UNITED STATES VIRGIN ISLANDS DEPARTMENT OF AGRICULTURE

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USDA AMS Agreement Number: ***12-25-B-1097***

Final Report

PROJECT TITLE

Propagation and Grafting of Highly Demanded Mango and Avocado Fruit Tree Varieties

Two tropical fruits, mango and avocado were selected for the study in the project. Virgin Islands Department of Agriculture (VIDA) will partner with the University of the Virgin Islands Agricultural Experiment Station Horticulture & Aquaculture program in collection of germplasm to establish rootstocks of mango & avocado and conduct grafting.

PROJECT SUMMARY

Grafting has been extensively used for centuries in horticulture to asexually propagate fruit trees. The benefit of applying grafting in fruit trees is to obtain a single tree that combines favorable characteristics of two or more cultivars. Mango (*Mangifera indica* L.) and Avocado (*Persea americana*) are popular fruit crops and great cultural significance in the U.S. Virgin Islands. The intent of this project was to develop a suitable procedure of successful grafting in mango and avocado, distribution and conduct training workshop. The University of the Virgin Islands Agricultural Experiment Station in collaboration with the Cooperative Extension Service and Virgin Islands Department of Agriculture conducted grafting of mango and avocado cultivars throughout the three USVI islands. Two prebachelorette students of the University of the Virgin Islands, six extension agents of UVI-AES, CES and VIDA were trained in horticulture and grafting mango and avocado. A poster paper on grafting of mango was presented at the Xth Mango Symposium in Dominican Republic in June 2013. Total 753 rootstocks (#489 mangos and #264 avocados) have been established and presently maintained in the UVI-AES nursery, St. Croix and VIDA nurseries in St. Thomas and St. John. Budwoods (#234) of 39 selected varieties of mango were procured from the USDA Sub Tropical Horticultural Station, FL and grafted on mango rootstocks as well as distributed to farming community in three islands. Side veneer and cleft grafting methods performed in mango and avocado. Total 106 farmers, backyard gardeners, and extension agents attended grafting workshops throughout the three islands of the USVI. Grafted plants of fruit trees are imported from the Puerto Rico and Florida in to the Virgin Islands. Successful production of grafted plants locally presents an economical, time saving and beneficial for the local farming community. Early and late-bearing cultivars exist in many areas of the USVI and grafting in early and late bearing trees of various varieties will also help provide a more consistent supply of fruits.

PROJECT APPROACH

A site at the University of the Virgin Islands Agricultural Experiment Station selected for the installation of nursery to establish rootstocks and conduct grafting in two fruit crops. A shade house (30'x40') and a fenced plot (30'x20') were cleaned, and irrigation installed. Nursery

supplies such as pots, potting mix, fertilizer, grafting tools and kits procured locally or off-island. Rootstocks of mango were collected from VIDA orchard and farmers' fields in diverse locations throughout St Croix and avocado seeds collected from the UVI-AES orchard. Blow-Molded Nursery Containers 1600 were used to plant the seedlings. Utilizing a combination of one part Pro-Mix one part local soil the mango seedling were replanted and allowed to recuperate for four months. During which time the seedlings were fertilized on a bi-weekly basis with 4lbs of Chem-Sol water soluble fertilizer (12-48-8). Grafting in rootstocks performed in two fruit crops throughout the three islands. Project Director travelled to St Thomas and St John to conduct workshops in collaboration with CES and VIDA personnel. Water soluble fertilizer, pots and grafting kits were provided to the collaborators from VIDA to establish rootstocks in their nurseries.

GOALS AND OUTCOMES ACHIEVED

Budwoods (#234 nos.) of selected 39 varieties of mango procured from the USDA Sub Tropical Station, FL grafted and distributed throughout the three islands. Total 753 rootstocks established in two crops and delivered to V.I. Dept. of Agriculture. Over 320 grafts (side veneer and cleft) performed in two crops in rootstocks and mature trees at VIDA and farmers' field (Table 1). One hundred six (106) trainees including farmers, backyard gardeners, hobbyists and agriculture extension agents participated in three grafting workshops organized in three islands (St. Croix, St. Thomas and St. John) and provided grafting knives and tools.

