Minimizing Contamination and Phenolization in the Establishment *in vitro* of Dwarf Cavendish Banana (*Musa* spp.)

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Submitted by

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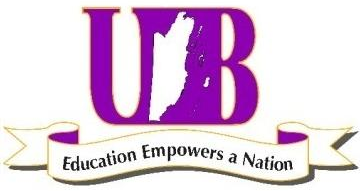

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Abstract

Micropropagation is described as the practice of growing plants like banana from meristematic tissue or somatic cells of superior plants *in vitro*. The use of field banana plants as a direct source of explant material to produce clean *in vitro* plantlets presents two major problems, contamination and phenolization. The present study was done on shoot tip culture to minimize the contamination and phenolization in Dwarf Cavendish Banana (*Musa* spp.*)* by determining the optimum time, sterilizing agent, and explant size for the cultures. The study was done at the University of Belize during the period March to May, 2015. Banana explants were micropropagated through shoot tip culture; a form of micropropagation that assures high quality planting material on a regular basis. This is important for agrarian societies as well as world food security since banana serves as a major source of food and income. The Belize banana industry employs about 2% of the labor force and contributes approximately USD 30 million to foreign exchange. The effectiveness of two different sterilization agents, 70% ethanol and 3% sodium hypochlorite were tested in relation to sterilization time and size of explants. The effectiveness of 5 different methods for limiting phenolization was tested; activated charcoal, coconut water, ascorbic acid, physical state of the culture media and incubation in total darkness. From the experiments it was concluded that 3% sodium hypochlorite at 30 minutes on larger explants is the most effective sterilizing agent for banana explants. In regards to phenolization, none of the techniques proved effective.

Acknowledgements

Firstly, I recognize and give thanks to God for giving me the strength and determination needed to see this project through. I extend heartfelt gratitude to my family and friends who provided not only moral support but ensured that I had all the materials needed to complete this project. Thanks to the people at Farm 14 and Farm 4 who willingly donated plant material. Last but not least, special thanks to Dr. Dion Daniels for the concept applied to this research and the guidance and the support he willingly provided. Without his idea, knowledge and vast experience this project would not have been such a success.

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Introduction

Agriculture and food security are among the chief issues of international apprehension. Universal governments and heads of states are concerned with sustainable agriculture, horticulture and aquaculture practices that can provide enough protein, fats and carbohydrates for the local, regional, national and global food and nutrition insecurity (Nagarajaia & Prakash, 2006). Bananas, belonging to the genus Musa and family Musaceae is known as one of the most important source of tropical fruits in the world market as it is a significant staple food as well a major export commodity (Rahman et al., 2013). Bananas and plantains are the fourth most important crop in developing countries, with a worldwide production of about 100 million tons. Bananas provide a starch staple across some of the poorest parts of the world including Africa (with consumption up to 400 kg per person per year) and Asia (FAOSTAT, 2007).

Banana is of great nutritional value. It has a rare combination of energy value, tissue-building elements, protein, vitamins and minerals. It is a good source of calories since it is rich in solids and low in water content as compared to any other fresh fruit. Bananas are a good source of vitamin C which helps to rebuild the immune system. Bananas are also relatively easy to digest as compared to other foods and so they are invaluable to those with compromised immune systems. Vitamin C also increases the absorption of iron and increases the formation of blood, these two health benefits of bananas make it ideally suited for those with anemia or blood related problems. Bananas can also be included in a diet for high blood pressure as they contain potassium which helps to reduce and control high blood pressure. Furthermore, bananas do not contain even trace amounts of fat, cholesterol, or sodium which makes it a healthy food option even for restrictive diet plans (Sampath et al., 2012).

Apart from its nutritional benefit, the banana industry is important to Belize's economy through its contributions to GDP, foreign exchange earnings, direct injection of money into the local economy of southern Belize through wages, and by creation of spin-off enterprises for a multiplier effect. Its contribution to GDP rose from USD17.5 million in 1990 to USD41.6 million in 2008. The industry employs about 2% of the labor force and contributes approximately USD30 million to foreign exchange. It is concentrated in the southern part of the Stann Creek and the northern part of the Toledo Districts, the so-called "Banana Belt".

