UNIVERSITY DEVELOPMENT LINKAGE PROJECT
ANNUAL ACTIVITY REPORT
OCTOBER 1, 1998 to SEPTEMBER 30, 1999

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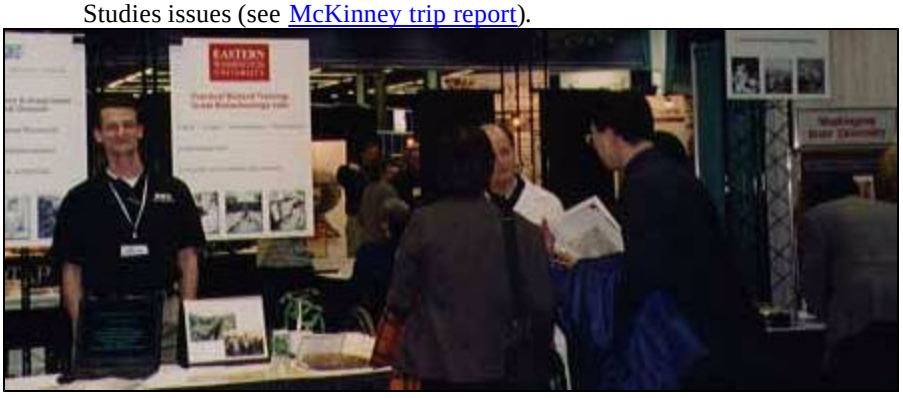
Cooperative Agreement: PCE-5063-A-00-3040-00
Grant Web Site: http://146.187.224.89/cehd/faculty/ntodd/GhanaUDLP/home.html

A description of the past year's activities under each linkage objective:

Objective 1: To implement a post-graduate training program which will provide current University of Cape Coast (UCC) faculty with the opportunity to enhance their professional knowledge and skills in fields identified as critical to UCC and to provide focus to the development of master's level education at UCC.

During this past fall 1998, three faculty from Eastern Washington University were at the University of Cape Coast to work with graduate faculty at UCC. This group was the last group of EWU exchange faculty participating at UCC.

- Dr. Carlos Maldonado taught a course, "Ethnicities in the Americas," to about 60 students (see Maldonado trip report). As a Chicano-American professor, he was able to dispel many myths held by his Ghanaian students about stereotypical perceptions of minority cultures in the United States.
- Dr. Steve Stein taught Biology courses (see Stein trip report). His contribution was an important continuation of our exchange of Botany faculty visiting each other's campuses.
- Dr. Karen McKinney provided consultation and gave several presentations regarding Women's Studies issues (see McKinney trip report).



During this past year we have focused on developing the biotechnology collaboration between Eastern Washington University and the University of Cape Coast. Eastern Washington University purchased a Helios Gene Gun System. Dr. Lightfoot is developing a user protocol for the gun so that UCC Biotechnology faculty will be able to do their own trouble- shooting. Dr. Lightfoot was asked to provide an update on the use of this piece of equipment. The update appears below. During May 1999, the EWU program presented their progress at International Biotechnology conference held in Seattle, Washington. (See photo at left Noah Pefaur with name tag & Dr. Don Lightfoot in white coat:) During July 1999, Dr.

Eric Quaye, Chair of the Faculty of Botany at the University of Cape Coast, visited Eastern Washington University while in the U. S. on a Fulbright Exchange at Arizona State University. During his time at EWU, Dr. Quaye received training on the use of the Helios Gene Gun System. Dr. Quaye was also given a laptop computer with modem for the Biotechnology program.

Objective 4: To implement a 'training of the trainers program' at UCEW

During February 1999 Dr. Todd met with the faculty from the University College of Education at Winneba who are graduate students participating in the field-based masters program sponsored by EWU and UCC. She advised on their progress toward completion of their masters theses. A series of seminars were set up by Dr. Jophus Anamuah-Mensah and Dr. Joseph Tufuor to enable these faculty/students to discuss their work and prepare for the oral defense of their theses.

A description of progress toward the completion of each linkage objective

Objective 1: To implement a post-graduate training program [which will] upgrade UCC faculty skills...

