Establishment in vitro of Cocoyams

(*Xanthosoma sagittifolium*)

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: Allen Miguel

Under the Supervision of

Dr. Dion Daniels

Department of Science

Faculty of Science and Technology

University of Belize

Abstract

Establishment of *in vitro* Cocoyam *(Xanthosoma sagittifolium)*

Allen C. Miguel

Biology Program, Department of Science, Faculty of Science and Technology, University of Belize, Belmopan, Belize

Cocoyam *(Xanthosoma sagittifolium)* contribute a significant portion of the carbohydrate content of the diet to Belizeans and many other people in developing countries and provide edible starchy storage corms. Despite cocoyam's nutritional qualities and its importance to the livelihoods of many small farmers, this crop has received little attention in the field of research. The experiment done was conducted to find out what was the effect of different surface sterilization time periods on the cocoyam explants grown *in vitro*. The first step in this research was to grow the coco plants, after the plants were extracted from the soil and transported to the laboratory. The ex-plants were prepared (removal of excess pseudo stem and micro corm was squared) to be sterilized in a sodium hypochlorite solution (4%). After 15 minutes in the surface sterilization solution, half the amount of the explants were removed, reduced in size and inoculated into test tubes containing culture medium. After a further 15 minutes the remaining explants were also removed, reduced and placed into the test tubes containing the same culture medium. These cultures were then incubated in the growth room with artificial light and a temperature of 27^{o} C. Two weeks later the cocoyam plants were observed and evaluated for differences between the two treatments. Preliminary data showed that there were no differences between the two treatments. Based on the findings of this research cocoyam could be produced by *in vitro* methods, which would yield more plants, genetic stability, and better cocoyam tubers.

Acknowledgements

First I would like to thank God for allowing my plants to grow, second I would also like to thank my parents for financial and moral support throughout my research. I would also like to thank Dylan and Deanah for tending my plants while I was at school. Last but not least I would also like to thank Dr. Dion Daniels for his guidance throughout this project and for critiquing and reviewing my abstract.

Table of Contents

Abstract…….. Pg. 2

Acknowledgement……. Pg. 3

Introduction…….Pg. 6

Literature Review……Pg. 8

Materials and Methods……...Pg. 13

Results and Discussion……...Pg. 15

Conclusion and recommendations……..Pg. 19

Bibliography……Pg. 20

Appendix……..Pg. 22

List of Figures

Figure 1: Explants 15 minutes……...Pg. 15

Figure 2: Explants 30 minutes……...Pg. 15

Figure 3: Plant Height……Pg. 16

Figure 4: Contamination Distribution……....Pg. 16

List of Tables

Table 1: Production of Cocoyam in Belize……Pg. 12

Table 2: Height of explants……Pg. 17

Table 3: Number of Leaves on explants……Pg. 17

Table 4: Contamination Distribution……Pg. 17

Introduction

Cocoyam (*Xanthosoma saittifolium*) is a common name for the corms and tubers of several plants in the family Araceae. It is a perennial, tropical plant primarily grown as a root vegetable for its edible starchy corm, and as a leaf vegetable and is considered a staple in the Central American, African, Oceanic and Asian cultures. It is believed to have been one of the earliest cultivated plants. Its corms are an important source of starch. Cocoyam can be grown in paddy fields where water is abundant or in upland situations where watering is supplied by rainfall or by supplemental irrigation (Wang 1983).

Xanthosoma is a genus of flowering plants in the arum family, Araceae. The genus is native to tropical America but widely cultivated and naturalized in other tropical regions. Several are grown for their starchy corms, an important food staple of tropical regions, known variously as cocoyam, otoy, otoe, cocoyam , tannia, tannier, yautía, macabo, ocumo, macal, taioba, dasheen, quequisque, ‘ape . Many other species including *Xanthosoma roseum* are used as ornamental plants, and in popular horticultural literature are known as ‘ape or elephant ear from the resemblance of the leaf to an elephant's ear, although the latter name is sometimes also applied to members with similar appearance and uses in the closely related genera Caladium, Colocasia and Alocasia. The leaves of most Xanthosoma species are 40-200 cm long, arrowhead-shaped or subdivided into three or as many as 18 segments. Unlike the leaves of Colocasia, those of Xanthosoma are usually not peltate- the upper v-notch extends into the point of attachment of the leaf petiole to the blade (FAO 2013).