Due to its importance as both a food crop and a commodity for export much effort has been placed in developing the most effective cultivation methods for banana crop. Mass

production of a crop that does not reproduce by means of seed permits only vegetative propagation methods. Previously this would entail using only banana suckers. However, with advance in technology and science microprogation has become a popular form of propagation. This technique gives growers advantages such as clones and production of disease-free planting material. The technology also rejuvenates the plants resulting in more vigorous growth, higher yields, better quality fruits, earlier fruiting and more uniform crop than those produced by conventional means. These plants are relatively inexpensive to ship and easier to be certified by plant inspectors since they are packaged in sterile containers (Kahangi, 2008).

The variety of banana that is predominantly produced in Belize is the Cavendish variety. The bananas belonging to the Cavendish subgroup are the most cultivated around the world and they represent an important source of jobs and income for the exporting countries, mainly in Latin America and the Caribbean, as well as in Africa and Asia. In 2005 this crop occupied about 34,000 ha in Venezuela, yielding 15.3 ton ha which meant an overall production of 520,000 ton and the worldwide 23rd position, as it represented 4.9% of the total (Villalobos & Garcia, 2008).The objective of this paper is to evaluate the effectiveness of two different sterilizing agents, 70% ethanol and 3% sodium hypochlorite, on different size of explants at varying time intervals and to minimize phenolization of explants *in vitro* of Dwarf Cavendish Banana *(Musa* spp.*).*

Literature Review

Classification

Kingdom: Plantae
Division: Magnoliophyta
Class: Liliopsida
Order: Zingiberales
Family: Musaceae
Genus: *Musa*
Species: *M. spp.*

As herbaceous plants bananas are thought to be one of the oldest cultivated plants. They are described as monocotyledonous plants which are sterile and parthenocarpic so the fruit develops without seed. Bananas trace their roots back to the jungles of Malaysia, Indonesia, the Philippines, and northern Australia. They have been in cultivation since the time of recorded history and are mentioned in ancient Hindu, Chinese, Greek, and Roman texts. The first Europeans to refer to bananas were the armies of Alexander the Great during their conquest of India in 327 B.C. (Gulick, 2006).

Early migrants from Southeast Asia carried the roots of banana plants with them to the Middle East and Africa. From there, Portuguese traders carried the plants to the Canary Islands off the northwest coast of Africa, where bananas are grown commercially today. According to Spanish history, Friar Tomas de Berlanga sailed to the Caribbean in 1516 and introduced bananas to the New World. It wasn't until the 1876 Philadelphia Centennial Exhibition that the American public received its first taste of banana (Gulick, 2006).

The cultivated hybrids and species are mostly triploid and most have been propagated from mutants found in the wild (Harrison & Schwarzacher, 2007). All the above ground parts of a banana plant grow from a structure commonly referred to as a corm. Plants are usually tall and fairly sturdy, and are often mistaken for trees, but what appears to be a trunk is actually a psuedostem. The leaves of banana plants are composed of a petiole and a lamina. The base of the petiole broadens to form a sheath; the tightly packed sheaths make up the pseudostem, which is

all that supports the above ground part of the plant. The edges of the sheath meet when it is first produced, making it tubular. As new growth occurs in the centre of the pseudostem the edges are forced apart (Stover & Simmonds, 1987). Cultivated banana plants vary in height depending on the variety and growing conditions. Most are around 5 m (16 ft) tall, with a range from 'Dwarf Cavendish' plants at around 3 m (10 ft) to 'Gros Michel' at 7 m (23 ft) or more (Stover & Simmonds, 1987).

One major problem with banana propagation is that most of the horticultural and forest crops are infected by systemic disease caused by fungi, viruses, bacteria, mycoplasma and nematode. While plants infected with bacteria and fungi may respond to treatments with bactericidal and fungicidal compounds, there is no commercially available treatment to cure virus infected plants. It is possible to produce disease free plants through tissue culture (Agricultural Information, 1914). Tissue culture means cloning and micropropagation of tissues of the selected elite plants and daughter suckers. Shoot tip tissue culture is the method of choice to eliminate viruses and other pathogens from many plant species. The excised shoot tip and meristem can be cultured aseptically on agar solidified simple nutrient medium or on paper bridges submerged into liquid medium, both methods are used in this experiment. Under appropriate conditions the explants will grow out directly into a small leafy shoot or multiple shoots (Agricultural Information, 1914).

