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In vitro Growth of Dwarf Cavendish Banana Plantlets in Different Culture Vessels and Light Intensities

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ABSTRACT

The growth and multiplication of a local Cavendish banana called "Kwadu Pa" was investigated under three different light intensities and three types of culture vessels. The light intensities were 4000, 5000 and 6000 Lux with glass, plastic bottles and conical flask as the vessel types. Each of the treatments for light intensity and vessel type were replicated 50 times and cultures were arranged in a completely randomized fashion. They were cultured for 16 weeks and data was taken on mean shoot fresh weight, number of shoots per plantlet, shoot dry weight, leaf chlorophyll content and shoot height. Plantlets that were cultured in conical flask under the three different light intensities had higher values for all the parameters measured under the different light intensities. Among the light intensities, plantlets that were cultured under 5000 Lux showed superior growth and multiplication compared to those cultured under 4000 and 6000 Lux. At light intensities above 5000 Lux, growth characteristics and chlorophyll content declined significantly. Conical flask as a culture vessel and photon flux of 5000 Lux were optimal for the *in vitro* growth and multiplication of banana.

Key words: Cavendish banana, chlorophyll, vessel type, photon flux

INTRODUCTION

Banana (*Musa* spp.) is an important fruit crop of the Musaceae family. It is widely grown in developing countries and is the second largest fruit crop in the world, after citrus (Madhulatha *et al*., 2004). It forms an important component in the diet of tropical third world countries because of its nutritional composition and wholesomeness (Thangavelu *et al*., 2007; Creste *et al*., 2004).

In Africa, only five countries namely, Côte d'Ivoire, Cameroon, Somalia, Ghana and Cape Verde, export approximately 427,000 t of banana and plantain (IITA., 2008). In Ghana, the banana industry is made up of small holders who usually grow banana in backyard gardens, in mixed field cropping, in association with tree crops and sometimes in intensive monocropping systems. Banana cultivars are vegetatively propagated through corms and suckers which usually habour pests such as fungi, nematodes viruses and insects and spread through the vegetatively propagated materials. Commercial banana production and export in Ghana started in 2000 (MoFA., 2011). The export trend has not improved much in recent years, because growers have experienced problems such as nematodes, viruses and fungal diseases. However, the introduction of Dwarf Cavendish and adoption of new growing techniques have recently made banana production more profitable in Ghana. For example, the production of Dwarf Cavendish under plastic covers and protected cultivation was found to be 53% greater than the open-field production (65.5 vs. 42.8 t ha^{-1}) (Gubbuk *et al*., 2004). Despite this effort, the demand for banana on the local and foreign markets out weighs the production. One of the constraints that accounts for the low production of the crop is the inadequate supply of healthy planting material for large-scale production.

Various innovations have evolved in trying to solve the problem of the shortage of planting material. Some of these innovations are the split-corm, tissue manipulation and the decapitation techniques and all these have yielded some good results (Swamy *et al*., 2010).

Tissue culture technique of growing banana plants *in vitro* has become a major innovation which has been employed in other countries like the Philippine and Netherlands to produce large amounts of planting materials for banana production. Propagating bananas through tissue culture provide excellent advantages over traditional propagation, including a high multiplication rate, physiological uniformity, the availability of disease-free material all year round, rapid dissemination of new plant materials throughout the world, uniformity of shoots, short harvest interval in comparison with conventional plants and faster growth in the early growing stages compared to conventional materials (Vuylsteke, 1989; Daniells and Smith, 1991; Arias, 1992).

Similar efforts have been directed in the last 25 years to propagate other crops, especially in tissue culture of clonally propagating rootstocks of apple, pear and cherry (Dennis and Hull, 2003). Many studies have reported on the clonal propagation of *Musa* spp. and the factors for the optimal growth of the plantlets (Akin-Idowu *et al*., 2010; Garcia-Gonzales *et al*., 2010; Nandwani *et al.,* 2000; Kodym and Zapata-Arias, 2001; Muhammad *et al*., 2004).

