

Surface Sterilization for the Establishment In-Vitro of Cassava (*Manihot esculenta*)

A Thesis Submitted to the University of Belize in Fulfillment of
BIOL 4992 - Independent Research

As Part of Bachelors of Science in Biology

Submitted by Lijia Manzanilla

Under the Supervision of
(Dr. Dion Daniels)



Department of Science
Faculty of Science and Technology
University of Belize

Table of Contents

Abstract -----	1
Acknowledgments -----	1
List of figures -----	2
List of tables -----	2
Introduction -----	3-4
Literature Review -----	5-10
Materials & Methods -----	11-14
Results & Discussion -----	15-17
Conclusion -----	18
Recommendations -----	18
Bibliography -----	19-20
Appendix -----	21-22

Abstract

A study was carried out to investigate the effect of surface sterilization and *in vitro* propagation of Cassava (*Manihot esculenta*). Two different sterilization treatments were used during this process: two treatments; 4% sodium hypochlorite, in which explants were immersed into the solution for four minutes and 70% ethanol was used in which the explants were immersed into the solution for twenty minutes during the first trial. The second trial, one surface sterilizing treatment was used which consisted of using 100ml of 4% Sodium Hypochlorite only, in which the explants were immersed into the solution for 30 minutes. Cassava stem nodes were used as explants for both trials and were cultured on Magar. After several weeks, data determined that a 4% sodium hypochlorite solution with immersion for 30 minutes showed the best results in which only 21% of the explants were contaminated. The 70% ethanol only treatment had the most contamination with 100% of all explants being fully contaminated. Sodium hypochlorite (4%) alone, immersed for 30 minutes will give better results as a surface sterilizing technique.

Acknowledgments

I would like to extend my gratitude to Dr. Dion Daniels for his time, patience and academic guidance during these past couple months in working throughout this research. I would like to thank my colleagues Ms. Juliany Chi in taking some of her quality time in assisting throughout the inoculation process. I would like to thank Ms. Marla Magana in assisting during the culture media preparation.

List of Figures

Figure

- 1 Showing Countries with Top Production of Cassava Worldwide.
- 2 Showing Countries with the Highest Value of Cassava Production Worldwide.
- 3 Showing 100% contamination of explants using 4% sodium hypochlorite immersed for 20 minutes.
- 4 Showing contamination of explants using 4% sodium hypochlorite immersed for 30 minutes.
- 5 Showing results of the types of surface sterilization treatments used.
- 6 Showing effect of 4% sodium hypochlorite surface sterilization on cassava explants at different time durations.
- 7 Showing callus formation 2 weeks after inoculation.

List of Tables

Table

- 1 Showing Top 20 Countries with Top Production of Cassava Worldwide.
- 2 Showing different surface sterilization treatments used along with times in which they were immersed, amount of explants used and contamination percentage.

Introduction

Tissue culture is the *in vitro* aseptic culture of cells, tissues, organs or whole plant under controlled nutritional and environmental conditions (Thorpe T, 2007) often to produce the clones of plants. The resultant clones are true-to type of the selected genotype. The controlled conditions provide the culture an environment conducive for their growth and multiplication, these conditions include proper supply of nutrients, pH medium, adequate temperature and proper gaseous and liquid environment. Plant tissue culture technology is being widely used for large scale plant multiplication. Apart from their use as a tool of research, plant tissue culture techniques have in recent years, become of major industrial importance in the area of plant propagation, disease elimination, plant improvement and production of secondary metabolites. Small pieces of tissue (explants) can be used to produce hundreds and thousands of plants in a continuous process. A single explant can be multiplied into several thousand plants in relatively short time period and space under controlled conditions, irrespective of the season and weather on a year round basis (Akin-Idowu et al., 2009). In addition, plant tissue culture is considered to be the most efficient technology for crop improvement by the production of soma clonal and gameto clonal variants. The micro-propagation technology has a vast potential to produce plants of superior quality, isolation of useful variants in well-adapted high yielding genotypes with better disease resistance and stress tolerance capacities (Brown and Thorpe, 1995).