By the close of year five, twenty-five participants from the University of Cape Coast had participated in the faculty development objective. The purpose of this activity is to enhance the skills of UCC faculty in graduate education.

There has been continued collaboration between faculty from both universities with a particular emphasis on biotechnology.

Objective 2: To internationalize EWU's curriculum by providing faculty with a first-hand opportunity to experience culture and educational process in a developing nation, and by utilizing the expertise of UCC faculty in integrating materials on development-related issues into EWU curriculum...

To date, we have had twenty-four EWU faculty who have visited UCC and participated in teaching in the graduate program, consulting, and/or collaborating on a research project. All faculty have returned with a heightened sense of awareness and enthusiasm about the need for internationalizing the EWU curriculum. EWU grant participants have incorporated their learning experiences from grant participation in Ghana into their professional roles.

Objective 3: Collaborative Research...There are two research projects that are actively being pursued.

Objective 4: To implement a 'training of the trainers program' at UCEW

The eighteen candidates are in the last stages of defending their research projects or have completed the degree.

A description of any problems or barriers affecting the progress toward achieving the linkage objectives.

Completion of the masters program by UCEW faculty continues to be slow. I understand that some of their theses have been collected to be sent over, to EWU, but this hasn't yet happened. Communication has been compromised because the modem in the UDLP office is again dysfunctional.

Quantitative outputs of the linkage activities

Outputs include the following:

(a). Twenty-six UCC Faculty have traveled to EWU to address objective one of the project--to enhance the skills of UCC faculty in fields identified as critical the UCC's graduate education.

(b). Eighteen faculty from the University College of Education at Winneba have been enrolled full time in the field-based masters degree program. These participants are now pursuing their final major research projects or theses.

(c). Twenty-three different EWU professors have participated in the linkage, returning with ideas for internationalizing the EWU curriculum.

(d). A total of nine African Studies courses have been taught at EWU during the past five years. These include: African Resources for the Curriculum, The Black Family, African Music, African Literature in English, African Religions, Problems of Aging in Africa, and African Culture in Transition, African History, and African Economic and Political Geography. The African History courses has been officially approved as a requirement for the international education component of the undergraduate degree at EWU. Additionally, a tenth course, a education workshop for teachers, African Resources in the Curriculum was also taught.

Description of progress toward ensuring the sustainability of the Linkage.

This part includes two reports. One report is by Dr. Don Lightfoot who oversees the Biotechnology Program at EWU and the other is by Noah Pefaur the research assistant who is doing most of the leg work.

Dr. Lightfoot:

I am pleased to report progress on several fronts over the past two months of work on the EWU -UCC UDLP collaboration. The portion of the linkage project, which I am directing, is aimed to develop a useful, practical plant biotechnology activity. The UCC Botany faculty co-teachers have identified this activity and they have participated in each step of the way and have developed their skills and materials in parallel with, and with the direction of our work. The goal is to have the UCC Biotechnology laboratory functioning on its own for some years to come in tissue culture and genetic transformation of cassava plants and to develop a variety of economic value in Ghana that has increased disease resistance.

Principally, Mr. Isaac Galyuon, and secondarily Dr. Eric Quaye, head of the Botany Department at UCC, is using the plant biotechnology lab at UCC to make and maintain callus cultures of cassava (Manihot esculenta), the main starchy food source for 500 million equatorial people, world-wide. They are replicating the media and tissue culture methods we use and are getting to maintain the right kinds of callus for gene. We are very fotunate that Dr. Quaye was able to arrange to come to EWU to be trained in our current version of cassava tissue culture. He also successfully went through a mock tissue transformation using the BioRad Helios biolistic gene gun.

We have contacted and developed working relations with other professionals in support of this cassava development project. Dr. Isao Morishima, at Tottori University in Japan, has agreed to supply us with a proprietary material only licensed out. Dr. Brian Fritzinski in Alberta, Canada, has offered to consult on construction of a new set of genes that will 1) benefit cassava agronomics, and 2) have marker genes to tell us how the plant transformation process is going. Dr. Nigel Taylor at ILTAB has been, and will continue to be, extremely important in guiding us through the 1) tissue culture, 2) the transformation, and 3) the plant regeneration phases of the project. The fourth phase we focus on, and which we will provide a service to UCC in the future, is in preparation of useful genes and gene combinations to be Îshotâ into the cassava tissue. We will scrape together the needed funds to send Noah Pefaur to ILTAB this fall to get first hand training. I plan to visit ILTAB, at my own cost, in the spring. Noah and I will use this knowledge to help transfer skills and plans and to advise our colleagues at UCC. Recently we developed a friendly, working relation with Dr. Sui-Chang Sun, director of Research at Jacklin Seed in Post Falls, ID (the world âs largest blue grass seed producer and marketer). Dr. Sun assists us on tissue and organ culture and on use of plant hormones in these steps.