Culture conditions as well as the type and size of vessel can affect the growth of plants *in vitro*. Various researchers have used different type of containers for the *in vitro* culture of bananas (Kane and Philman, 1992; Preeti *et al*., 2009). It is believed that the type and size of culture vessel and light intensity are some of the important factors in micropagation because they have tremendous effect on plantlet growth (Amoo *et al*., 2009; Munoz *et al*., 2009; McClelland and Smith, 1990). The natural conditions under which plant growth occurs allow for the dissipation of secondary products into the soil or atmosphere, while tissue culture systems lack an effective method of secondary product disposal. This results from the use of enclosed containers to prevent microbial contamination and to retard desiccation which interrupts the flow of secondary products away from the developing plant. This interruption can have a variety of consequences, both harmful and beneficial, depending on the type of tissue being cultured, the species and the culture vessel type. The type of vessel used in micro propagation and the type of closure employed have been shown to have a variety of effects on culture growth and development (Islam *et al*., 2005). Some of these effects are that itaffects the gaseous composition inside the vessel as well as light penetration. Therefore, the growth of tissues in culture (shoot elongation, proliferation, fresh weight increase and possibly the hyperhydric degradation processes) is also affected by vessel type (Islam *et al*., 2005). Amoo *et al*. (2009) have reported that various vessel types and the amount of light that reaches the tissues in the culture vessel affect the growth of *in vitro* cultured plants (Amoo *et al*., 2009). No such work has however been carried out to investigate the *in vitro* growth performance of the local cavendish "Kwadu Pa".

This study was therefore, aimed at assessing the growth of banana plantlets *in vitro* in different culture vessels and under different light intensities to optimize the *in vitro* protocol for the local Dwarf Cavendish "Kwadu Pa" plantlets.

MATERIALS AND METHODS

Plant materials and explant preparation: A local variety of Dwarf Cavendish (*Musa* accuminata) called "Kwadu Pa" in Ghana was used as the explantsource. Plant materials were taken from a farmer's field in the western region of Ghana. Before washing with running tap water, the roots were trimmed off and the top of the shoot was removed. The sheaths that formed

Fig. 1(a-c): Culture vessel types used for the culture of plantlets (a) Glass bottle, (b) Plastic bottle and (c) Conical flask

the pseudostem were carefully removed to reduce the size of the material to about four leaf sheaths. They were then sterilized with 70% ethanol for 3 min and washed three times in sterilized distilled water (Buah *et al*., 2010). More leaf sheaths were then removed aseptically in a clean bench until about two leaves covered the shoot meristem. This process was followed by sterilization with 1% sodium hypochlorite solution containing a drop of polyoxyethylenesorbitan monolaurate (Tween 20) for 5 min with occasional shaking and there after washed three times with sterilized distilled water. Prior to their inoculation on the medium, each shoot tip (about 1 cm) was longitudinally divided into two halves and again sterilized with 1% sodium hypochlorite (NaClO) as above for 1 min.

Media composition: The MS medium (Murashige and Skoog, 1962) supplemented with 4.5 mg L^{-1} 6-Benzylaminopurine was used. Thirty grams (30 g L^{-1}) sucrose and 6 g L^{-1} agar were used because they had been the optimal concentrations from previous work with *Musa* species (Buah *et al*., 2010). The pH of the media was adjusted at 5.8 with HCl and NaOH before autoclaving for 15 min at 121°C. Forty milliliter of the medium was then dispensed into the various culture vessels, which were plastic, glass and conical flask. The glass and plastic bottles had covers while the conical flasks were clogged with sterilized cotton wool and aluminum foil (Fig. 1). They were then placed on shelves and allowed to cool and solidify before being used. The explants were inoculated into the medium in the various culture vessels and kept under a temperature of 26°C, 16 h photoperiod and different light intensities of 4000, 5000 and 6000 Lux and a relative humidity of 60%. Each type of culture vessel was replicated 50 times under each of the different light intensities. The culture vessels were placed in a completely randomized fashion. The initial sub

culturing was done 4 weeks after placing the explants in the media and subsequently at two weeks interval. During sub culturing, materials that had formed multiple shoots were separately removed and placed into additional vessels. In all, seven subcultures were done during which data were taken on mean number of shoots, mean plantlet height, mean fresh weight, mean dry weight and leaf chlorophyll content.