Cassava is a crop of great importance in the tropics because it is a readily available staple food, ease of cultivation and the ability to be transformed into different forms and stored as food for several years (Nassar et al., 2009). Cassava is a valuable source of calories especially in countries where malnutrition is widely spread, and ranks fourth as a source of energy, after rice,

sugar cane and maize (Scott et al., 2000). It is predominantly grown by subsistence and commercial farmers in the sub-Saharan countries of Africa such as Nigeria, Ghana, Burundi, Democratic Republic of Congo, Cameroon, Congo and host of others (Oyewole, 2002). Cassava is a vegetative propagated crop and its multiplication is generally tedious and slow (Taye, 1998). Inadequate high yielding varieties and susceptibility to diseases were identified as the key challenge to the expansion of cultivation of the crop. These were compounded by the bulkiness and high distribution costs, low multiplication rates and poor storage quality of planting materials (Mahungu et al., 2004). Therefore, any effort geared towards removing or reducing these constraints will be worthwhile. Plant tissue culture carried out under aseptic conditions has important applications in plant biotechnology. However, microbes are a common cause of contamination in tissue culture. Sterilization is one of the reliable means to control the pathogenic effect of microbes. The cost of producing plantlets through this technique can be minimized by optimizing sterilization conditions by increasing the rate of growth, thus reducing the rate of mortality (De Almeida et al., 2007). There is the need therefore, to optimize or modify the use of existing protocols that will be more cost effective and efficient for surface sterilization of explants to establish a healthy culture.

Literature Review

Classification

Kingdom: Plantae

Phylum: Magnoliophyta

Class: Magnoliopsida

Order: Euphorbiales

Family: Euphorbiaceae

Genus: Manihot

Species: *M. esculenta*

Numerous research has been conducted in Cassava cultivation via tissue culture. Cassava is an important crop in the tropics because it is a readily available staple food, ease of cultivation and the ability to be transformed into different forms and stored as food for several years (Nassar et al., 2009). It is a valuable source of calories especially in countries where malnutrition is spread, cassava ranks fourth as a source of energy, after rice, sugar cane and maize (Scott et al., 2000). A study completed by (Ogero et al., 2002) was based on using locally available fertilizers as alternative nutrient sources for cassava micro propagation. Results determined that there was a reduction of 95.5% in nutrient cost meaning that the cost of cassava cultivation does not have to be an expensive process, thus being able to cultivate the crop to feed many.

The cultivation of cassava is affected by many problems including diseases, pests, high cyanide content of the roots, low nutritional quality and commercial value of the starch, the

allopolyploidy nature of the plant, long growth cycle (8-24 month), physiological deterioration of roots after harvest and the low fertility of cassava (Munyikwa et al., 1998).

The importance of cassava has generated intensive efforts in breeding programs to develop better varieties. Genetic modification techniques are now widely active in cassava improvement (Acedo and Labana 2008). A study conducted by (Mapayi et al., 2012), used three cassava genotypes which were examined in sixteen different culture media with different concentration of growth hormones. The responses of the explants were evaluated by nodal length, survival, leaf and root formation, plant height, root and leaf number. Results determined that Medium composition 1 (Murashige and Skoog (MS) were observed to give 100% plantlets survival. Genotype 92/0326 had the highest mean plant height (1.49 cm) and mean root number (0.82). As the best performer, full strength MS is recommended in a semi-solid medium.

Plant tissue culture has emerged as one of the major components of plant biotechnology. It's techniques has been demonstrated in rapid clonal propagation, regeneration and multiplication of genetically manipulated superior clones, production of secondary metabolites and ex-situ conservation of valuable germ plasms (Gupta and Ibaraki, 2006).

In vitro propagation is a common method for vegetative reproduction, it is a technique of biotechnology which uses plant tissue culture and has a number of advantages over traditional methods of plant propagation. One major advantage is that exceptionally large numbers of seedlings can be produced in a very small amount of space (Hopkins, 2007).

Sterilization is one of the reliable means to control the pathogenic effect of microbes. The cost of producing plantlet through this technique can be minimized by optimizing sterilization conditions by increasing the rate of growth, thus reducing the rate of mortality (*De Almeida et al., 2007*). A study conducted by (Neama et al., 2013), was completed by using stem nodes of

cassava, these nodes were treated with different concentrations of Clorox at different times. The concentrations used were 10, 20 and 30% with different times of 5, 10 and 15 minutes. Results determined that the best treatment was 30% Clorox for 15 min, which recorded the lowest contamination percentage. The highest contamination percentage was 97.8% was represented by 10% Clorox for 5 min. it was determined that the contamination percentage was decreased with increasing Clorox concentration and sterilization time.

