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5

Establishment of Functional Biotechnology Laboratories in Developing Countries

Marian D. Quain, James Y. Asibuo,
Ruth N. Prempeh and Elizabeth Y. Parkes
Council for Scientific and Industrial Research, Crops Research Institute, Kumasi, Ghana

1. Introduction

Traditionally, biotechnology is defined as making use of living organisms or genetic material from living organisms to provide new products for agricultural, industrial, and medical uses. This definition includes the use of fermentation in the leavening in the 10000 BC. This technology over the years has advanced into Modern Biotechnology. According to the Cartagena protocol (Secretariat of the Convention on Biological Diversity, 2000), Biotechnology is defined as any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or process for a specific use. According to the Convention on Biological Diversity, Art.3 (i), "Modern biotechnology" means the application of:

a. *In vitro* nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles, or
b. Fusion of cells beyond the taxonomic family, that overcome natural physiological reproductive or recombination barriers and that are not techniques used in traditional breeding and selection.

Plant Biotechnology encompasses tools such as tissue culture and molecular biology which are used in crop improvement. Although these technological tools are applied in advanced countries, their use in agricultural research and development in developing countries is limited. However, these countries need to enhance the utilization of tissue culture and molecular biology to increase agriculture productivity. The prospects of biotechnology as a modern tool for addressing various productivity problems and challenges in agriculture in the face of present day changing climatic conditions and starvation are now well known. Agriculture accounts for about 40% of Ghana's GDP, contributes 35% of foreign exchange earnings, and provides employment for over 60% of the population. More than 80% of the rural populations depend on it for their livelihood.

In recognition of the need to use biotechnology tools in agriculture in sub-Sahara Africa, in June 2003 at a Worldwide Ministerial Conference, in Sacramento, USA 112 Ministers of Science and Technology from 117 countries recommended the facilitation of access of Developing countries to Science and Technology innovations as means to reach Millennium

development goals (MDGs). As a follow-up to that, in June 2004, a West African Ministerial Conference on "the use of Science and Technology to improve agricultural productivity in Africa" was held in Ouagadougou (Burkina Faso). At this meeting, participants recognised the need to:

- Develop an information strategy on new technologies and especially on biotechnology
- Establish a strong scientific partnership between research institutions of Africa and developed countries
- Put in place a Regional Biotechnology Centre

Subsequently, in November 2004, there was the Economic Community of West African States (ECOWAS) Ministerial Conference on "Agriculture and Biotechnology" in Abuja (Nigeria) the meeting recommended the following:

- The need to establish Regional Biotechnology Centres of Excellence in countries having comparative advantages
- The promotion of *in situ* Research and Development activities on priority areas to support the emergence and growth of the biotechnology industry in West Africa
- The transfer of biotechnology product packages and their commercialisation in relevant areas
- The reinforcement of private-public sectors collaboration in order to boost the local Biotechnology industry
- The reinforcement of regional and national capacities in Biosafety

There was thus adoption of the Biotechnology and Biosafety Programme (BBP) Action Plan by the ECOWAS Ministerial Conference in Accra (Ghana), in May 2007. The BBP general objective is to use the development and exploitation of biotechnology products as means to increase agricultural productivity and competitiveness in West Africa

The Specific Objectives are to:

1. Promote the use of Biotechnology in agriculture
2. Develop a regional approach for Biosafety
3. Establish and make effective at the regional level, a mechanism for coordination of initiatives, fund raising and communication in the field of Biotechnology and Biosafety.

Thematic areas addressed in priority being:

- The application of Molecular Markers
- The application of Genetic Engineering
- The application of Molecular Diagnostics for animal and plant diseases
- Plant tissue/cell culture and micro-propagation techniques
- Vaccines for livestock production
- Animal Reproduction Technologies

This meeting was a significant landmark in the application of Science and Technology for improving performance in the agribusiness sector in the West African sub-region. This is because the meeting brought together biotechnology and biosafety experts, as well as the ECOWAS Ministers of Environment, Science and Technology, and Agriculture. At the end of the meeting, the communiqué issued indicated that the Ministers fully support the

application of biotechnology in addressing some of the numerous problems facing agriculture in Africa, particularly towards improvement in production, competitiveness and sustainable management of natural resources. Although the Ministers pledged their support, they stressed the need to have in place safety measures to ensure effective and sustainable application of the technologies.

All these initiatives have harnessed the application of modern techniques, however, in Ghana, utilization of biotechnology tools in agricultural research and development is associated with several setbacks: which may lead to nonfunctional and unsustainable laboratories. This paper thus focuses on how functional laboratories have been established in Ghana, with specific reference to some research output by the Biotechnology Unit of the Council for Scientific and Industrial Research (CSIR) – Crops Research Institute (CRI).