The shoot tip culture technique has the advantage of regenerating a single plant from a single, small shoot. The combination of low hormone levels combined with a minimum time in culture reduces the chance of mutation and regeneration of an off type plant. At the same time, many pathogens, including viruses, are eliminated by this technique (Goswami & Handique, 2013). The use of meristematic tissue is important as this is one of the few parts of the plant where viruses do not move readily as there is no vascular system in this region. A high metabolite activity in the actively dividing meristematic cells does not allow virus replication and a high endogenous auxin level in shoot apices may inhibit virus multiplication. Meristem tip cultures have also enabled plants to be freed from other pathogens including viroids, mycoplasmas, bacteria and fungi. Therefore, main objective of shoot-tip and meristem tip culture is the production of disease free plants through micro propagation (Agricultural Information, 1914). The process consists of five important steps: Initiation, Multiplication, Shooting &

rooting, Primary Hardening in green houses and Secondary Hardening in shade houses. Strict adherence to aseptic standards and micro-climatic conditions and care during the hardening process alone can ensure success.

Microbial contaminations are the major hurdle to the initiation and maintenance of viable *in vitro* cultures. Explant contamination occurs due to several plant and environmental related factors such as plant species, age of the plant, explant source and prevailing weather condition. Despite the best timing and selection efforts it is difficult to eliminate contamination from *in vitro* grown plants. Losses due to contamination in *in vitro* condition average between 3 and 15% at every subculture in the majority of commercial and scientific plant tissue culture laboratories (Goswami & Handique, 2013). The majority of which is caused by fungal, yeast and bacterial contaminants. Sterilization is the process of making explants contamination free before in vitro establishment of cultures. Various sterilization agents are used to decontaminate the tissues. These sterilants are also toxic to the plant tissues, hence proper concentration of sterilants, duration of exposing the explants to the various sterilants and the sequences of using these sterilants have to be standardized to minimize the injury to the explants for achieving better survival rate (Goswami & Handique, 2013).

During sterilization, the living materials should not lose their biological activity and only contaminants should be eliminated; therefore explants are surface sterilized only by treatment with disinfectant solution at suitable concentrations for a specified period. The disinfectants widely used are sodium hypochlorite (which dates back to the mid 18th century), calcium hypochlorite, ethanol (or isopropyl alchohol), mercuric chloride, hydrogen peroxide, silver nitrate and bromine water (Oyebanji, et al., 2009). Hypochlorite is known to be a very effective killer of bacteria, even micromolar concentrations are enough to reduce bacterial populations significantly. However, little is known about the exact mechanisms of its bacteriocidal activity. When diluted in water the hypochlorite salts (NaOCl, $Ca(OCl)_2$) lead to the formation of HoCl whose concentration is correlated with bactericidal activity (Oyebanji, et al., 2009). Sodium hypochlorite, usually purchased as laundry bleach is the most frequent choice for surface sterilization *in vitro* because it is readily available and can be diluted to proper concentrations.

A balance between concentration and time must be determined empirically for each type of explant because of phytotoxicity. Calcium hypochlorite is used mostly in Europe and the concentration generally used is 3.25%, it may be less injurious to plant tissues than sodium hypochlorite (Oyebanji, et al., 2009). Ethanol is a powerful sterilizing agent but also extremely phyto-toxic. Therefore, the explant is typically exposed to it for only a few seconds or minutes. Explants such as seeds or dormant buds can be treated for longer periods of time since the tissue that will develop is actually within the structure that is being surface sterilized. To enhance effectiveness in sterilization procedure, a surfactant like Tween 20 is frequently added to the sterilizing solution (and in some laboratories a mild vacuum is applied during the procedure); in general, the sterilizing solutions containing the explants are continuously stirred during the sterilization period (Oyebanji, et al., 2009).

Apart from contamination, another major problem in banana tissue culture is phenolization. Described as the blackening and browning of explants shortly after isolation, it is caused by the release of phenolic compounds into the media where they are immediately oxidized by peroxidases or polyohenoloxidases and form quinines (highly reactive compounds that finally polymerize to melanins) (Fatemh et al., 2008) which are highly reactive and toxic to the tissue as it prevents it from absorbing nutrients. Phenolics are compounds containing one or more aromatic rings with one or more hydroxyl group. More than 8,000 phenolic structures are known, ranging from the simple molecule phenolic acid to much more complex substances like tannin. Banana plants cause activation of oxidative enzyme when explants are cut (El-Shafey et al., 1999). Work done on banana browning indicates that dopamine, one of the catecholamines, plays a major role in the browning of banana leading to inactivation of the growth of banana tissues in culture (Fatemh et al., 2008). Different attempts have been made to alleviate the problem of phenolization in banana. These manipulations of cultures depend on the variety of banana, the explants type and the size of the explants. Pretreatment of explants with antioxidants and incorporation of antioxidants in culture medium is one of the most common techniques used. Ascorbic acid is also an antioxidant used to control oxidation of phenols (Ngomuo et al., 2014). It is able to scavenge oxygen radicals produced when plant tissue is wounded, therefore protecting the cells from the damage resulting from the injury. The detoxification of the free radicals by ascorbic acid produced through oxidation of the phenolic compounds reduces the