Contamination by bacteria and fungi make it difficult to establish axenic cultures (Cassells and Doyle, 2005). Since the use of antibiotics and fungicides are not encouraged, development of any effective sterilization protocol which would effectively eliminate fungi and bacteria without damaging the explant is a basic pre-requisite.

Origin:

Cassava, which may be the most widely grown root crop in the world, originated in western and southern Mexico and tropical South America (likely Brazil). Archaeological evidence suggests that it was cultivated in Peru 4,000 years ago, and in Mexico by 2,000 years ago. It was introduced to West Africa in the 16th century, and became a major food crop there and in Asia. Total 2010 global production was 228 million metric tons, harvested from 18.4 million hectares, with Nigeria, Thailand, and Brazil producing the largest amounts. In optimal conditions, cassava may yield up to 68 tons per hectare in a year, but typical yields are 10 tons/hectare. In addition, cassava is often intercropped with maize, vegetables, legumes, cocoa, and coffee (Sadik. 1988).

Uses:

Cassava is grown for its enlarged starch-filled roots, which contains nearly the maximum theoretical concentration of starch on a dry weight basis among food crops. Fresh roots contain about 30% starch and very little protein. Roots are prepared much like potato. They can be peeled and boiled, baked, or fried. It is not recommended to eat cassava uncooked, because of potentially toxic concentrations of cyanogenic glucosides that are reduced to innocuous levels through cooking. In traditional settings of the Americas, roots are grated and the sap is extracted through squeezing or pressing. The cassava is then further dried over a fire to make a meal or fermented and cooked. The meal can then be rehydrated with water or added to soups or stews. In Africa, roots are processed in several different ways. They may be first fermented in water. Then they are either sun-dried for storage or grated and made into a dough that is cooked. Alcoholic beverages can be made from the roots (O'Hair, 1995).

Young tender leaves can be used as a potherb, containing high levels of protein. Prepared in a similar manner as spinach, care should be taken to eliminate toxic compounds during the cooking process. One clone with variegated leaves is planted as an ornamental (O'Hair, 1995).

Production in Belize:

According to the United Nations Food and Agricultural Organization of the United Nations (FAO), Belize ranked 89th in world production of Cassava, producing 442 tons.

Global production:

Global production for 2012, Nigeria ranked number 1 in production of Cassava by producing 54,000,000 metric tons at a value of Int \$5,641,002. Thailand ranked 2nd in production with

29,848,000 metric tons at a value of Int \$ 2,212,526. Indonesia ranked 3rd in production with 24,177,372 metric tons at a value of Int \$ 2,448,829. Figure 1 shows Nigeria as being the highest global producer of Cassava in 2012.