Table 1: No. of rootstocks and grafted plants in mango and avocado in different locations. No. of rootstocks (no. of grafted plants)

Fruit Crop	AES STX	VIDA STX	VIDA STT	VIDA STJ	No. of grafted Plants	Total (rootstocks + grafted)
Mango	324 (13a)	26 grafts on mature trees	105	60	39	489
Avocado	110 (20a)	98* (30a)	33	23(4a)	54	264
					93	753

BENEFICIARIES

Farmers, backyard gardeners, hobbyists and agriculture extension agents participated in three grafting workshops organized in three islands (St. Croix, St. Thomas and St. John). Participants were provided grafting tools, mango and avocado rootstocks and budwoods to practice grafting. Not only did the farmers involved on this grafting project benefit but the entire Virgin Islands community. Farmers and backyard gardeners have embraced the training and skills learned in the workshops. Bdwoods of new varieties distributed, skill and knowledge in grafting fruit trees. There has been tremendous enthusiasm observed in the Virgin Islands community on grafting and demanded for more training workshops. The increase in knowledge and grafting skills learned eventually shall help in producing grafted fruit trees locally reduce off-island import of

grafted fruit plants and benefit economically local nurseries and entrepreneurs. Information on grafting fruit trees was delivered to visitors during the Annual Agriculture Fair in St. Croix in Feb. 2013. Two prebachelorette students of the University of the Virgin Islands, six extension agents of UVI-AES, CES and VI Department of Agriculture were trained in horticulture and grafting mango and avocado. A poster on grafting of mango was presented at the Xth Mango Symposium held in Dominican Republic in June 2013.
Participants in three training workshops: 20+72+14=106
Total no. of beneficiaries (all three islands of the USVI): 250 (including ag fair)

LESSONS LEARNED

Project initiated in November 2012, therefore, hiring of student aid delayed until January 2013 due to holidays in UVI. November is off-season for two fruit crops and collection of seeds in mango and avocado was a challenge to establish rootstocks. No avocado budwoods were provided by USDA Germplasm Repository due to disease issue. Rootstocks need to be a certain age of growth before grafting is performed (6 months or so), and short duration of project was a challenge. Grafting requires some skill to make the successful `take' and increased chances of success acquired through practice.

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Grafting tools

Mango and Avocado seeds kept for drying in the greenhouse before planting

Mango rootstocks in AES nursery

Avocado rootstocks

UVI students grafting fruit trees

Grafting workshop, St. Croix (03/21/2013)

Grafting workshop, St. Croix (03/21/2013)

Mango plants grafted by trainees in the workshop

Grafting workshop, STJ (03/26/2013)

Mango rootstocks in STJ nursery

Grafting site 1 (Farmer Denise Nash)

Graft on mature tree of mango at VIDA Orchard

Grafting workshop in St Thomas (03/25/2013)

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Final Performance Report

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Project Title

On-Farm Evaluation and Breeding Sorrel to Enhance Marketability

VIDA will partner with the University of the Virgin Islands Agricultural Experiment Station Biotechnology & Agro forestry to conduct trials to micro propagate and grow varieties of virus and disease free sweet potatoes in farmers' fields and compare them to the cultivars that farmers are presently growing

Amount Budgeted: $50,000.00

ACTIVITIES PERFORMED

Project Summary

Sorrel (*Hibiscus sabdariffa L.*) has a calyx that develops into a fleshy tissue used for making nutritious juices, teas and desserts. The propose of the project was to conduct on-farm evaluation and plant breeding to combine beneficial attributes into new sorrel varieties with year-round production potential. Plant breeding was used to combine beneficial characteristics of sorrel varieties to develop new highly marketable types. This allowed farmers to extend the market season and consumers to obtain the beneficial health characteristics of fresh sorrel all year-round. Two Universities of the Virgin Islands students were trained in Biotechnology, sorrel breeding and production practices and presented the results of their research locally, nationally and internationally. During the two years of the project, the US Virgin Islands experienced two of the wettest years on record. However, sorrel was established in six farmers' fields and at UVI from late summer through early spring. Sorrel, a self-pollinated inbred, exhibited hybrid vigor in the first generation hybrid populations. Hybrid sorrel had taller plants with more fruiting branches, larger fruit and earlier production. Farmers were able to market all the sorrel produced outside the traditional season indicated a strong market demand for the fruit.

Project Approach

Sorrel seeds were planted into seedling trays in a greenhouse and germination recorded. Sorrel germinated within a range of 2-6 days. After 2-4 weeks, seedlings were established in four farmer's fields in diverse locations throughout St Croix and at UVI. Drip irrigation and water soluble fertilizer was provided to the farmers. One farmer had problems with the sorrel wilt disease. This has been reoccurring since they have been growing sorrel in the same location for three years compounded with the wet soil conditions. A second farmer had deer foraging and eating the sorrel plants. This farmer obtained a dog that was tied near the field which limited the deer damage. A third farmer had problems with weed control in the sorrel.