extent of browning. Activated charcoal's use in micro propagation was first reported by Lu *et al*. (1990) and it was suggested that charcoal was responsible for adsorption and desorption which controlled the release of nutrients in the production of synthetic seeds. Activated charcoal has a very fine network of pores with large inner surface area on which many substances can be adsorbed (Ngomuo et al., 2014). Other methods include; incubation of cultures in the dark which reduces the production of secondary metabolites and frequent subcultures to fresh medium decreasing the accumulation of metabolites in the media.

Material and Methods

This research was conducted at the University of Belize's Micropropagation Laboratory at Central Campus in Belmopan City under the Faculty of Science and Technology. The research was carried out from March to May 2015.

Plant Material

Banana sword suckers of Cavendish variety, genus Musa, were obtained from the banana field of Farm 14 Farm 4 in Southern Belize. Suckers ranging between 1-3 feet were dug up and excised from the mother plants. They were washed with tap water and cut down to an approximate size of 6 inch in length and 4 inches in diameter with a machete. Explants were then transported in plastic bags to the lab where experiments were done.

Figure 1. Dwarf Cavendish Banana (*Musa* spp.) sword sucker

Culture medium

Murashige and Skoog (1962) salts was used at full strength. It was supplemented with 2mg/L 6-Benzylaminopurine (6-BAP), 0.11 mg/L Indole-3-Acetic Acid (IAA), 3 % sucrose and 50 mg/L ascorbic acid. The pH of the culture medium was adjusted to 5.8 using Sodium Hydroxide and/or Hydrochloric acid.

Filter paper was cut, formed into an M shape and placed to the bottom of test tubes with an approximate capacity of 1.5cm x 15cm. 20 ml culture media was then poured into the test tube such that the wedge of the M was submerged approximately 5mm. Test tubes were then covered and placed in the basket of a vertical autoclave. Culture media were autoclaved at a temperature

of 121^0C and a pressure of 1.2 kg/cm^3 for 15 minutes. Test tubes were taken out and placed in a rack to cool.

For the 3rd experiment, culture media of Murashige and Skoog (1962) salts at full strength was used. It was supplemented with 2mg/L 6-Benzylaminopurine (6-BAP), 0.11 mg/L Indole-3-Acetic Acid (IAA) and 3 % sucrose. This basic formula was modified to make 4 distinct culture medium; 1.) 18% coconut water

2.) 50 mg/L activated charcoal

3.) 7 g/L agar

4.) 50 mg/L ascorbic acid which was used in the total darkness treatment

Instruments

Scalpels, forceps and tiles were sterilized in a vertical autoclave for 25 minutes at 121^0C and 1.2 kg/cm^2. Scalpels were submerged in 70% ethanol and were flamed before use.

Experiment 1

Sterilizing agent ethanol 70% and sodium hypochlorite 3% were tested to ascertain the most effective in establishment of banana shoot tip *in vitro*. The laminar floor chamber was turned on and the inside was wiped down using 70% ethanol. Explants were cut done to approximately 2 cm^2. On the table of the laminar floor chamber explants which consisted of the shoot tip and microcorm were divided into groups of five and taken to the inoculation room. Five explants were placed in a jar of previously prepared 70% ethanol for 30 seconds and another 5 in another jar for 60 seconds. After the time was up explants were rinsed 3 times in distilled sterilized water. They were then further cut down to 1.5cm^2. Once explants were cut to approximate sizes they were inoculated in liquid establishment culture media in individual test tubes. Test tube lips were flamed, covered, sealed and labeled. Five explants were placed in a jar of previously prepared 3% sodium hypochlorite for 15 minutes and another 5 were placed in another jar of 3% sodium hypochlorite for 30 minutes. A drop of dishwashing liquid was added to each jar. Jars were shaken for the duration of the sterilization period. When the time was up explants were rinsed 3 times with distilled sterilized water and cut down and placed in test tubes in the same

manner as the ones in ethanol were. The entire procedure was aseptic (Hands washed with ethanol for every two explants, forceps and scalpels flamed and put in ethanol for each explant).