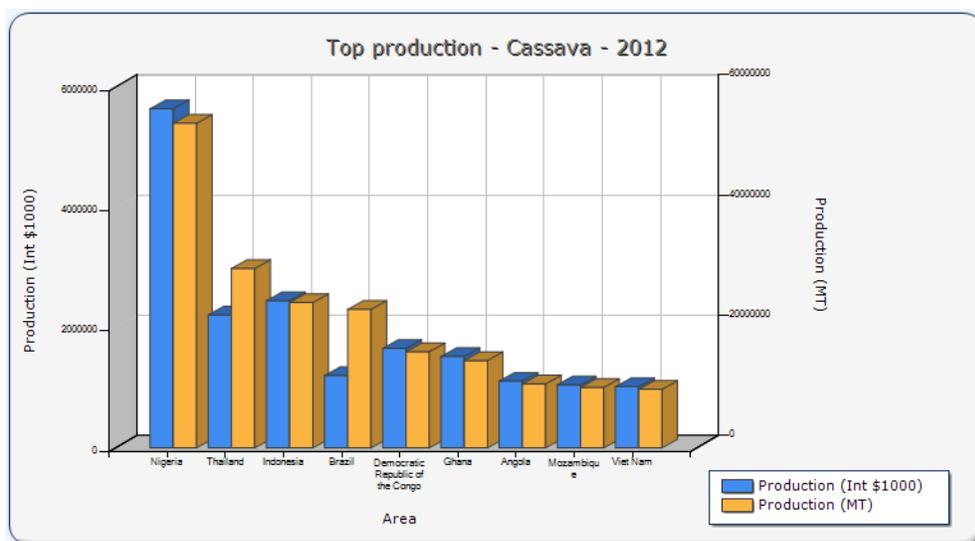


Figure 1: Showing Countries with Top Production of Cassava Worldwide

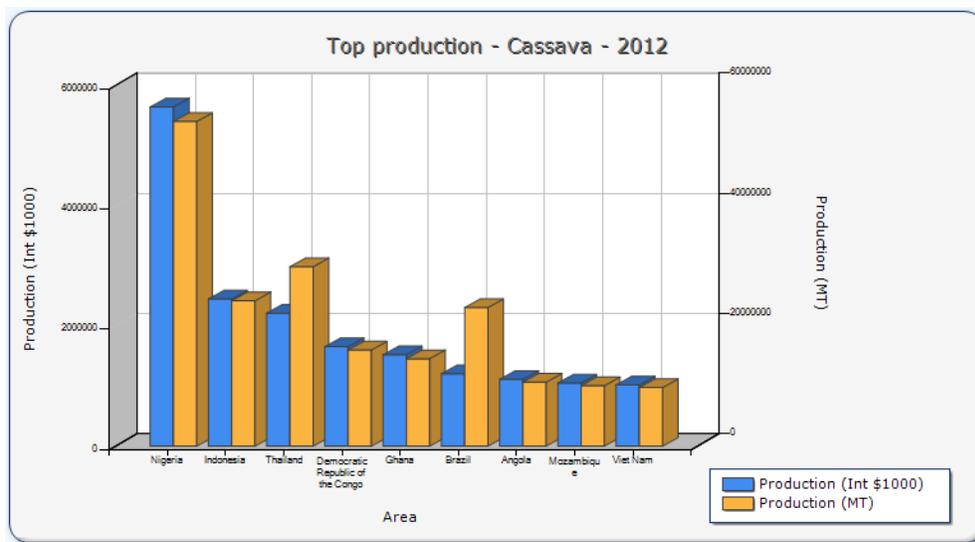


Figure 2 Showing Countries with the Highest Value of Cassava Production Worldwide

Rank	Area	Production (Int \$1000)	Production (MT)
1	Nigeria	5641002	54000000
2	Indonesia	2448829	24177372
3	Thailand	2212526	29848000
4	Democratic Republic of the Congo	1654693	16000000
5	Ghana	1519652	14547279
6	Brazil	1203651	23044557
7	Angola	1111110	10636400
8	Mozambique	1049995	10051364
9	Viet Nam	1018048	9745545
10	India	848239	8746500
11	Cambodia	795349	7613697
12	United Republic of Tanzania	570624	5462454
13	Uganda	514434	4924560
14	Malawi	490161	4692202
15	China, mainland	428716	4560000
16	Cameroon	394870	4287177
17	Sierra Leone	367709	3520000
18	Madagascar	365620	3621309
19	Benin	344287	3295785
20	Rwanda	283765	2716421

Table 1 Showing Top 20 Countries with Top Production of Cassava Worldwide

Material & Methods

The research was carried out at the Micro-propagation Lab at the University of Belize Preschool Compound, Belmopan Campus, during the months of March to May 2015.

Preparation of Explants:

Cassava plants were obtained from the University of Belize, Belmopan Campus. The leaves of the cassava plant were removed leaving only the stem. The stem nodes were cut off of the cassava plant. For the first trial a total of 42 explants were obtained, 21 explants were used for each of the two treatments. Under the laminar flow, for first treatment; 21 explants were rinsed three times with sterile distilled water and immersed in 4% sodium hypochlorite for 20 minutes, stirring constantly until the 20 minutes were up. For the second treatment 41 explants were rinsed 4 times in sterile distilled water and immersed in 70% ethanol for 4 minutes, stirring constantly until the 4 minutes were up.

The second trial consisted of obtaining a total of 14 explants. The explants by rinsing 3 times with sterile distilled water and immersed in 4% sodium hypochlorite for a total of 30 minutes, stirring constantly until the 30 minutes were up.