In Belize, cocoyam are grown all over the country in every district, coco is grown by local farmers as way to provide their families with food and to sell excess produce as a means of income. This crop is very profitable due to the minimal investments required, the high yield and relatively easy maintenance. Recently there has been an increased interest in increasing production of this crop.

Since there is an asserted effort to increase production, this is where culturing these plants *in vitro* would be beneficial. Growing these plants *in vitro* is an efficient method to increase yield which would be plants of better quality and genetic stability, and better coco tubers in less time when compared to conventional propagation methods. The objective of this experiment was to find out what was the effect of different surface sterilization time periods on the cocoyam explants grown *in vitro*.

Literature Review

Kingdom	Plantae
Sub Kingdom	Tracheeobionta
Super Division	Spermatophyta
Division	Magnoliophyta
Class	Liliopsida
Order	Arales
Family	Araceae
Genus	Xanthosoma
Species	*X. Sagittifolium*

Origin:

Cocoyams is native to tropical Central and South America and the Caribbean where it has been cultivated and consumed since the pre-Columbian times. Many sources have the Cocoyams to be a native Tropical or Caribbean plant while other sources state that the plant originated in West Africa (Purseglove 1985).

Cocoyams are herbaceous perennial plants belonging to the family Araceae and are grown primarily for their tubers, although all parts of the plant are edible. Cocoyams that are cultivated as food crops belong to either the genus Colocasia or the genus Xanthosoma and are generally comprised of a large spherical corm (swollen underground storage stem), from which a few large

leaves emerge. The petioles of the leaves stand erect and can reach lengths in excess of 1 m .The leaf blades are large and heart-shaped and can reach 50 cm (15.8 in) in length (Onwueme 1994).

The corm produces lateral buds which give rise to tubers or cormels and suckers or stolons. Cocoyams commonly reach in excess of 1 m (3.3 ft.) in height and although they are perennials, they are often grown as annuals, harvested after one season. Colocasia species may also be referred to as taro, old cocoyam, arrowroot, eddoe, macabo or dasheen and originates from Southeast or Central Asia. Xanthosoma species may be referred to as tannia, yautia, new cocoyam or Chinese taro and originates from Central and South America (NAS 1975).

Habitat:

Cocoyam grows best in fertile, well-draining, sandy loam soil with a pH between 4.2–7.5.It can be grown in a wide variety of conditions including paddies in wetland areas using a system similar to that of rice. Xanthosoma species require temperatures above 21°C (69.8°F) to grow properly. Unlike Colocasia, they will not tolerate waterlogging and grow best in deep, well-draining loams with a pH between 5.5 and 6.5 in partial shade. Cocoyam will thrive when planted in full sunlight or partial shade. The plants can survive for short periods at temperatures of 10°C (50°F) but will be damaged or killed by lower temperatures. Cocoyam is a fast grower in the presence of warm, wet conditions and will rapidly spread over an area with appropriate wet soil. The natural habitat of Xanthosoma is under the forest canopy, under cultivation they are usually sown with full exposure to sunlight. They require well-drained soils and do not tolerate the permanent presence of water (Anon 2001).

Uses:

The usable parts are the subterranean tuberous stems which have a nutritional value comparable to the potato and are probably easier to digest. A secondary use is of consumption of the young leaves, similar to spinach. Leafy species of Xanthosoma not grown for tubers are called belembe and calalous. There are actually two different cocoyams: Cocoyam (*Xanthosoma sagittifolium*), considered the true cocoyam, which grows on dry land. The other is cocoyam amarillo (Colocasia esculenta) which grows in wet bog-like areas. Generally, cocoyam resembles dasheen (taro) and the elephant-ear plant with large green leaves about 2 feet wide by 2 1/2 feet long (NAS 1975). The cocoyam plant may attain a height of 5 feet or more. Edible tubers (cormels) are formed in the soil at the base of the plant. A central large tuber (corm) is formed, with a cluster of cormels, grayish brown to black lateral tubers, around it. Cocoyams are about the size and shape of a regular white potato; they look a little like an overgrown gladiola bulb, because the outside skin of the cocoyam is brown and somewhat hairy. It generally weighs 1/2 to 2 pounds, but can be heavier. The interior has an extremely crisp texture and can vary in color from cream, yellow or pink (Wilson 1987). Cocoyam is often milled into flour, since cocoyam is probably the most hypoallergenic food in the world. Even persons with extensive allergies should do very well with this flour. The starch grains are the smallest and most easily digested of all complex carbohydrates. Cocoyam flour is used as a substitute for wheat flour to make cookies, quick breads, and similar items. Cocoyam is most commonly grown for its starchy edible roots. Colocasia, a relative to the Xanthosoma is grown for its corm which is consumed after boiling, frying or roasting. The corms can be dried and used to make flour or sliced and fried to make chips. The leaves of the plant are