Rainforest location with deer

VI Dept. Ag farmer plot

Commercial large farmer with weed issues

Goals and Outcomes Achieved

Seeds were produced on a large scale and supplied to the VI Dept. of Agriculture who grew seedlings for farmers and backyard gardeners. Over 265 small packets of seeds were also directly distributed to farmers and local gardeners upon request. Local growers have incorporated the new sorrels and the traditional late sorrel of four years ago can no longer be found. Growers have replaced the traditional sorrel with the new more productive varieties and darker red color. Growers are also able to plant at three month intervals to ensure continuous production. Consumers buy the fresh sorrel whenever they see it available

Beneficiaries

Four (4) local farmers participated and were provided with land and water to assist in this sorrel research. Not only did the farmers involved on this sorrel project benefit but the entire Virgin Islands community. Sorrel varieties grown have completely changed to the new earlier and darker types. Fruit can now be observed on the roadside and in the farmer's market from October through April instead of just December and January just four years ago. Farmers and backyard gardeners have embraced the new varieties with the extended fruiting season, larger fruit and increased fruit color from white, striped red/white, pink, red and deep burgundy. The general public has bought up the supply during the off-season and increasing the demand for the sorrel. The increase in sorrel production has benefited the public with the nutrient and bioflavonoid rich fruit which will benefit the overall health of the Virgin Islands community.

Two University of the Virgin Islands prebachelorette students were trained in Biotechnology, sorrel breeding and production practices and presented the results of their research locally at the University of the Virgin Islands Spring Research Symposium, the

American Society for Horticultural Science and the Caribbean Food Crops Society. As a result of the presentations in a regional Caribbean conference, inquiries for seed have been obtained from six islands. This has motivated the students to inquire about graduate research studies in the agricultural and biotechnology field.

Rainforest location with deer

VI Dept. Ag farmer plot

Commercial large farmer with weed issues

Goals and Outcomes Achieved

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Lessons Learned

Initiating a plant breeding program of a new crop requires a good initial stock of plant germplasm to isolate the potential lines from weedy wild spiny types. The initial screening of plant germplasm requires a larger quantity of land than originally expected. Because

sorrel sheds pollen in the middle of the night and prior the flower opening, Breeding was not possible at university plots and potted plants needed to be grown in the yard of the PI so late night floral emasculations could occur and early morning crosspollinations obtained.

Farmers and consumers will change their sorrel preference when they have available larger and darker fruit. Sorrel was consumed outside the normal Christmas holiday season and consumers now expect to see it on the market throughout most of the year.

USDA AMS Agreement Number: *12-25-B-1097*
SCBG-FB 2010
Final Report
Submitted on January 11, 2013

Institutional Sub-Grantees
Project Title: Expanding the Availability of Improved Disease-free Sweet Potato to Increase Production and Marketability

Institution Name: University of the Virgin Islands Agricultural Experiment Station (UVI AES)

Project Participants:

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Project Summary

Sweet potato is an important staple food to the Virgin Islands and many other parts of the world. Viruses of the sweet potato plants can tremendously decrease the yield farmers receive from cultivating the tuberous roots of this plant. To determine if viruses were present in local sweet potato crops, ELISA kits were used to screen for the presences of viruses in the local sweet potato crop on St Croix. The ELISA kit used antibodies to detect the presence of a specific protein produced by a virus; in this case from sweet potato leaf samples. Leaf samples were collected from seven locations around the island of St. Croix

and tested for three different viruses. The data was then plotted and graphed onto a map. Forty-two (42) samples of sweet potato leaves were collected. An in vitro virus-free variety, obtained from the USDA Germplasm Repository, was used as a negative control. Most of the sweet potato plants on the island of St. Croix were positive for at least one virus. ELISA tests indicated that 15 samples were positive for Potyvirus, 12 samples were positive for Cucumber Mosaic Virus and 22 samples were positive for Zucchini Yellow Mosaic Virus. Of these results, 16 sweet potato samples were infected with multiple viruses. Six (12%) of sweet potato samples tested negative for all three of the virus tests performed. The ELISA test was an effective way to detect the presence of Potyvirus, Cucumber Mosaic Virus and Zucchini Yellow Mosaic Virus in sweet potatoes grown on the island of St. Croix.

Virus-free sweet potato plants, obtained from the USDA Germplasm Repository, were maintained in culture to supply clean plantlets to local growers. However, between requests for plant material a system was needed to control growth and increase the intervals between transfers. Long-term in vitro maintenance, on MS medium containing 0 to 12% sucrose, was used to evaluate shoot growth over time on four sweet potato cultivars. Sucrose levels above 6% were expected to impose an osmotic stress to suppress growth. However, sucrose levels from 2 to 12% had no influence on controlling in vitro growth and development over time. Having no sucrose in the medium resulted in minimal growth but was lethal to 50% or more of the cultures. The rate of root growth and leaf development was significantly reduced on sucrose levels from 0.1 to 0.3%. These low sucrose levels controlled the rate of growth and extended the interval between transfers from monthly, on 3% sucrose, to 8 to 12 months on 0.1 to 0.3% sucrose containing media. Shoots actively grew after one year when transferred back to a 3% sucrose medium. Low sucrose concentrations can be used to control growth of sweet potato and extend the intervals between transfers in vitro for long-term in vitro storage.