Instruments:

Forceps and scalpels were wrapped in aluminum foil, cutting tiles were wrapped separately with aluminum foil. All three instruments were placed in the vertical autoclave. They were sterilized at 121°C at a pressure of 1.2 Kg/cm² for a period of 15 minutes.

Culture media:

One hundred ml of distilled water was added to beaker of 1L capacity. Ten ml of Murashige and Skoog Salts stock solutions (MS Salts, 1,2,3,4 and 5) was added to the 1L beaker. The salts were accompanied by adding 0.5mg/L of Thiamine, 50mg/L of Myoinositol, 15g/L of Sucrose,

0.5mg/L of 6-Benzylaminepurine (6-BAP), 0.5mg/L of Naphthalene Acetic Acid (NAA). Sterile Distilled Water was then added to the beaker in order for it to be filled to the final volume mark of 500 ml. The contents were then stirred in order for them to be dissolved and the pH was then adjusted to 5.7. The beaker was then placed on a hot plate, when the temperature reached approximately 50°C, 3.5g/L of agar was added slowly and cooked until the agar dissolved completely. The beaker was then removed from the hotplate and 40ml/L of the culture media was placed into 12 sterile 100 ml glass jars. The 12 glass jars were then placed into the autoclave at a temperature of 121°C at a pressure of 1.2Kg/cm² for a period of 15 minutes. The jars containing the culture media were then removed from the autoclave and stored in the inoculation room.

The second trial consisted of making a total of 300ml/L of culture media. One hundred ml of distilled water was added to a 1L beaker. Six ml of Murashige and Skoog Salts stock solutions (MS Salts, 1,2,3,4 and 5) was added to the 1L beaker. The salts were accompanied by adding 0.3mg/L of Thiamine, 30mg/L of Myoinositol, 6g/L of Sucrose, 0.3mg/L of 6-Benzylaminepurine (6-BAP), 0.02 mg/L of Naphthalene Acetic Acid (NAA). Sterile Distilled Water was then added to the beaker in order for it to be filled to the final volume mark of 300 ml. The contents were then stirred in order for them to be dissolved and the pH was then adjusted to 5.7. The beaker was then placed on a hot plate, when the temperature reached approximately 50°C, 2.3g/L of agar was added slowly and cooked until the agar dissolved completely. The beaker was then removed from the hotplate and 40ml/L of the culture media was placed into 7 sterile test tubes. The 7 test tubes were then placed into the autoclave at a temperature of 121°C

at a pressure of 1.2Kg/cm² for a period of 15 minutes. The test tubes containing the culture media were then removed from the autoclave and stored in the inoculation room.

Treatment preparation:

For the first and second trial 4% of sodium hypochlorite was used as a surface sterilization treatment. Twenty three point eight ml of sterile distilled water was added to large 500ml beaker, 76.5 ml of 5.25% Clorox was added to the beaker for a total volume of 100 ml. A total of 100ml of 70% ethanol was used for the first trial only.

Inoculation:

The laminar flow was cleaned with 70% ethanol before commencing the following procedures. The sterile forceps and scalpels were immersed in 70% ethanol and heated over a Bunsen burner flame before trimming any excess tissue from the explants. The buds from each explant were trimmed on top of sterile tiles as close to the rear of the laminar flow wall in order to avoid contamination. For the first trail the mouth of each glass jar was heated over the Bunsen burner flame and a total of 3 explants were placed into each glass jar containing the culture media. Each explant was placed in an upright position just deep enough to be covered by the culture media but also that buds would stick out. The same procedures were repeated until all 12 jars contained 3 explants. After placing the explants into the jars, the mouth of the jars were then heated once more over the Bunsen burner flame and covered with their respective lid. All 12 jars were labeled and then stored in the inoculation room for 1 week to obtain results.

The second trial; the laminar flow was cleaned with 70% ethanol before commencing the following procedures. The sterile forceps and scalpels were immersed in 70% ethanol and heated

over a Bunsen burner flame before trimming any excess tissue from the explants. The buds from each explant were trimmed on top of sterile tiles as close to the rear of the laminar flow wall in order to avoid contamination. One explant was placed in an upright position into a test tube containing the culture media. The same procedures were repeated until all 14 test tubes contained one explant. After placing the explants into the test tubes, the mouth of the test tubes were then heated over the Bunsen burner flame and covered with their respective lid. All 14 test tubes were labeled and then stored in the inoculation room for 1 week to obtain results.