These cultures were then incubated in the growth room at a temperature of 27+/-2 ^{0}C at a photoperiod of 12 hours light. Contaminants were evaluated 10 days after inoculation and the effects of the sterilizing agents at different duration were identified.

Experiment 2

The objective of this experiment was to determine if there is significant deference between the size of the explants and the level of contamination. Explants were obtained from the field and cut down to approximately 1.5cm^2. The laminar floor chamber was turned on and the interior was wiped down with 70% ethanol. Explants were then taken into the inoculation room where they were sterilized with 3% sodium hypochlorite for 30 minutes. A drop of dish washing liquid was added to the jar which was then shaken for the duration of sterilization time. Explants were then rinsed 3 times with distilled sterilized water. Explants were then cut down to 1.0cm^2. Once explants were cut to approximate sizes they were put in previously prepared ascorbic acid solution for 5 seconds before they were inoculated in liquid establishment culture media in individual test tubes. Test tube lips were flamed and covered. The entire procedure was aseptic (Hands washed with ethanol for every two explants, forceps and scalpels flamed and put in ethanol for each explant).

These cultures were then incubated in the growth room at a temperature of 27+/-2 ^{0}C at a photoperiod of 12 hours light. Contaminants were evaluated 10 days after inoculation and the effects of the sterilizing agents at different duration were identified.

Experiment 3

The objective of this experiment was to minimize phenolization in Cavendish Banana *in vitro*. The laminar floor chamber was turned on and the inside was wiped down using 70% ethanol. Explants were cut down to 2cm. Explants were then taken into the inoculation room where they were sterilized with 3% sodium hypochlorite for 30 minutes. A drop of dish washing liquid was added to the jar which was then shaken for the duration of sterilization time. Explants were then rinsed 3 times with distilled sterilized water and further cut down to 1.5cm^2. Groups of 5 were

established in previously prepared media; 50mg/L activated charcoal, 18% coconut water, semi-solid media and 50mg/L ascorbic acid.

The first 3 cultures were then incubated in the growth room at a temperature of 27+/-2 ^{0}C at a photoperiod of 12 hours light. The culture in ascorbic acid was incubated in the dark. Contaminants and phenolization were evaluated 10 days after inoculation.

Results and Discussion

Experiment 1

The purpose of this experiment was to determine the best treatment combination for surface sterilization of banana explants with sterilizing agent 70% ethanol and 3% sodium hypochlorite at variable sterilization time. As can be seen in Figure 2, the most effective surface sterilant was 3% sodium hypochlorite. Unlike 70% ethanol where there was total loss of sample population due to contamination, 3% sodium hypochlorite had survival at both of its treatment combination after 10 days. The most effective of the two treatment combination with 3% sodium hypochlorite was the 30 minutes treatment. This treatment had no loss of explants material due to contamination. Majority of the contamination observed was fungal in nature.