also edible and are usually consumed as a vegetable after cooking in dishes such as stews. Xanthosoma species produce tubers much like potato and are boiled, baked, steamed or fried prior to consumption. The corm of some varieties is also consumed. Young leaves are eaten as a vegetable (Wilson 1987).

Propagation:

Cocoyam is propagated by vegetative means from headsets tops or suckers which establish quickly and give the highest rate of survival. Larger headsets and suckers tend to produce larger corms and higher yields but the size of the planting material may be determined by the particular cultivar being grown. Some varieties will produce two heads from the same corm if the sucker that is planted is too big and therefore medium sized suckers are selected when growing tubers for export. Headsets and suckers should only be taken from healthy plants in order to protect yields and prevent the spread of diseases (Anon 2001).

Production:

Production of Cocoyam in Belize

Table 1.0

Production of Cocoyam in Belize		
Year	**Production, tonnes**	**Yield, tonnes/ha**
1989	1056	11.68
1990	346	7.36
1991	301	4.36
1992	789	10.52
1993	1050	11.54
1994	204	2.72
1995	582	7.37
1996	173	7.52
1997	225	4.59
1998	557	7.33
1999	932	9.41
2000	918	7.85
2001	1289	9.77
2002	854	6.33
2003	469	6.25
2004	264	6.6
2005	281	6.24
2006	261	6.21
2007	156	10.4
2008	167	7.26
2009	197	5.47
2010	201	6.7
2011	274	8.84
2012	203	9.23
2013	238	9.15

Materials and Method

This research was conducted at the micro propagation lab at the University of Belize Central Campus Belmopan. The research was carried out during a period from March to May 2015.

Culture Medium Preparation

The culture media was composed of 100% Ms salts. Which after 1mg/L of thiamine , 100mg/L of myoinositol, 20g of sucrose, 4 ml of 6 –benzylaminepurine (6BAP), 0.1 mg/L of indolebutyric acid (IBA),and 50 mg/L of ascorbic was added to the mix. It was gelled with agar at a concentration of 7g/l added and stirred. When the media was finished 20ml was added to each test tube. The test tubes were placed into a rack and then autoclaved and sterilized at 121o C at pressure of 1.2kg/cm-2 for 15 minutes. After 15 minutes the pressure from the auto clave was gradually released and the culture medium was placed in another room to become semi solid.

Procedures:

The best cocoyam explants were removed from the soil and transported to the micro propagation lab located at the UB preschool campus. During preparation of the explants the laminar flow chamber was turned on and sterilized with 70% ethanol. The explants were cut down to approximately 1 cm (removal of pseudo stem and micro corm was squared) with a knife. After the appropriate size was achieved the explants were washed with liquid soap to remove excess dirt. After this brief wash a sterilization solution was prepared with 4% sodium hypochlorite and distilled water. The coco explants were then added to the jar containing the solution of sodium hypochlorite and distilled water, a drop of liquid soap was added to reduce surface tension. The

jar containing the explants were transported to the inoculation room with the laminar flow chamber, Prior to working under the laminar flow chamber hands were washed with soap.