Results & Discussion

The purpose of this research was to find out which surface sterilization treatment worked best at producing the least amount of contamination for Cassava explants. As seen from figure 3, the first trial determined that the Surface sterilizing treatment (4%) sodium hypochlorite resulted in 100% contamination. The surface sterilizing treatment (70%) ethanol also resulted in 100% contamination.



Figure 3 Showing 100% contamination of explants using 4% sodium hypochlorite immersed for 20 minutes.

Sterilization of explants with 4% sodium hypochlorite at different time intervals of 20 minutes and 30 minutes resulted in different levels of contamination. The percent of contamination was high with those explants that were immersed for 20 minutes. As seen from figure 4 the explants from trial 2 that were immersed in 4% sodium hypochlorite for 30 minutes had the least amount of contamination with 3 test tubes being contaminated out of 14.



Figure 4 showing contamination of explants using 4% sodium hypochlorite immersed for 30 minutes.

Based on the time differences as seen from figure 6, the explants that were immersed for 30 minutes produced little contamination, resulting in 21% contamination as opposed to the explants that were immersed for 20 minutes, with 100% contamination of explants. In general, percentage contamination decreased as the time of exposure to the sodium hypochlorite increased. This suggests that the decontamination process by using surface sterilization 4% sodium hypochlorite, immersed for 30 minutes was effective against reducing contamination.

Sodium hypochlorite is usually purchased as laundry bleach, it is the most frequently used product for surface sterilization. Plant material is usually immersed between 10-20 minutes. It is important to note that surface sterilization is toxic towards plant tissue. The concentration of the sterilizing agent and the duration of the treatment should be chosen in order to minimize tissue death. Based on the results 4% sodium hypochlorite proved to produce minimum contamination but did not in particular eliminate it by 100%. One of the main differences between using test tubes instead of glass jars is the test tubes contained one explant as opposed to the glass jars that

contained three explants. Those explants in the test tube that were contaminated did not have another explant to contaminate compared to the glass jars that had three explants. Once one explant in the glass jar got contaminated it infected the other explants thus 100% contamination occurred.

Seventy percent ethanol proved to be one of the least effective surface sterilization agents known to be effective. Ethanol is known to be powerful sterilizing agent but it is also phytotoxic. It is recommended that plant material be exposed to it for seconds or several minutes the most.

During this research the explants were immerse in 70% ethanol for 4 minutes. The time in which they were immersed may have been too long. The tenderer the plant tissue is the more it can be damaged by alcohol, thus it might have been possible that some of the tissue from the explants may have been damaged during the trimming process.



Figure 7 showing callus formation 2 weeks after inoculation.

Two weeks after inoculation of the second trial, the remaining eleven test tubes that were not contaminated began callus formation on the plant tissue. As seen from figure 7 that callus formation commenced, by week 3 after inoculation callus formation remained the same for the remaining eleven explants. There was no initiation of plant growth after the third week of inoculation for the second trial.

Conclusion:

The best surface sterilizing treatment that can be used to produce cassava plants via in-vitro and contain the least amount of contamination is using 4% sodium hypochlorite with the explants rinsed three times with sterile distilled water and immersed into 4% sodium hypochlorite for a total of 30 minutes. The explants that were surface sterilized with (4%) sodium hypochlorite for 30 minutes resulted in 21% contamination that is 3 out of the 14 test tubes were contaminated. If carried out properly plant tissue culture can make a difference in the production of Cassava in Belize, since Belize does not mass produce Cassava for export, plant tissue culture can be a step forward towards this process.

Recommendations:

If this research was to be carried out again, I would recommend following the same procedures as in trial 2 of surface sterilization treatment. By using 4% sodium hypochlorite with explants immersed for 30 minutes it was an effective method to use thus resulted in less contamination as opposed to 100% contamination. Future research should take into consideration using 5% sodium hypochlorite with explants immersed for 20 minutes and 30 minutes and see if there is any difference between concentration and time duration.