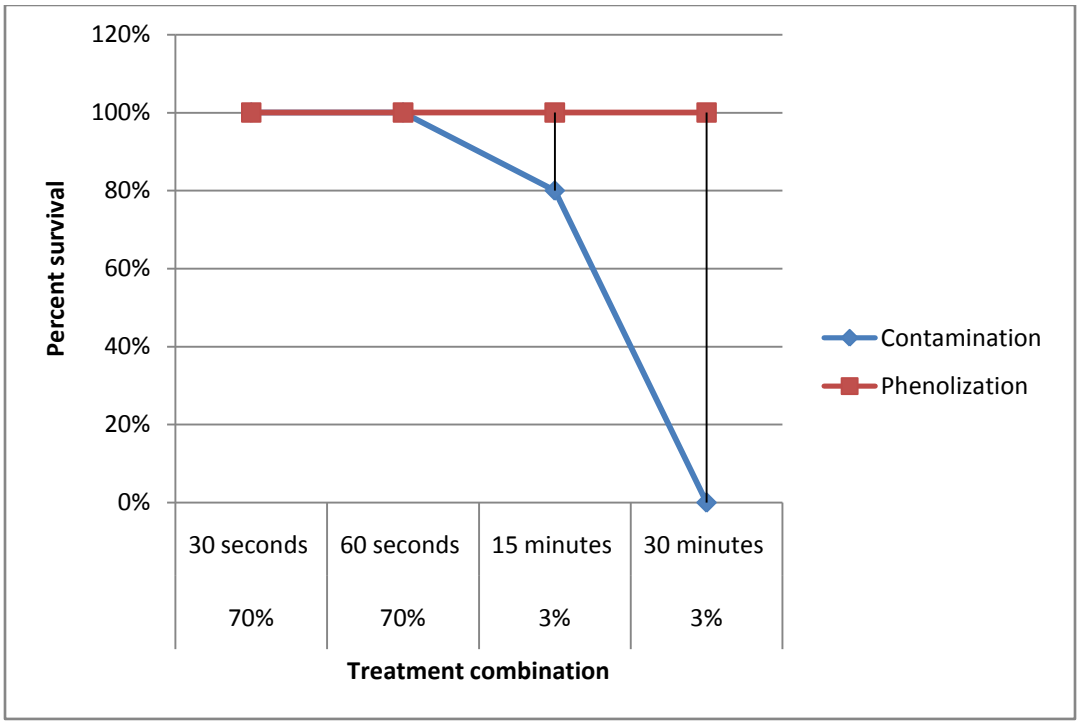

Figure 2. Results of surface sterilizing treatment *in vitro* of banana explants with sterilizing agents 70% ethanol and 3% sodium hypochlorite at different sterilization time period

70% Ethanol may not have been as effective a surface sterilizer because the explants were immersed in it for only a short period of time (30-60 seconds). It is a powerful sterilizing agent but also extremely phytotoxic. Therefore, plant material was not exposed for long period

so as to prevent damage to the tender tissue of the explants. Using 70% ethanol prior to treatment with other compounds may have produced less contamination in explants, as according to Ahmed et al. (2014) considerable reduction in contamination of explant was obtained by treating the explants with sodium hypochlorite 5% for 10 minutes after rapid rinsing in 70% ethanol for 30's. In the same study Ahmed et al. (2014) concluded that sterilization of explants with 5% sodium hypochlorite alone was ineffective resulting in very high contamination.

In regards to phenolization, all samples in the 4 treatment combinations became phenolized. The 2 treatments with 70% ethanol started phenolization during the surface sterilization period when all cut surfaces appeared to oxidize phenols. The treatments with 3% sodium hypochlorite became phenolized during the incubation period. On day 10 phenols were observed in the culture medium of all the explants.

Experiment 2

The present study was conducted to evaluate the response of different explant sizes on *in vitro* propagation of *Musa* spp. Two different explants sizes were used for the study. As is seen in Figure 3 explant size of 1.5cm^2 showed the best result. The use of smaller explants resulted in survival of fewer explants. This may be attributed to smaller explants having tissue damage upon excision and treatment with sterilizers during the process of sterilization of the explants (Goswami & Handique, 2013). Source of explant material and the time the explants were collected are also factors that must be assessed in regards to why larger explants had higher survival rate. The explant in this experiment was obtained four weeks after the first experiment was conducted. These explants were obtained from a different area on the farm and as such may have had more spore fungal contamination than the other explants.