Project Approach

Virus-free sweet potato plants, obtained from the USDA Germplasm Repository, were grown and maintained in vitro. The four sweet potato cultivars used were 'Francia', 'Mojave', 'Papino' and 'Viola'. Single nodal cuttings from these plants were transferred to fresh Murashige & Skoog media (MS) (1962) containing 0, 3, 6, 9 and 12% sucrose with 0.8% agar. Shoots were grown in a 28°C growth room with a 16 hour photoperiod. Data on root and leaf development were collected twice weekly for 45 days. A second trial involved sucrose levels from 0, 0.1, 0.3 and 1% on MS medium gelled with agar after the higher sucrose levels didn't retard the rate of sweet potato growth in vitro. Data was again collected twice weekly for 45 days and included number of leaves and roots for the lower

sucrose trial. Data was analyzed with ANOVA and averages plotted over time. We were trying to suppress sweet potato growth by increasing sucrose content as was possible with the work done on cassava. However, increasing the sucrose content which raises the osmotic potential of the medium, did not suppress growth in these sweet potato varieties. Leaf development over time was similar for all four varieties containing sucrose from 3-12% (Figure 1). Shoots grown without sucrose was lethal to 15-50% of the shoots. Those that survived without sucrose did have reduced shoot growth and root development. The high rate of shoot death with no sucrose doesn't make it a practical to use to suppress growth for long term in vitro maintenance. Root initiation and development at all sucrose levels was similar and the average after 45 days which ranged from 5.2 to 5.9 roots. There was no significant difference between varieties or the media containing sucrose (Figure 2). It was concluded that sweet potatoes aren't influenced by the osmotic potential of the medium with sucrose levels between 3% and 12% over 45 days of growth in vitro.

In the second trial, the lower levels of sucrose were able to suppress shoot and leaf development. After 45 days in vitro, the size of the plant reflected the sucrose concentration it was grown on with increasing sucrose levels resulting in more growth and development but at a slower rate than previously observed on high sucrose (Figure 3). At the lowest sucrose level of 0.1%, all explants survived. The rate of leaf development at 1% sucrose was similar to that obtained at the 3-12% levels (Figure 1 and 4). However, the rate of leaf development was reduced at sucrose levels below 1% (Figure 4) indicating a suppression in growth as was desired.

Root growth and development was reduced at all the low sucrose concentrations with averages between 1.6 and 3.2 for 0% and 1% respectively (Figure 5). The low sucrose levels were able to suppress root initiation and root growth. The average rooting for the 0.1 - 1% range of sucrose concentration was between the 0% - 3% rate previously observed in the high sucrose study (Figure 5).

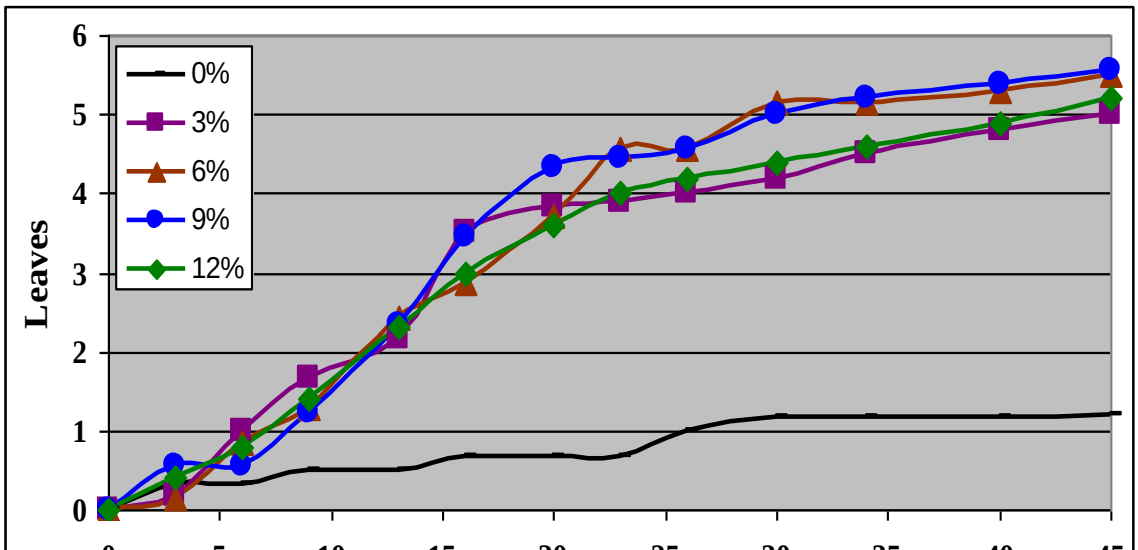

Figure 1. Average leaf development of four sweet potato varieties grown in vitro on 0-12% sucrose over 45 days.