Bibliography:

- Acedo, V.Z. and C. Labana, 2008. Rapid propagation of released Philippines cassava varieties through tissue culture. *J. Root Crops*, 34(2): 108-114.
- Akin-Idowu PE, Ibitoye DO, Ademoyegun OT (2009) Tissue culture as a plant production technique for horticultural crops. *Afr. J. Biotechnol.* 8(16): 3782-3788.
- Brown DCW, Thorpe TA (1995) Crop improvement through tissue culture. *World J. Microbiol& Biotechnol.* 11: 409-415.
- Cassells, A.C. and B.M. Doyle, 2005. Pathogen and Biological Contamination Management: The road Ahead. In: *Plant Cell Culture Protocols*, Loyola. Vargas, V.M. and F. Vasquez.Flota (Eds.). Vol. 318, Humana Press, New York, USA. ISBN: 978-1-58829-547-7, pp; 35-50.
- Ceballos, H., Iglesias, A. C., Perez, J. C., & Dixon, A. (2004). Cassava breeding: opportunities and challenges. *Plant. Mol. Biol.*, 56, 506-516.
- De Almeida, W.A.B., A.P. de Matos and A.S. da Sousa, 2007. Effects of benzylaminopurine (BAP) on in vitro proliferation of pineapple (*Ananas cosmseus* (L) Merr). *Acta Horticut. (ISHS)*, 425:242-245.
- Escobar, R., Hern, A., Larrahondo, N., Ospina, G., Restrepo, J., Mu-Noz, L. Roca, W. (2006). Tissue culture for farmers: Participatory adaptation of low-input cassava propagation in Colombia. *Exper. Agric.*, 42, 103-120. <http://dx.doi.org/10.1017/S001447970500311X>
- Mahungu, N. M. (2004). Contribution of SARRNET (Southern African Root Crops Research Network) to food security in the SADC (Southern Development Community) region. *African Crop Science Journal*, 12(3), 312.
- Munyikwa, T.R.I., K.C.J.M. Raemakers, M. Schreuder, R. Kok, M. Schippers, E. Jacobsen
- Nassar, N. M. A., Junior, O. P., Sousa, M. V., & Ortiz, R. (2009). Improving Carotenoids and Amino-Acids in Cassava. *Recent Patents on Food, Nutrition & Agri.*, 1(1), 32-38.
- O'Hair, S. K. (1995). Cassava. Retrieved from Purdue University.
- Oyewole, O. B. (2002). The Power at the Tubers: Food and Its Microbial Allies. An Inaugural Lecture Delivered At the University of Agriculture Abeokuta, Nigeria. October 09, 2002.
- R.G. F. Visser, 1998. Pinpointing towards improved transformation and regeneration of cassava (*Manihot esculenta* Crantz). *Plant Science*, 135: 87.

- Sadik, S. 1988. Root and tuber crops, plantains and bananas in developing countries: challenges and opportunities. Rome: Food and Agriculture Organization of the United Nations. 83 p
- Scott, G. L., Best, R., Rosegrant, M., & Bokanga, M. (2000). Roots and tubers in the global food system: A vision statement to the year 2020. A co-publication of the International Potato Center, Centro Internacional de Agricultura Tropical, and International Food Policy Research institute, Intl. Institute of Tropical Agriculture and International Plant Genetic Resources Institute. Lima, Peru.
- Taye, B. (1998). Cassava Africa's food security crop. International Institute of Tropical Agriculture (IITA), Ibadan.
- Thorpe T (2007) History of plant tissue culture. *J. Mol. Microbial Biotechnol.* 37: 169-180.

Appendix

Treatment	Trial	Time left immersed	Total explants	Contamination %
70% ethanol	1	4 minutes	21	100
4% sodium hypochlorite	1	20 minutes	21	100
4% sodium hypochlorite	2	30 minutes	14	21

Table 2 showing different surface sterilization treatments used along with times in which they were immersed, amount of explants used and contamination percentage.

