose (2%)	3.11 ± 0.27^{b}	1.01 ± 0.23^{bc}	0.007 ± 0.006^{a}	0.003 ± 0.005^{a}
Maltose (4%)	2.93 ± 0.12^{ab}	0.86 ± 0.20^{b}	0.006 ± 0.005^{a}	0.002 ± 0.004^{a}
Maltose (2%)	2.84 ± 0.12^{a}	0.53 ± 0.44^{a}	0.005 ± 0.005^{a}	0.002 ± 0.004^{a}

Mean values in the same column with same superscripts are not significantly different (P<0.05)

Table 1 showed that the control had the highest mean in shoot length though it did not significantly differ ($P \leq 0.05$) from 4% glucose. The glucose-based medium generally exhibited higher mean value in root length and shoot length when compared to the maltose-based medium. Similar results of inefficiency of maltose-based medium was however observed by Samoylov *et al*. (1998) when they analyzed the effect of carbohydrate on embryo histo-differentiation and maturation on liquid medium using FNL medium supplemented with 3% sucrose and 3% maltose. The result obtained also supported the study done by Tiexeira da silva (2004), who reported that glucose as carbon increased the growth of both the shoots and roots in *in vitro* organogenesis of Chrysanthemum. However this study did not agree with the findings of Sridhar and Naidu (2011), who reported that in *in vitro* shoot regeneration of *Solanum nigrum,* maltose at both at 2% and 4% concentration gave more number of shoots and shoot length than glucose at same concentrations. While on the contrary, maltose at 2% and 4% had less shoot regeneration frequency than glucose at same concentrations. The 4% glucose in shoot dry weight was significantly different from other treatments, though the control had the highest mean value. A 0% (control) gave more root dry weight than other treatments and differed significantly ($P \leq 0.05$) from other treatments except 4% glucose.

The growth induction of embryo was monitored and expressed in percentage as shown in Fig 1. Glucose at 4% level showed highest embryo growth induction of 80% from the second day of inoculation, likewise the control, and both differed significantly($P \leq 0.05$) from glucose at 2% level with 70% induction. Maltose at 4% and 2% started also from the second day, but with percentage induction of 60% each. Cuenca and Vitez (2000), also reported a positive effect of glucose on leaf proliferations of Beech *in vitro.*

Plate 1 revealed faster growth of plantlets in 4% glucose based medium compared to the control, thereby revealing the importance of carbon source in a basal media for proper plant growth. Comparing the two different carbon sources, the glucose-based medium showed greater efficiency both in shoot length and embryo growth induction than the maltose based medium. However, as shown in the analysis of the growth parameters, glucose at 4% level of concentration is preferable to that at 2% level. The possible explanations of the variation between the two carbon sources in the result could be because, compared to maltose, glucose is a monosaccharide and therefore easy to decompose, hydrolyze and metabolize than maltose

that belong to disaccharides family. Hydrolysis of maltose is slower, therefore, its metabolism and absorption takes more time than that of glucose hence, plant cultures on glucose based medium get energy for growth faster than those in maltose based medium. (Blanc *et al.,* 2002).

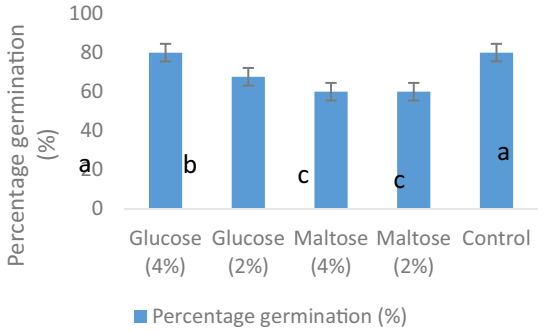

Figure1: ***Percentage growth induction of Glycine max under varying concentrations of different carbohydrate sources***

Plate 1: (A)-Ten-day old plantlet of Glycine max in control medium (Mag ×0.8). (B)- Ten-day old plantlet of Glycine max in 4% level glucose concentration

Conclusion

It is evident from this study, that the choice of carbon source is an important consideration in the *in vitro* growth of *Glycine max.* From the results obtained from this work, glucose at 4% level of concentration is the most preferable sugar concentration for the *in vitro* propagation of *Glycine max.* From the percentage germination figure, glucose based medium produced a significant effect on the germination induction when compared to maltose based medium. Maltose at 2% level had the lowest value in the growth characters and in the embryo growth induction. This justifies the choice of glucose over maltose for the propagation of soybean *in vitro.*

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