Determination of chlorophyll pigment: Leaf samples were taken from the *in vitro* plantlets for the estimation of total chlorophyll content according to the method of Razani *et al.* (2012) and the amount of chlorophyll was expressed as mg g^{-1} fresh weight of sample.

Data was analyzed with Genstat version 7.1 for analysis of variance and Excel 2007 for the plotting of graphs (Hilbe, 2007).

RESULTS

The growth and other physiological parameters were affected by type of vessel and the light intensity.

Fresh weight (g) of plantlets: The fresh weight of plantlets varied with the different vessels and light intensities after 16 weeks of culture. Plantlets that were cultured in conical flask recorded the highest fresh weight values of 16.0 g under 5000 Lux light intensities, followed by those in glass bottles which had mean fresh weight of 14.0 g while those in plastic culture vessel recorded least values of 7.0 g (Fig. 2). Generally, plantlets that were cultured in conical had better growth under the different light intensities, with those in plastic bottles recording the least. The difference between fresh weight of plantlets cultured in conical flask at 5000 Lux was significantly different from the other treatments. Fresh weight of plantlets showed a decrease at light intensities above 5000 Lux for plantlets in all the culture vessel types.

Height of plantlet: Even though plantlets that were cultured in the conical flasks grew taller than those cultured in the other vessel types, the height of the plantlets decreased with increase in the light intensity. Plantlets in conical flask had 18.7, 17.7 and 14.9 cm under 4000, 5000 and 6000 lux

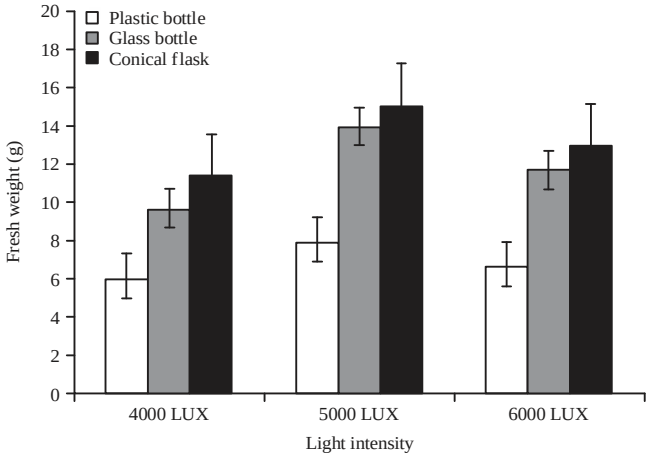

Fig. 2: Fresh weight of banana plantlets in different culture vessels and under different light intensities 16 weeks after culture

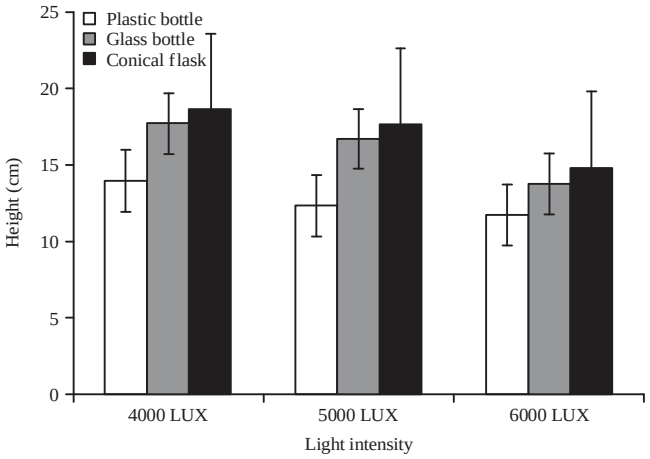

Fig. 3: Plantlet height of banana plantlets in different culture vessels and under different light intensities16 weeks after culture