2. Establishment of biotechnology laboratories and some research output

2.1 Tissue culture

Tissue culture is the most applied biotechnology tool and its establishment is vital for the application of various techniques. By means of definition, plant tissue culture is the growth or maintenance of plant cells, tissues or organs or whole plant on a nutrient culture medium under aseptic conditins *in vitro*. Tissue culture employs the principle of "Totipotency" which is the cell characterestics in which the potential for forming all the cell types in the adult organsim is retained, or the capacity of differentiated cells to retain their full genetic potentialities and express them under appropriate conditions, or potential of cells or tissues to form all cell types and/ or to regenerate plants (Murch & Saxena 2005).

Listed below are applications and advantages of plant tissue culture

- Production of clones
- Large-scale plant multiplication
- Mutation-assisted breeding
- Induction of genetic variability- somaclonal variation
- *In vitro* selection
- International germplasm exchange
- All year round availability of tissue culture derived plants
- High commercial prospects – floriculture and vegetative crops
- Plants as a bioreactor for producing vaccines, and chemicals
- Saves time and space
- Long-term storage of elite genetic material
- Establishment of germplasm bank
- Labour oriented- employment generation, socio-economic impact
- Secondary metabolite production- medicine

Plant tissue culture techniques include:

- Anther/pollen/microspore/ovary culture
- Protoplasts
- Embryo rescue
- *In vitro* fertilization

- *In vitro* micro-grafting
- Micropropagation
- Somatic embryogenesis
- Callus and cell suspension
- Cryopreservation
- Cold storage
- Encapsulation
- Bioreactor
- Gene transfer

In Ghana, one of the first agricultural based research organizations to set up a tissue culture laboratory was the Ghana Atomic Energy Commission (GAEC) in the mid 1980s. This was followed by the Department of Botany of the University of Ghana, Legon in 1988, to train students. Subsequently, the Council for Scientific and Industrial Research (CSIR) Crops Research Institute (CRI) also established a tissue culture laboratory in 1996. All these set ups had to cope with interrupted supply of water and electricity, however, putting in place efficient water storage systems as well as bore hole and also standby generator for electricity helped solve these problems. Other setbacks included lack of regular and reliable source of consumables, glassware, equipment, equipment maintenance, not to mention source of funds, since limited funds were received from central government. However, it is worth mentioning that the CGIAR centers have been instrumental in training human resources and assisting with supplies through projects. The Consultative Group on International Agricultural Research (CGIAR) centers include the International Institute for Tropical Agriculture (IITA), International Center for Tropical Agriculture (CIAT), International Crops Research Institute for the Semi-Arid-Tropics (ICRISAT), just to mention a few.

When setting up the tissue culture facility of the CSIR-CRI, the laboratory initially used to share laminar flow cabinet with the microbiology research group. This was very frustrating since it took us a while to establish clean cultures. Once we established our ability to produce results in tissue culture, the laboratory in 1998 collaborated in research activities sponsored by German Technical Cooperation (GTZ), West Africa seed Development Unit (WASDU) for the production of clean plating materials of yam and cassava in West Africa (Quain, 2001). Another project with IITA with funding from GATSBY UK, produced clean *Musa* planting material for field evaluation and selection of hybrids with tolerance to the Black Sigatoka Disease in Ghana which started in 1998 this project resulted in the selection an release of two hybrid Musa species for release and utilization in Ghana. Also, in 1999, as the institute's sweetpotato breeding group worked towards the selection of varieties to be released to farmers under the Root and Tuber Improvement Project (RTIP), the laboratory with assistance from IITA, produced clean planting materials for multilocational trial. The sweetpotato varieties cleaned through tissue culture techniques in the laboratory, when established in the field was highly accepted by Agriculture extension officers and farmers. The tuber yield resulted in a 30% increase when compared with the conventional planting material (Otoo & Quain 2001).

Subsequently, the CSIR-CRI tissue culture laboratory has optimized existing protocols for local crop varieties, some of these recent research outputs include publications on the following: Multiple Shoot Generation Media for *Musa sapientum* L. (False Horn, Intermediate French Plantain and Hybrid Tetraploid French Plantain) Cultivars in Ghana

(Quain et al., 2010a). This paper considered plantains (*Musa sapientum*), a major staple in Ghana, which encounter several production constraints including availability of adequate healthy planting materials at the time the crop needs to be planted. In attempts to improve production, tissue culture methods were employed, using one medium. It was however realized that optimization of *invitro* rapid propagation protocol for mass production of different accessions of *Musa* was paramount. Excised buds from cultures with proliferating buds were used as explants in this experiment. The cultures of proliferating buds had been generated from excised apical meristem of four *Musa* varieties (False Horn; local names – Osoboaso and Apantu, intermediate French plantain; local name – Oniaba and FHIA 21, which is a hybrid tetraploid French plantain) which were cultured on Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) containing indole-3-acetic acid (IAA), citric acid, and 0-20 µM benzyl amino purine (BAP). The most popular local plantain variety, Apantu, only produced proliferating buds profusely when placed on routine medium (MS medium containing IAA, citric acid and 20 µM BAP). Reducing the concentration of BAP generated an average of more than 4 shoots/culture in 8 weeks. Medium not supplemented with any plant growth regulators also generated an average of less than 2 shoots/culture in 8 weeks. The other three *Musa* varieties generated 4-8 shoots/culture from proliferating buds, indicating that each cultivar has optimum concentrations at which rapid plantlet formation can be optimized to meet growing demands for planting material. This protocol has presently been adapted by the laboratory which produces about 3000 musa plants yearly through tissue culture for farmer, NGOs and interested organizations.