At this point, practically all of the laboratory work has been accomplished by Noah Pefaur, a graduate student, and, before him, Kristin Suprak, an undergraduate. Several senior biotechnology students, principally Willi-Jo Williams, have also contributed. Noah now has each of the important stages of cassava tissue culture on hand and growing consistently. He is now producing suspended cell masses, ready for gene bombardment. He has trained Willi-Jo in maintaining all of the cultures and producing enough (over 200 containers worth) to support the gene gun work. We may also try to do this with West African cultivars of cassava. We have on hand the pLAU2-GUS gene, a marker to tell us how well the gene shooting is going. This gene is combined with other markers in a form provided by ILTAB. In the next two weeks Noah will make the first Helios gene gun optimizing experiments. He has exchanged
the original gun for a 22 volt model. We also have on hand the special gasses and equipment needed to conduct gene transformations. You, Dr. Todd, have been invaluable in all the excellent help and reminders you provided, to obtain and coordinate the arrival of this Helios gun. Many thanks to you for your excellent organization and encouraging help.

We are delighted to make this report and to relay two months of progress in 1) the UCC collaboration, 2) other contacts and professional help, and 3) our own lab progress. We expect that our skills are now sufficient to add value to the UCC lab. We are actively working with Isaac and will guide them as we close in on the remainder of the needed skills. We plan to ship the Helios gene gun to UCC - Botany and Isaac Galuyon in early summer of 2000. At that time the UCC lab should have the tissue culture, plant regeneration, and gene preparation skills on hand. They will then use them with the Helios gene gun to improve cassava, the goal of this project.

Mr. Pefaur:

Cassava Improvement Project:

It is estimated that by the year 2050 the earth will have to support 11 billion people, 9 billion of whom live in countries around the equator . Cassava is a clonal shrub, grown throughout the equatorial world, which produces the starchy tuber upon which 500 million of these people depend for basic sustenance . Crop losses of 70-80% for Cassava are common and are caused by bacterial

blight, viruses, fungi and insect attack both in the ground and post harvest1 . The purpose of the Cassava Transformation Project, described here, is to provide Ghanaian researchers in the Department of Botany at the University of Cape Coast with the tools needed to improve their native Cassava cultivars (Manihot esculenta, Crantz). Only a few l laboratories worldwide are using molecular genetics to improve cassava. the laboratoriesâ goals for improvement are 1) resistance to West African Cassava Mosaic Virus3 and 2) reduction of cyanogenic content4 . Eastern Washington University has the only lab in the world attempting to transform Cassava with innate -immunity genes from insects as a method to increase the plantâs microbial resistance. This plant transformation task is my US thesis project and it is the culmination of a four year project funded by USAID and EWU through the university development linkages program between EWU and UCC in Ghana. The transfer of this technology to Ghanaian scientists will quickly bring them to the cutting edge of crop improvement capabilities. It is the foundation for future collaboration in biotechnology between UCC and Eastern, and other universities.

Specific Aims

Multiple skills are needed for the creation of transonic plants. The first is the development and maintenance of a comprehensive, plant tissue culture production system2,5. This is necessary for the development of target tissue, called Friable Embryonic Callus (FEC), and for regeneration of plants from this embryonic cell mass. Undergraduate researchers mastered these skills in 1997-98 and established a valuable collaboration with the leader in cassava tissue culture and transformation, Dr. Nigel Taylor. I expect to work with Dr. Taylor at the International Laboratory for Tropical Agricultural Biotechnology (ILTAB) in St. Louis later this year. During this visit, which is funded by an EWU Biotechnology travel grant, I will refine my skills in cassava tissue culture and in biolistic transformation.