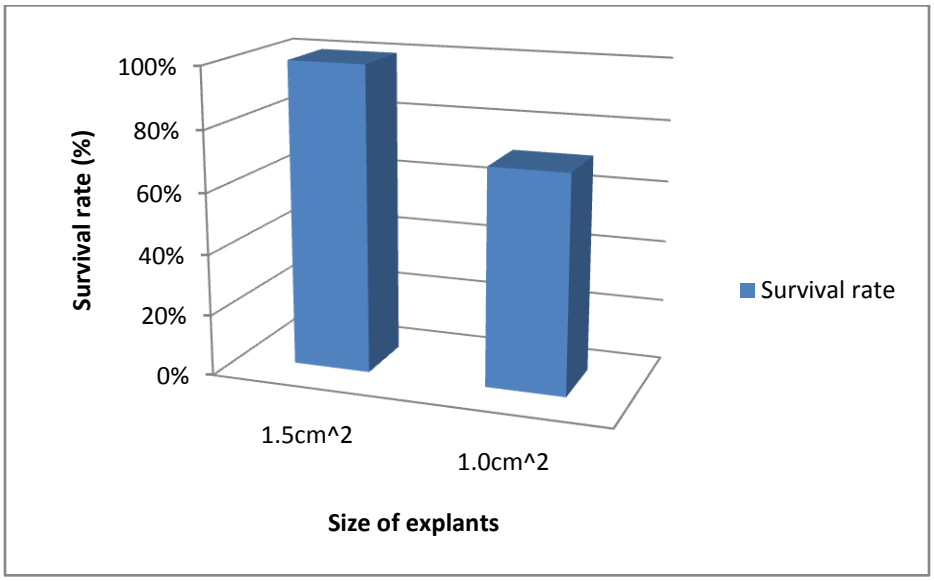

Figure 3. Survival rate of explants at two different size; 1.5cm^2 and 1.0cm^2

In this experiment, larger explants showed higher and more rapid percentage of shoot proliferation than smaller explants. Larger explants consisting of apical dome with 6-8 overlapping leaf bases, developed into multiple shoots more readily because they contained more lateral buds. According to (Vuylsteke, 1998), initiating cultures from such large explants increases explant and medium blackening therefore reducing their survival rate.

Experiment 3

Phenols cause very high explant mortality and have a negative effect on regeneration of explants. The explants in experiment 1 and 2 that were not contaminated with fungi grew at a very slow rate or were loss due to phenolization. The initial media for the first two experiments was supplemented with 50 mg/L ascorbic acid in an effort to limit the oxidation of phenols. The treatment was not effective thus the objective of this experiment was to limit phenolization in explants.

The result of the treatment with ascorbic acid, coconut water, activated charcoal and semi-solid media all proved to be ineffective. All treatment samples became phenolized at the same rate (Figure 4). In these treatment phenolization may have been at a lesser degree than in the other two experiments however there was no methodology to measure the rate of phenolization. Other literature reports that treatment combination Ascorbic acid (100mg/L)+ Citric acid for 1 hour before surface sterilization and 10 minutes after sterilization gave the best results with regard to number of days for initial browning of explants and days required for first subculture (Goswami & Handique, 2013).

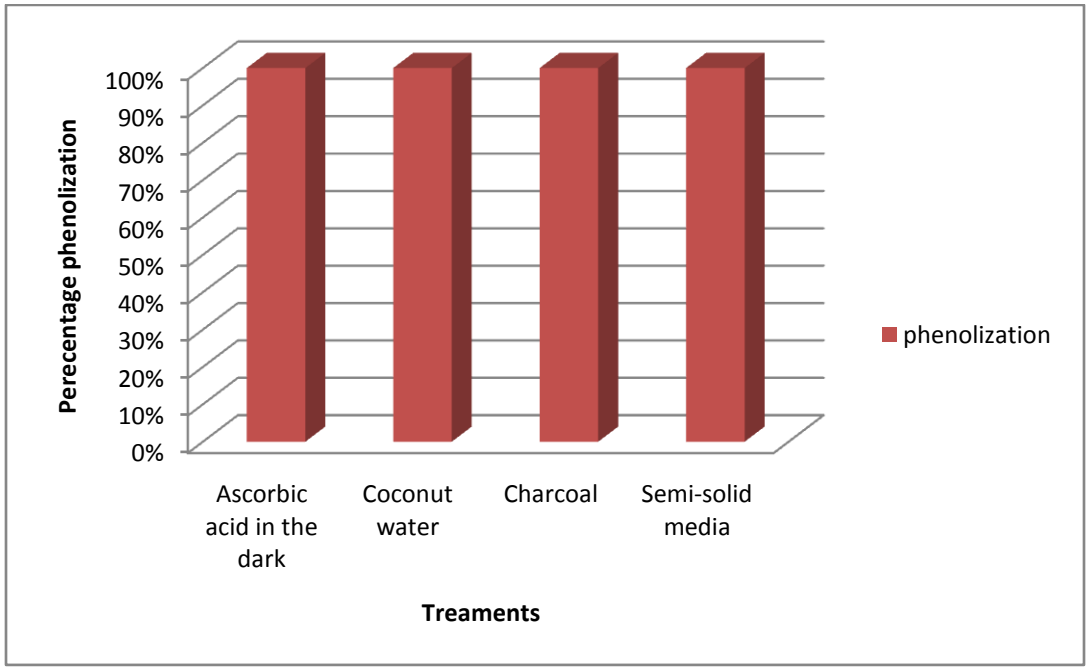

Figure 4. Results showing the effect of different phenolization treatment on Cavendish banana explants *in vitro*

Conclusion and Recommendations

The best treatment combination in regards to sterilizing agent and time is 3% sodium hypochlorite at 30 minutes. In this experiment smaller explants showed more contamination and less proliferation than larger explants. Other literature negates this result and show that the smaller the explant the lower the rate of contamination. None of the phenolization treatment proved more effective than the other.