Table 1: Mean number of shoots at 16 weeks under various light intensities and culture vessels

Mean shoot weight	Plastic CV	Glass culture bottle	Conical flask
4000 Lux	6.0±0.91	10.1±0.42	13.5±0.60
5000 Lux	11.3±0.83	16.0±1.12	18.4±1.50
6000 Lux	9.8±1.21	12.0±1.00	12.0±1.64
Total	27.1±2.95	38.1±2.54	43.9±3.74

CV: Culture vessel

Table 2: Mean dry weight of banana plantlet at 16 weeks under various light intensities and different culture vessels

Mean dry weight	Plastic CV	Glass culture bottle	Conical flask
4000 Lux	2.6±0.63	3.0±0.55	3.9±0.34
5000 Lux	3.0±0.21	4.6±0.30	5.4±0.17
6000 Lux	2.5±1.10	4.0±0.34	4.7±0.42
Total	8.1±1.91	11.6±1.19	14.0±0.93

CV: Culture vessel

intensities, respectively and this pattern was observed for plantlets in the other culture vessels. Thus plantlet height appeared to vary inversely with the light intensity. The variation in the plantlet height over the different light intensities was wider in the glass bottles and conical flask compared to those in the plastic bottles (Fig. 3).

Mean number of shoots and dry weight at 16th weeks: The number of shoots per explant varied with light intensity and culture vessel type. Explants that were cultured in conical flask recorded the highest number of shoots per explants (18.4±1) at 5000 Lux (Table 1). This was significantly higher than those recorded for plantlets under 4000 and 6000 Lux in conical flask (13.5 and 12.0 g), respectively. Plantlets cultured in plastic bottles had the least number of shoots under the three light intensities.

The dry weight values of plantlets followed a similar pattern as the fresh weight of plantlets cultured in conical flask at 5000 Lux light intensity having significantly higher dry weight values (Table 2).

Leaf chlorophyll content: There were significant variations among plantlets that were cultured in the various vessel types as well as light intensities in terms of leaf chlorophyll content. The highest chlorophyll content of 0.954 mg g^{-1} FW was recorded for plantlets cultured in conical flask

Table 3: Mean chlorophyll content (mg g^{-1} FW) of leaves Banana plantlets cultured *in vitro* for 16 weeks under different light intensities and culture vessels

Mean shoot weight	Plastic CV	Glass culture bottle	Conical flask
4000 Lux	0.613±0.91	0.741±0.62	0.763±0.06
5000 Lux	0.659±0.73	0.847±1.10	0.954±1.00
6000 Lux	0.578±1.51	0.610±1.00	0.687±1.34
Total	27.100±2.95	38.100±2.54	43.900±3.74

CV: Culture vessel

under light intensity of 5000 Lux. The least chlorophyll content values of 0.578 mg g^{-1} FW was obtained from leaves of plants cultured under a photon flux of 6000 Lux in plastic culture bottles (Table 3).