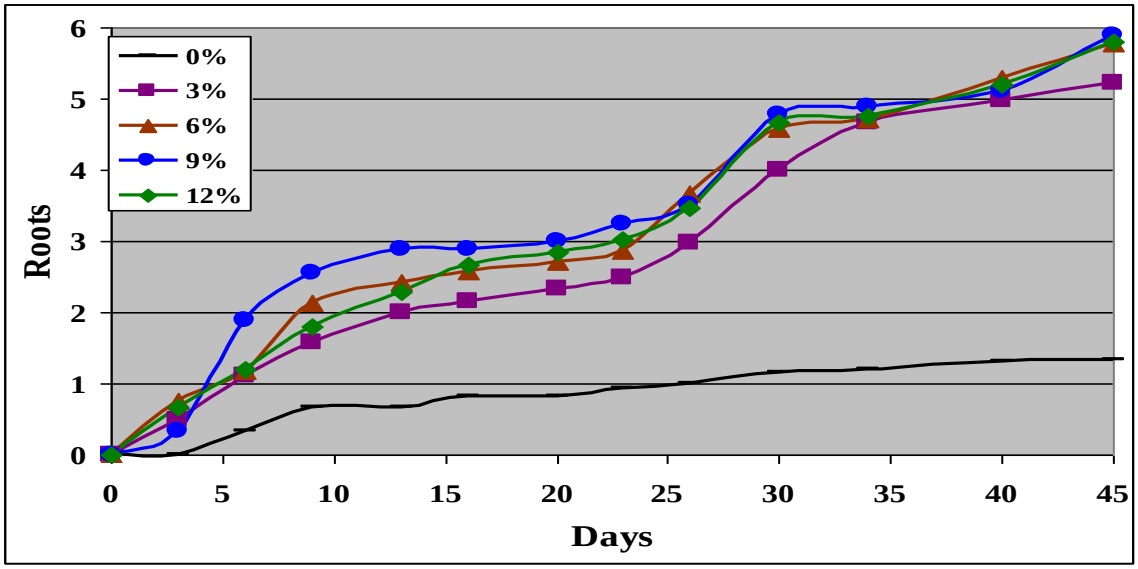

Figure 2. Average root development of four sweet potato varieties grown in vitro on 0-12% sucrose over 45 days.

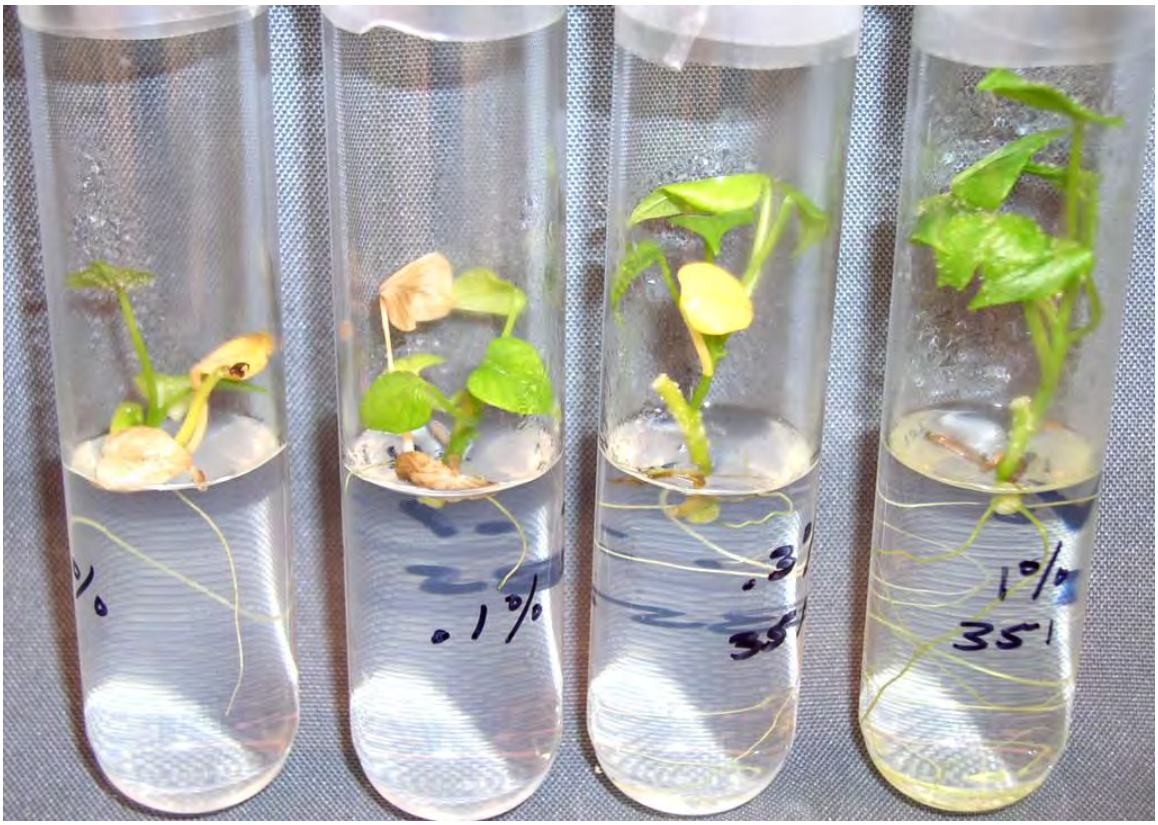

Figure 3. Growth of 'Mojave' sweet potato grown on 0% (left) to 1% (right) sucrose after 45 days in vitro.