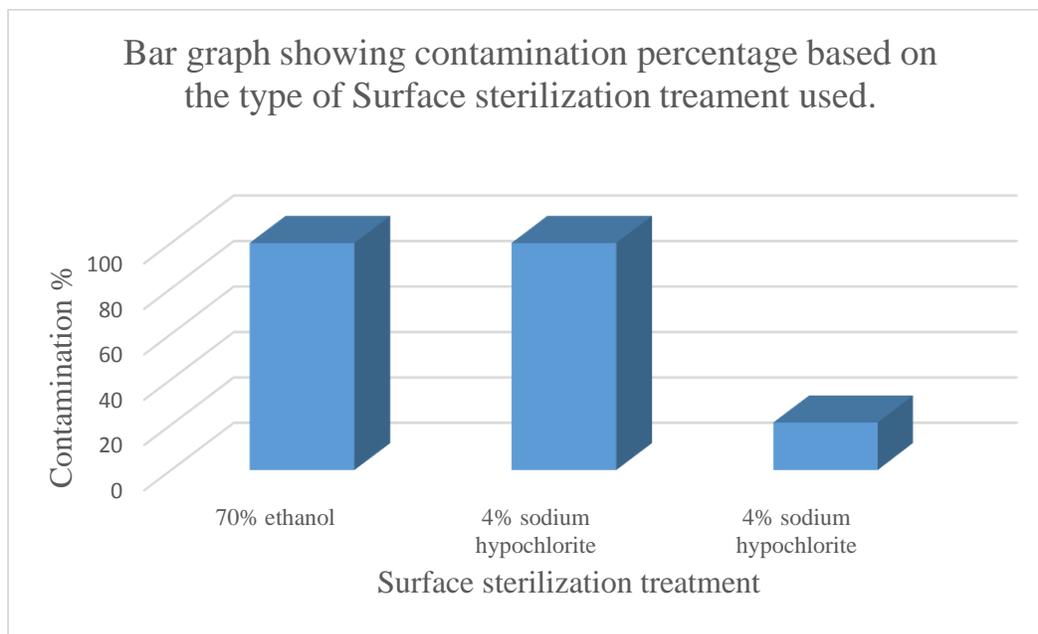


Figure 5 showing results of the types of surface sterilization treatments used.

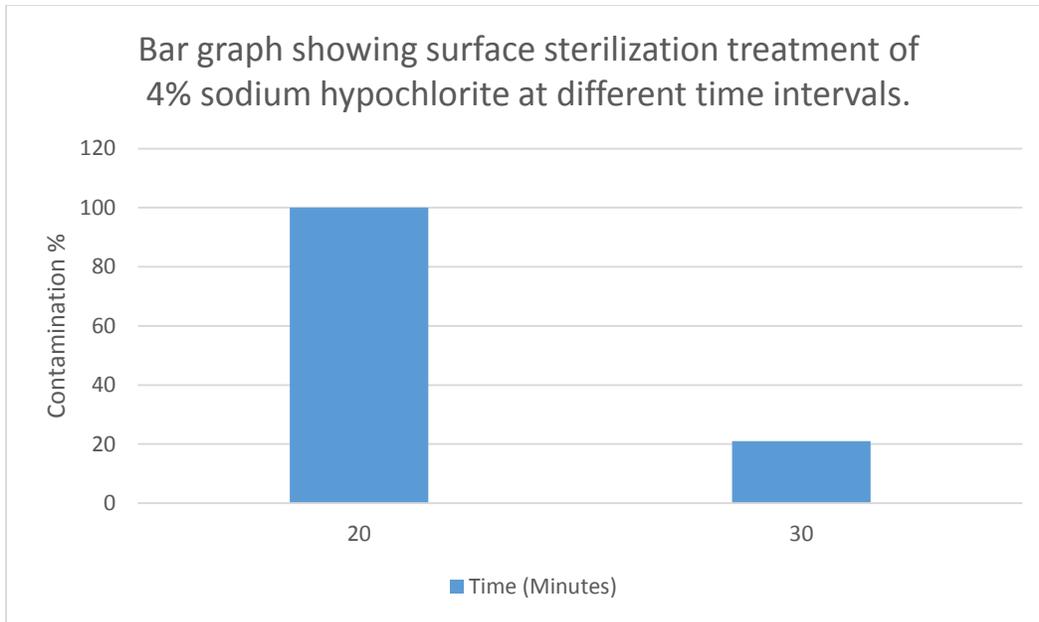


Figure 6 showing effect of 4% sodium hypochlorite surface sterilization on cassava explants at different time durations.