DISCUSSION

Controlled temperature, lighting, relative humidity and shelving are important in tissue culture. The type of culture vessel and light intensity affected the growth of plants *in vitro*. The plant materials that were cultured in different culture vessels and under different light intensities exhibited differences in their growth and leaf chlorophyll content. Shoot fresh weight, plant height, number of shoots and the other parameters measured increased with light intensity from 4000-5000 Lux and there after showed a decrease in values with the exception of plant height. Similarly, plantlets cultured in conical flask performed better at all the light intensities. The conical flask had larger volumes than the glass and plastic bottles and this could have contributed to the better growth performance of the plantlets. Many researchers like Kacar *et al*. (2010) and Amoo *et al*. (2009) have all reported the positive effect of larger culture vessels on the growth of Pontederia plants *in vitro*. Similarly, Swamy *et al*. (2010) and Amoo *et al*. (2009) reported that *Wrightia tomentosa* plants had better growth when cultured in larger culture vessels. Amoo *et al*. (2009) recorded increase in leaf area for all plant species tested when the volume of vessel used was increased. On the contrary however, Kane and Philman (1992) cultured pontederia plants in different culture vessels and reported that vessel size and type had no effect on the growth of the plants. Under *in vitro* conditions, there is limited gaseous exchange between the cultures and the external environment and this creates conditions in the culture bottles that can have some effect on the growth of the plants. Larger vessels allow for better gas exchange between the cultures and the external atmosphere and this reduces hyperhydricity and humidity, leading to healthy plant growth. This assertion has also been supported by Amoo *et al*. (2009), Munoz *et al*. (2009) and Preeti *et al*. (2009).

The plastic bottles were not very transparent as the conical flask and glass bottles and this could have reduced the amount of light reaching the plantlet. It is therefore possible that the amount of light that was available to the tissues were lower than the actual photon flux. This is also reported by Huang and Chen (2005), who observed that materials of glass have good penetration ability for irradiance of different wavelength.

Light is an important growth factor for plants because of its influence on photosynthetic activity. The observation from this work was that plantlets grew better up to 5000 Lux and at higher intensities of 6000 Lux, growth declined. This observation has also been made by other researchers. Navarro *et al*. (1994), Laforge *et al*. (1991) and Kitaya *et al*. (1995), who have all reported on growth of *in vitro* plants under different light intensities and observed that growth responded positively to light intensity and then declined as the intensity increased. Though, Gupta *et al*. (2000) on the contrary reported of better growth and enhanced shoot production in

cotton plants *in vitro* under low light intensities. Jackson *et al.* (1987) and Miller and Murashige (1976) have also reported of high biomass production of Dracaena plants *in vitro* under 10,000 Lux which is twice as much as the 5000 Lux optimum reported in this work. High light intensity results in increase in total number of leaves, dry matter, sugar content and nitrogen absorbed in plants while excess light causes stunting of stem and leaf (Soontornchainaksaeng *et al.,* 2001). This gives an indication that response of plants *in vitro* to light is dependent on the type of plant. Light helps in the accumulation of dry matter as well as enhance photosynthesis and also the production of chlorophyll but at very high intensities, these processes level off or begin to decline and this probably explains why the chlorophyll content of leaves under 6000 Lux was lower than those under 5000 Lux, consequently adversely affecting the growth of plants at high light intensity. This may be due to excessive light for the optimum growth regulation and photosynthetic activity. High light intensity has more violet and ultra violet radiation that can cause the production of excess phenolic compounds in plants like the banana and this phenolic compounds are known to change the quality of the culture medium as well as hinder the absorption of nutrients from the medium. Plant growth is related to the function of growth hormones which is sensitive to high light (Soontornchainaksaeng *et al*., 2001).

Though light is a substrate for photosynthetic energy conversion it can also harm plants. Higher light intensities cause photo oxidation which involves the destruction of chlorophyll, resulting in less biomass production (Gupta *et al.,* 2000). High light intensity is damaging to the water-splitting photosystem II leading to degradation of the reaction center and the frequency of the damage is high when light intensity is increased beyond a threshold optimal for a particular plant (Soontornchainaksaeng *et al*., 2001). This could have been a factor which affected the growth of the banana plant that were cultured under light intensity of 6000 Lux.

CONCLUSION

Growth of banana plantlets *in vitro* responded to vessel type and light intensities. Conical flask and glass type vessels gave better growth at all light intensities. Growth of plants was better at under 5000 Lux in all the culture vessels. For the multiplication of banana plants *in vitro,* 5000 Lux light intensity and a glass type culture vessel gives better growth performance. The results of this study therefore, throws more light on the performance of the local Cavendish banana "Kwadu Pa", *in vitro* which will improve upon the *in vitro* culture protocol for increased plantlet production.

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