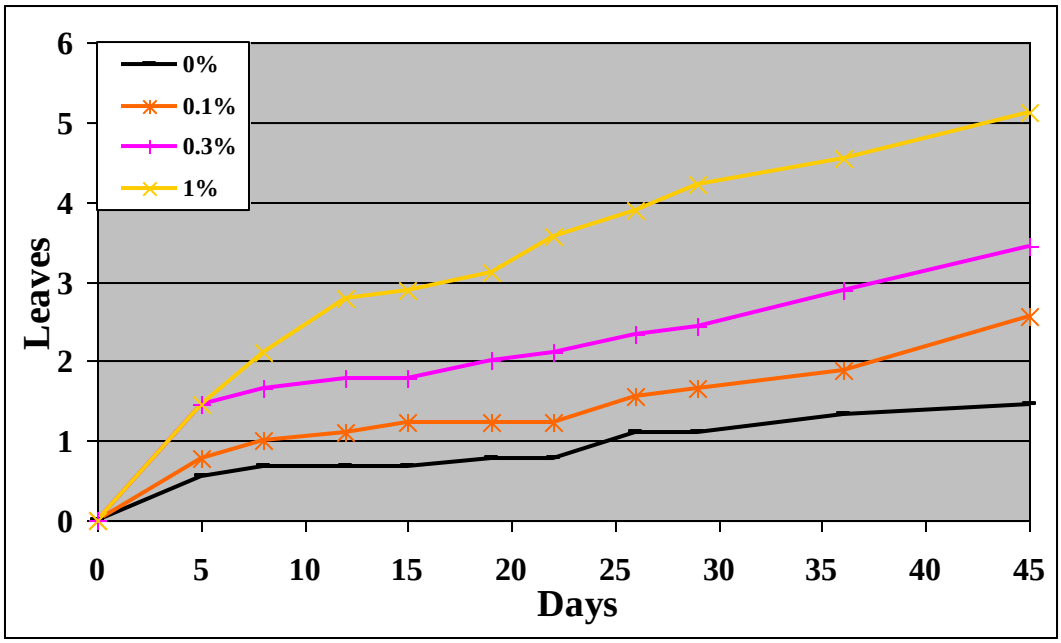

Figure 4. Average leaf development of four sweet potato varieties grown in vitro on 0-1% sucrose over 45 days.

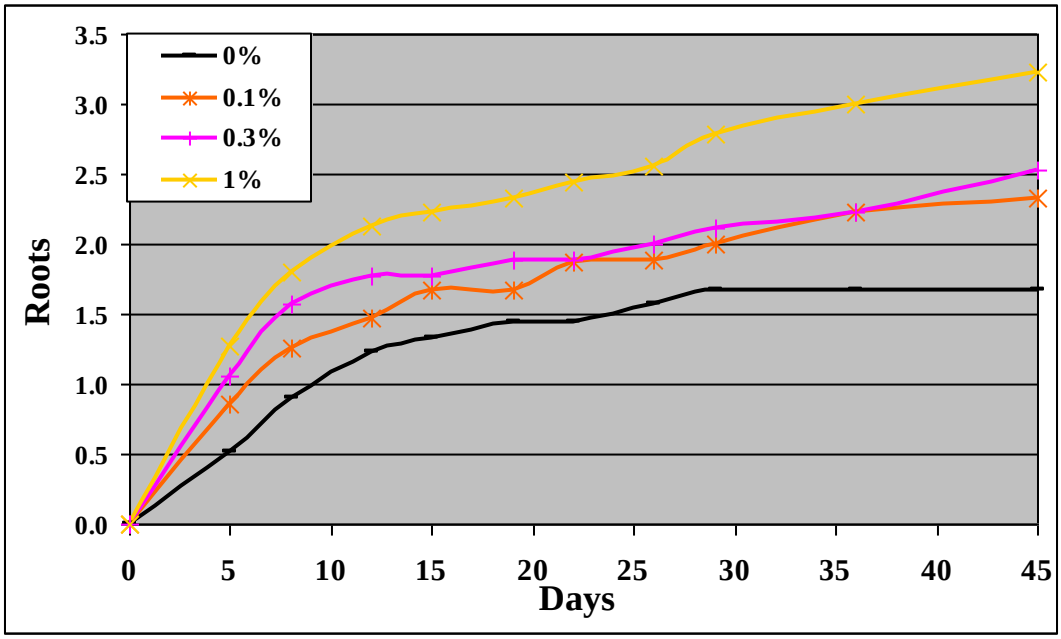

Figure 5. Average root development of four sweet potato varieties grown in vitro on 0-1% sucrose over 45 days.