The second aim of the project is the optimization of the transformation process, using the Helios biolistic gene gun from BioRad Corporation. Optimization of biolistic transformation, in general, is prerequisite to the efficient transformation of cassava callus with target genes6. Includes with the Helios gene gun, already purchased by USAID, is a small optimization kit with 250 mg each of l.0µm and 0.6µm diameter gold particles, and 50 feet of projectile cartridge tubing. This is only enough for 5 preparations of each size microcarrier, and will only allow 250 biolistic optimization events. Additional microcarriers are requested in the budget, allowing for the fine tuning of the optimization process, and the generation of transgenic plants.

The third aim of the project is the insertion of antimicrobial genes into cassava, and the verification of their stable transformation. The antimicrobial genes available are from the silk work, Bombyx mori, and the code for toxic peptides of about 35 amino acids7. Dr. Isao Morishima of Torrori University, Japan, has offered to provide these genes to us. He prepared Cecropin structural genes A and B as copy bacterium. The DNA genes will be re-cloned at EWU into plasmid constructs containing eletable markers of our choice. FEC tissue will then be bombarded with plasmid DNA-coated beads, using our optimized procedures. Following bombardment of cassava tissues by plasmid DNA containing Cecropin genes, the insertions of Cecropin copy-DNA genes into the plan genome will be verified by PCR.

Procedures and Rationale:

Tissue culture methods to produce adequate quantities of FEC use the recipes and methods of Dr. Taylor. He has advised us on a weekly basis and he trained our undergraduate, Kristin Suprak, last spring in his laboratory. Initial quantities of transformable have been produced in our labs and additional starts were received from ILTAB this month.

At each stage of the project, efficiency will be measured so an accurate representation of success can be made. This will start with survival and transformation efficiency at the FEC stage. Following biolistic transformation, the selected transformed FEC will be cultured to regenerate plants. Tissue culture success in this last phase of the project will be judged by the percentage of FEC embryos,

which regenerate plants, defined as organized structures bearing roots and cotyledons.

The biolistic process itself must be optimized prior to any attempt to insert genes. Optimization is the process of finding the gun settings that give the maximum number of FEC transformation events possible per bombardment. Transformation is a probability dependent event, in which only a fraction of successful bombardments result in selectable cells. This makes the determination of a global maximum
for the system variables essential for generation of transgenic plants.

The optimization of the Helios gene gun will be done according to the manual supplied. Five Helios gun variables will be used to optimize transformation efficiency of cassava FEC. Each variable will be tested over a working range of values, with replicates to increase statistical significance. The resulting efficiencies of the Helios gun will be compared to published transformation efficiencies for the older gun, the PDS-1000 system, currently in use by ILTAB. To our knowledge, we are the first laboratory in the world to use the Helios gun system for cassava callus transformation.

Transformation events are scored by histochemical staining of FEC cells expressing the ß-glucoronidase gene. ß-glucoronidase is not a naturally occurring enzyme in higher plants, so it is a good marker of successful biolistic transformation. The gene is inserted by bombardment with plasmid DNA containing the gene, which is followed by random homologous recombination events between the plasmid vector and the host genome. FEC is then soaked with a solution containing a substrate for the ß-glucoronidase, which colors the cells expressing the gene blue. These tissues are then embedded in phenolic glycerol gelatin to immobilize them, and the number of cells/cm2 expressing the gene are counted. in this manner it is possible to find optimal parameters to give the maximum number of transformants per event.

We have in our possession a plant plasmid called pLAU2-GUS, which is used for optimizing biolistic transformations. It contains two selectable marker genes coding for the NPT-2 gene, conferring antibiotic resistance, and the ß-glucoronidase gene. Previously published selection curves for antibiotic6 resistance in cassava culture will be used for positive selection of transformed cells, and success rate will be counted by gus staining. PLAU2-GUs is the same plasmid used by ILTAB for its cassava transformation events,.

The efficiency and degree of Cecropin gene expression in the transformed FEC will also be measured. This third gene in the transforming plasmid DNA is also the one giving useful, antimicrobial properties. Stable transformants, in this case, will be verified by DNA PCR, using Cecropin specific primers.