The explants in the sodium hypochlorite were in constant agitation (shaking). After 15 minutes five plants were removed from the solution with a forceps and rinsed three times with distilled water. These five plants were then cut down and squared some more with a scalpel to fit in the test tube that contained semi-solid culture medium. After another 15 minutes the other five remaining explants were removed and also rinsed three times, there after the pseudo stems and micro corms were reduced further and placed in test tubes with semi solid culture medium. After inoculation cultures were then incubated in a growth room with artificial light at a temperature of 27 ^{o}C. A second experiment was conducted, all the steps were similar to the first experiment except that all explants were sterilized for 30 minutes in a sterilization solution of sodium hypochlorite and distilled water. The explants were divided into groups with slit on the micro corm and those without.

Results and Discussion

Figure 1: Largest Explant (15 minutes)

Figure 1.1: Explants (15 minutes)

Figure 2: Largest Explant (30 Minutes)

Figure 2.1: Explants (30 Minutes)

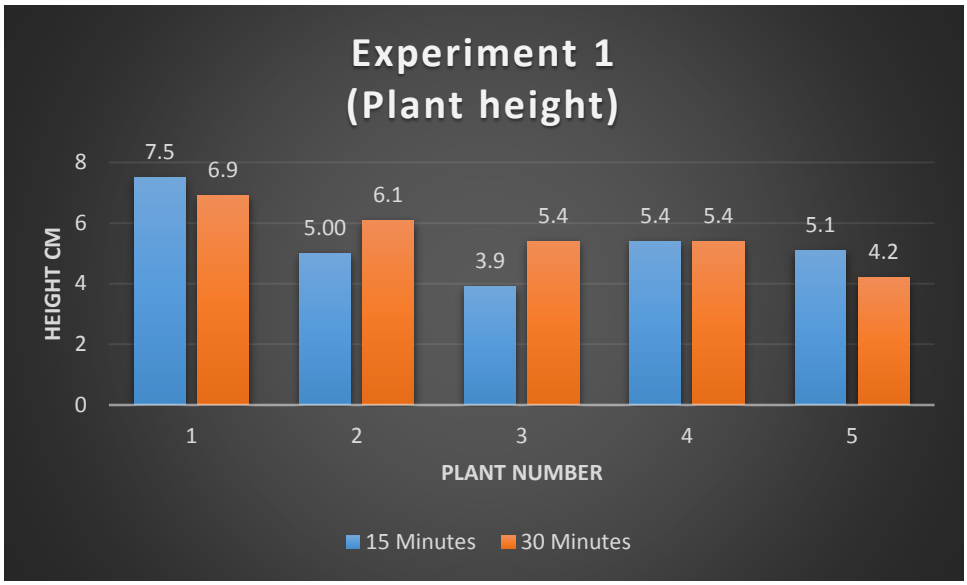

Figure 3: Plant Height

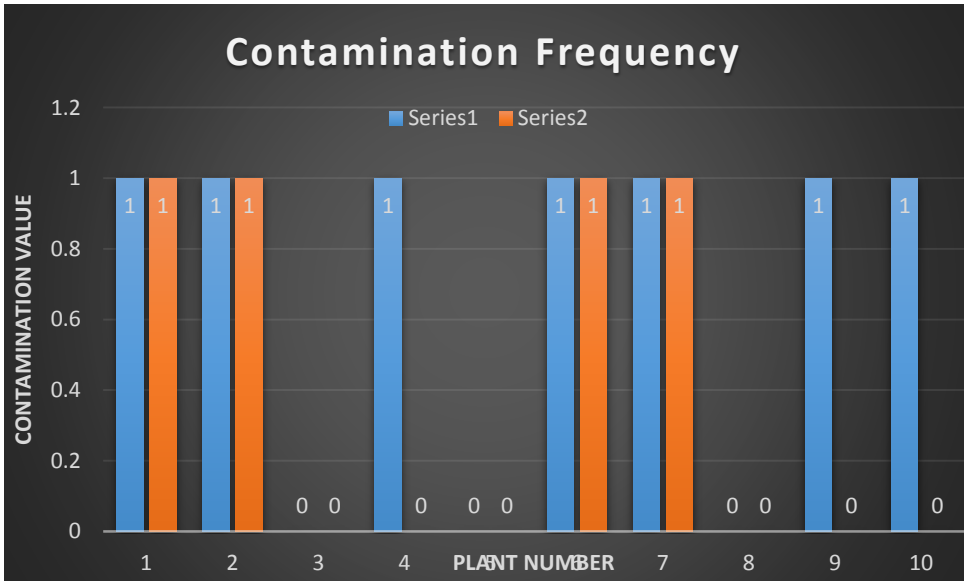

Figure 4: Contamination Frequency

Height of explants (cm)	
15 Minutes	30 Minutes
7.5	6.9
5.00	6.1
3.9	5.4
5.4	5.4
5.1	4.2