As banana is an important food and export commodity it is recommended that further research is done on shoot tip culture of banana *in vitro.* A combination treatment of 70% ethanol for 30 seconds and 3% sodium hypochlorite for 30 minutes may prove to be effective on explant material from more than one source. Another experiment can be conducted to determine if immersion in Ascorbic acid (100mg/L) + Citric acid for 10 minutes before and after sterilization treatment would be more effective on phenolization. In that experiment there should be a scale upon which the level of phenolization can be measured.

Bibliography

Agricultural Information. (1914). *Shoot-Tip and Meristem Culture.* Retrieved March 9, 2014, from Agriinfo: http://agriinfo.in/default.aspx?page=topic&superid=3&topicid=1914

El-Shafey, Y. E., Nesiem, M., Habib, M., & Abdel, M. (1999). Browning phenomenon: A seriouse problem in date palm tissue culture. *J. Agric. Sci. Mansoura Univ* , 1.

FAOSTAT. (2007). Retrieved February 4, 2015, from http://faostat.fao.org/

Fatemh, N., Kamahldin, H., Masoumeh, i. B., Ferdows, J., Omidreza, N., & Maedeh, Y. (2008). The Banana Pulp Polyphenol Oxidase is a Tyrosinase. *Journal of Biological Sciences* , 526.

Goswami, N. K., & Handique, P. J. (2013, August). Explants Size Response to in Vitro Propagation of. *Indian Journal of Applied Research* , 40-43.

Goswami, N., & Handique, P. (2013). In Vitro Sterilization Protocol for Micropropagation. *Indian Journal of Applied Research* , 51.

Gulick, A. (2006). *Gold of the Tropics:.* Retrieved March 9, 2015, from Dive Training: http://www.dtmag.com/Stories/What%20About/04-06-whatabout.htm

Harrison, H., & Schwarzacher, T. (2007). Domestication, Genomics and the Future for Banana . *Oxford Journals , 5* (1), 1073-1084.

Kahangi, M. (2008). *The Potential of Tissue Culture Banana (Musa spp.) Technology in.* Retrieved March 9, 2015 , from Jomo Kenyatta University of Agriculture and Technology : http://banana2008.com/cms/details/acta/879_28.pdf

Nagarajaia, S., & Prakash, J. (2006). *Chemical composition and antioxidant potential of peels from three varieties of.* Retrieved Febraury 3, 2015, from Asian Journal of Food and Agro-Industry: http://www.ajofai.info/Abstract/Chemical%20composition%20and%20antioxidant%20potential%20of%20peels%20from%20three%20varieties%20of%20banana.pdf

Ngomuo, M., Mneney, E., & Ndakidemi, P. (2014). The in Vitro Propagation Techniques for Producing Banana Using Shoot Tip Cultures. *American Journal of Plant Sciences* , 1616.

Oyebanji, B., Nweke, O., Odebunmi, O., Galadima, B., Idris, S., Nnodi, N., et al. (2009). Simple, effective and economical explant-surface. *African Journal of Biotechnology* , 5396.

Rahman, S., Biswas, N., Hassan, M., Ahmed, G., Mamun, A., Islam, R., et al. (2013). Micro propagation of banana (Musa sp.) cv. Agnishwar by In vitro shoot tip culture. *International Research Journal of Biotechnology , 4* (2141-5153), 83-88.

Sampath, K., Debjit, B., Duraivel, S., & Umadevi, M. (2012). Traditional and Medicinal Uses of Banana . *Journal of Pharmacognosy and Phytochemistry , 1* (3), 51-63.

Singh, A. K., Wali, V. K., Preeti, K., Ahmed, S., & Sharma, A. (2014). In vitro multiplication of banana (Musa sp.) cv. Grand Naine . *African Journal of Biotechnology* , 2696-2703.

Stover, R., & Simmonds, N. (1987). *Bananas (Tropical Agriculture Series).* Burnt Mill, Harlow: Wiley.

Villalobos, M., & Garcia, E. (2008). *Obtainment of embryogenic cell suspensions from scalps of the banana CIEN-BTA-03 (Musa sp., AAAA) and regeneration of the plants.* Retrieved March 26, 2014, from Electronic Journal of Biotechnology: http://www.ejbiotechnology.info/index.php/ejbiotechnology/article/view/v11n5-3/669#1

Vuylsteke, D. R. (1998). *Shoot-tip culture for the propagation, conservation and distribution of Musa germplasm.* Ibadan, Nigeria : Internation Institute of Tropical Agriculture .