To identify the virus level in locally grown sweet potato, twenty-nine samples of sweet potato were cut from 5 farmers around St. Croix, 12 samples were taken from the University of the Virgin Islands and 1 in-vitro sample as a control. Each sample contained 5 deformed, discolored and/or possibly virus infected leaves from a sweet potato variety. The scalpel blade used to cut the various sweet potato leaves was sterilized with 70% ethyl alcohol after each use to prevent contamination of samples. Each leaf was put into a separate zip lock bag, labeled and then into a cooler filled with ice immediately after it was cut. A survey was also done in order to collect data on the location, size, age and origin of the various sweet potato varieties.

An ELISA kit was used to determine viral infections. ELISA stands for Enzyme Linked Immunosorbent Serological Array. An ELISA test uses components of the immune system

and chemicals to detect immune responses in the body (for example, viruses). The ELISA kits being used in this study were manufactured by the Agdia, Inc. This experiment involves seven viruses: Sweet Potato Feathery Mottle Virus (SPFMV, potyvirus), Sweet Potato Shrunked Vein Virus (SPSVV, closterovirus), Sweet Potato Virus Disease (SPFMV+SPSVV-->SPVD), Sweet Potato Mild Mottle Virus (SPMMV, potyvirus), Sweet Potato Camilo-like Virus (SPCV), Cucumber Mosaic Virus (CMV, cucumovirus) and the Sweet Potato Chlorotic Stunt Virus (SPCSV, crinivirus). Most of the virus kits used in this study tested for the virus group, not the specific virus. The virus kits used in this experiment were: Potyvirus kit (SPFMV, SPMMV and SPVD), and a Cucumber Mosaic Virus kit (for CMV). ELISA kits are used to detect phosphotases that bind to the coat proteins of viruses. Apart from the items included in the ELISA kit (micro-titer plates, enzyme conjugate, PNP tablets, buffers, and stop solution), an airtight plastic bag, paper towels, distilled water, micro-pipetting equipment, mortar and pestle were used (Figure 6). A positive control was also included in the Agdia ELISA kit for the aforementioned viruses. One hundred microliters of the positive control was added to the microtiter well at the bottom left hand corner of the microtiter plate. Deionized water was used as a negative control. One hundred microliters of the negative control was added to the top right hand corner of the microtiter well. The controls were placed as far away from each other as possible in order to prevent contamination. The first step of the ELISA test process was grinding and diluting the leaf sample. The extraction buffer included in the ELISA kits were used to dilute the leaf samples. After this step, 100 µl of each sample was added to one microtiter well. After grinding, diltution and the addition of controls, the samples were incubated for one hour. Throughout this experiment, incubations were performed in the dark to prevent light contamination. After the first incubation, the samples were washed 6-8 times using the PBST (Phosphate Buffered Saline with TWEEN-20) wash buffer provided in the Agdia kit. After a thorough washing, 100 µl of the enzyme conjugate, provided by the Agdia kit, was added. The samples were then set to incubate again for 2 hours. After this incubation, the samples were once again washed 6-8 times with the PBST wash buffer. One hundred microliters of the PNP buffer, a detection solution that turned yellow if the specific virus protein was present, was then added to the microtiter wells. The wells were then set to incubate for another 2 hours. After this incubation period, samples were examined to see if any color change had occurred (Figure 7).

Figure 6. Leaf samples, ELISA kit and UVI student grinding samples for ELISA test.