Table 2: height of explants

Numbers of Leaves of explants	
15 Minutes	30 Minutes
7	7
4	6
3	5
4	8
5	5

Table 3: Number of leaves of explants

Experiment 2	
Contamination Distribution	
Slit	No Slit
1	1
1	1
0	0
1	0
0	0
1	1
1	1
0	0
1	0
1	1

Table 4: Contamination Distribution

Experiment 1

In experiment one based on the results obtained it showed that all plants survived and that there was zero contamination in experiment one which was the comparison of explants being sterilized with 4% sodium hypochlorite for time periods of 15 and 30 minutes respectively. Although there was no contamination in either group the plants that were sterilized for a longer period grew taller, averaged a height of 5.6 cm while the plants that were only sterilized for 15 minutes averaged 5.38 cm. the plants that were sterilized for 30 minutes also had more leaves per explant averaging 6.2 leaves while the 15minutes group averaged 4.6 leaves per explant. In terms of color and appearance both groups displayed a healthy ever green color. The plants that were in the sterilization solution for 30 minutes grew slightly taller than those that were in the solution for 15 minutes due to the longer exposure to the solution which completely rid the explants of any contamination or factors inhibiting growth, hence the explants that were sterilized longer were able to grow to full potential.

Experiment 2

The explants were grouped by plants that had slits on the micro corm and plants that had no slits. Based on the results of the experiment the explants with the slit on the micro corm had 30% more contamination than the group with no slits. The plants with the slits had more contamination due to the slits which potentially exposed the inner tissue of the micro corm to more pathogens.

Cocoyam is an under exploited crop which has untapped potential, with the aid of micro propagation through establishment *in vitro* , the production of cocoyams can be increased and improved with better, faster yields. This is a field that definitely needs more research and investment to enhance Belizean production of *Xanthosoma sagittifolium* (cocoyams).

Conclusion and recommendations

The best sterilization solution and procedure for Cocoyam is a concentration of 4% hypochlorite for a period of 30 minutes. The plants that were sterilized for 30 minutes showed the best results with the explants growing larger without contamination. The next best sterilization procedure was the 4% sodium hypochlorite in 100 ml of water for 15 minutes these plants showed no contamination but grew slightly smaller than the 30 minutes group.

In experiment 2 the explants without slits on the micro corm had 30% less contamination than those with slits on the micro corm. Cocoyam plants sterilized for 30 minutes in 4% sodium hypochlorite without any slits on the micro corm would be the best ex plants to micro propagate.

Bibliography

Ajijola, S. (2003). Efficiency of resource use among cocoyam farmers in Owo Local Government Area of Ondo State, Nigeria. Moor J. Agricultural Research, 4: 281-287.

Anon. (2001) Growing Cocoyams. International Institute for Tropical Agriculture. Commercial Crop Production Series. Available at: http://www.cassavabiz.org/agroenterpr....

Bradbury, J.H. and Holloway, W.D. (1988) Chemistry of tropical root crops: Significance for nutrition and agriculture in the Pacific. Australian Centre for International Research, Canberra.

CABI Crop Protection Compendium. (2008). Xanthosoma datasheet. Available at: http://www.cabi.org/cpc/datasheet/56986. Retrieved 4/28/15.

FAO. (2013)Pacific Root Crops. Pacific Food Security Toolkit. Available at: http://www.fao.org/docrep/013/am014e/.... Retrieved 4/28/15. Free to access.

Food and Agriculture Organization (2013).http://knoema.com/FAOPRDSC2013Aug/production-statistics-crops-crops-processed-august-2013?tsId=1220800
https://www.quandl.com/data/UNDATA/FAO_YAUT_BLZ

Galvez, B.E. (2012). A Study of the physical state of culture media in the Establishment in vitro of cocoyam. University of Belize.

Kushnir, I. (2012). agro economics/production/production_yautia_cocoyam_belize.html, http://kushnirs.org. Retrieved 4/28/15

National Academy of Science. (1975). Under exploited tropical plants with promising economic, report of hoc panel of the advisory committee on technology innovations, national academy of science, U.S.A. , Washington D.C

Onwueme, I.C. (1994). Tropical root and tuber crops - Production, perspectives and future prospects. FAO Plant Production & Protection Paper 126, FAO, Rome.

Purseglove, J.W. (1985) Tropical Crops: Monocotyledons, Longmans, London, UK,

Wang, J. (1983). (ed). Taro - a review of Colocasia esculenta and its potentials. University of Hawaii Press, Honolulu.

Wilson, J. W. (1987). Taro planting material. USP Institute for Research, Extension and Training in Agriculture (IRETA). Available at: http://www.adap.hawaii.edu/adap/Publi.... [4/28/15]. Free to access.

Appendices

Table 1.1

Production of cocoyam in Belize, averages in periods				
Period	**Production, th. tonnes**	**Share in, %**		
		Central America	Americas	World
1980s	2.1	26.4	1.1	1.1
1990s	0.52	1	0.24	0.24
2000s	0.49	0.66	0.12	0.12
2010s	0.23	0.29	0.059	0.059

Table 1.2

Yield of cocoyam in Belize, averages in periods				
Period	**Yield, tonnes/ha**	**Comparative with**		
		Central America	Americas	World
1980s	11.68	6.96	5.82	5.82
1990s	7.55	13	6.48	6.48
2000s	7.36	11.81	9.21	9.21
2010s	8.4	10.51	8.79	8.79

Comparison of production of cocoyam in Belize and the neighbors

Table 2.1

Production of cocoyam in Belize and its neighbors, th. tonnes, averages in periods		
Period	Mexico	**Belize**
1980s		2.1
1990s	0.48	0.52
2000s	1.3	0.49
2010s	14.5	0.23

Table 2.2

Yield of cocoyam in Belize and its neighbors, tonnes/ha, averages in periods		
Period	Mexico	**Belize**

1980s		11.68
1990s	4.45	7.55
2000s	9.99	7.36
2010s	44.48	8.4

Comparison of production of cocoyam in Belize and the leaders

Table 3.1

Production of cocoyam in Belize and leaders, th. tonnes, averages in periods						
Period	Cuba	Venezuela (Bolivarian Republic of)	El Salvador	Peru	Dominican Republic	**Belize**
1980s	63.7	48.7		24.8	34.7	2.1
1990s	26.2	62.4	54.9	32.2	35.3	0.52
2000s	193	68.2	43.8	33.9	43.7	0.49
2010s	152.3	87.8	42.3	32	31.2	0.23

Table 3.2

Yield of cocoyam in Belize and leaders, tonnes/ha, averages in periods						
Period	Venezuela	El Salvador	Cuba	**Belize**	Dominican Republic	Peru
1980s	5.92		5.86	11.68	5.57	5.14
1990s	7.14	19.4	3.19	7.55	6.97	5.14
2000s	10.74	14.24	9.52	7.36	8.14	5.47
2010s	10.93	9.53	8.99	8.4	6.34	5.61

Table 2.1

Rating of production of cocoyam in World, averages in 2010s

rank	regions, sub regions, countries	Production, th. tonnes	share, %
World		390.7	100.0
regions			
1	Americas	390.7	100
subregions			
1	Caribbean	192.2	49.2
2	South America	119.8	30.7
3	Central America	78.7	20.1
countries			
1	Cuba	152.3	39
2	Venezuela (Bolivarian Republic of)	87.8	22.5
3	El Salvador	42.3	10.8
4	Peru	32	8.2
5	Dominican Republic	31.2	8
6	Costa Rica	16.3	4.2
7	Mexico	14.5	3.7
8	Panama	5.2	1.3
9	Dominica	5.2	1.3
10	Trinidad and Tobago	1.8	0.46
11	Puerto Rico	1.7	0.44
12	Belize	0.23	0.059

13	Saint Lucia	0.04	0.01