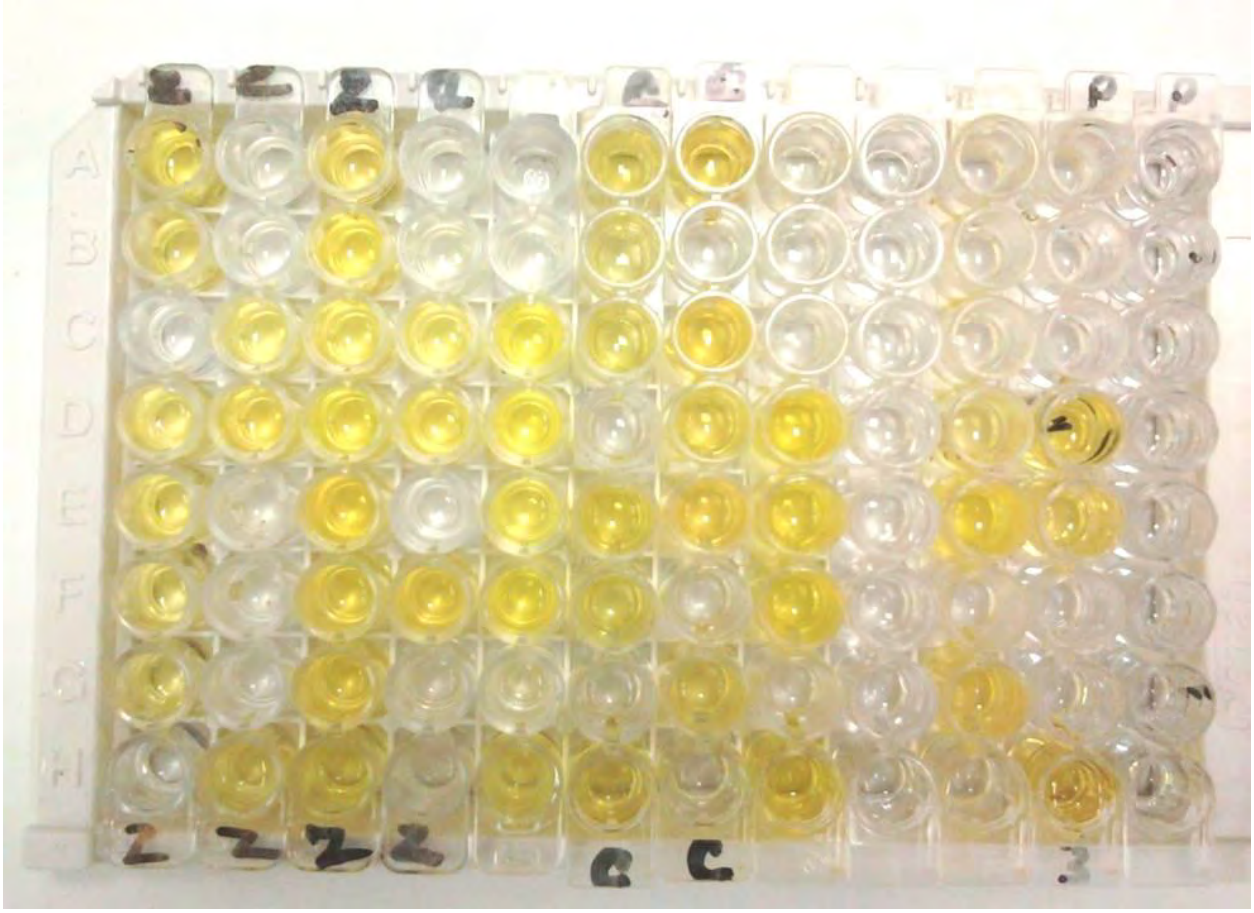

Figure 7. ELISA results of micotiterplate were yellow indicates presence of sweet potato virus.

Figure 8. Distribution of sweet potato viruses on St Croix, VI.

Goals and Outcomes Achieved

A system was developed for long-term in vitro storage of virus-free sweet potatoes in the Virgin Islands that local growers have access to. Sucrose levels between 3%-12% had no influence in suppressing growth due to increased osmotic potential and plants grew similarly at these levels for the four sweet potato varieties while 0% sucrose was lethal to 15-50% of the explants. However, using a sucrose concentration in the media at 0.1% - 0.3%, significantly reduced the rate of leaf and root growth yet sustained the plants. For long-term sweet potato in vitro maintenance, a sucrose level between 0.1% and 0.3% is recommended.

The long-term vitro storage has enabled UVI and VIDA project staff to increase the availability of disease free sweet potatoes to local farmers and backyard gardeners. VIDA will continue to monitor the production and success of these sweet potatoes.

Beneficiaries

Virus-free sweet potatoes will be maintained in vitro and under greenhouse conditions until the screen house is repaired. Shoots were propagated from nodal cuttings and acclimated in situ after 4 weeks. Acclimated shoots were transferred to an antivirus screen house for grow out. Plants were maintained in the antivirus screen house where cuttings were distributed to eight growers. Stakeholders are still able to obtain sweet potato cuttings from greenhouse grown plants upon request.

Two UVI undergraduate students were employed and conducted research relating to this project. They learned both tissue culture techniques as well as using biotechnology to verify presence of viruses in sweet potato plant tissues. Results were presented at a campus-wide research symposium and at the Caribbean Food Crops Society meeting. The research training he obtained on this project stimulated his interest biotechnology. One of the students is completing his undergraduate training at Boston Medical School and plans to go on to obtain a Ph.D.

Lessons Learned

Sucrose levels between 3%-12% had no influence in suppressing growth due to increased osmotic potential and plants grew similarly at these levels for the four sweet potato varieties while 0% sucrose was lethal to 15-50% of the explants. However, using a sucrose concentration in the media at 0.1% - 0.3%, significantly reduced the rate of leaf and root

growth yet sustained the plants. For long-term sweet potato in vitro maintenance, a sucrose level between 0.1% and 0.3% is recommended. The ELISA test indicated that sweet potatoes on the island of St. Croix are positive for Potyvirus, Cucumber Mosaic Virus and Zucchini Yellow Mosaic Virus. ELISA tests are effective in determining viruses of sweet potato in the Virgin Islands.