Other research activities have also established *in vitro* manipulation protocols for *Dioscorea* species (yam), which is a vital staple. In the yam tissue culture research, effect of various hormonal (growth regulators) combinations on *in vitro* sprouting of various species of *Dioscorea* spp under light and dark conditions (Ashun, 1991). *In vitro* studies on micropropagation of various yam species (*Dioscorea* species) (Ashun, 1996), indicated that where various concentrations of phytohormone Naphthalene Acetic Acid (NAA), 2,4-dichlorophenoxycetic acid (2,4-D), and BAP are used to culture *Dioscorea spp in vitro* using complete Murashige and Skoogs medium, the concentrations of 0.5µM and 5µM NAA, enhanced plantlet development. BAP concentrations of 5µM and above were lethal to explant development whereas 5µM and above NAA enhanced callus development in *D. alata* cv. 145 used. These studies also established that during yam explants initiation in culture with explants derived from vine, the age of the explants is critical. Explants aged two to 20 weeks were used in this study and for the different *Dioscorea* species used, the highest growth for *D. bulbifera* was in 2 week old explants, *D. dumentorum* were six weeks and four week old explants for *D. alata* (Quain & Achempong, 2001). *Dioscorea* species produce tubers and it is an important staple in Ghana and sub-saharan African countries. The above mentioned tissue culture studies therefore provide the basic tissue culture tools applicable in modern biotechnology, that can be used in the improvement of the crop in the sub-region.

Current tissue culture research activities are aiming at producing protocol for the *in vitro* manipulation of local bananas, plantains as well as various local root and tuber crops. The aim of these is to establish schematic mass production systems to benefit the commercial farmer. Protocols for long-term *in vitro* conservation of germplasm are also being optimized. The development of all these protocols will facilitate the adaptation of other modern biotechnology tools for the maintenance and improvement of local crop varieties to meet agricultural production constraints.

2.2 Molecular biology

Molecular biology is the aspect of biology that deals with the molecular basis of biological activity. This aspect of science is related with other areas of biology, chemistry, genetics and biochemistry. Mostly, molecular biology chiefly covers interactions between the various systems of a cell, namely between the different types of DNA, RNA and protein biosynthesis, and how these interactions are regulated. In 1961, William T. Astbury, made a statement that "molecular biology implies not so much a technique as an approach, an approach from the viewpoint of the so-called basic sciences with the leading idea of searching below the large-scale manifestations of classical biology for the corresponding molecular plan. It is concerned particularly with the *forms* of biological molecules and with evolution, exploitation, and ramification of those forms of the ascent to higher and higher levels of organisation. Molecular biology is predominantly three-dimensional and structural—which does not mean, however, that it is merely a refinement of morphology. It must at the same time inquire into genesis and function" (Astbury, 1961).

Molecular biologists have since the late 1950s and early 1960s learned to characterize, isolate, and manipulate the molecular components of cells and organisms. These components include firstly, DNA, which is the storehouse of genetic information. Secondly is RNA, which is a close relative of DNA with functions ranging from serving as a temporary working copy of DNA to actual structural and enzymatic functions, as well as a functional and structural part of the translational apparatus. Thirdly, are proteins; the major structural and enzymatic type of molecule in cells (Molecular Biology *Source*: http://en.wikipedia.org/w/index.php?oldid=417169395).

The aspects of biology listed below can all be found under Molecular Biology:

- Genomics
- Proteomics
- Molecular Microbiology
- Genetic transformation
- Molecular modelling
- Molecular breeding
- Molecular marker selection
- Mutation
- Bioinformatics
- Genetic fingerprinting

Following the successful establishment of tissue culture facility at the CSIR – CRI, it became apparent that a molecular biology laboratory be establish to complement the biotechnology activities. Project proposals developed under the then Agricultural Services Sub-Sector Investment Programme (AGSSIP) project secured the necessary basic equipment for molecular biology research. The project also contributed toward the training of two researchers; one in marker assisted breeding and another in genetic transformation in advanced laboratories. These scientists returned to Ghana, with the basic laboratory consumables to initiate the molecular biology laboratory. With assistance from a research assistant who had just completed using molecular techniques in disease diagnosis in a Ghanaian university, the molecular biology laboratory started operation in 2006 supported with inputs from the Generation Challenge Programme (GCP), CIAT and International Atomic Energy Agency (IAEA). In that same year, funds were also secured from the Government of Ghana to provide more equipment and consumables for the laboratory.

These gave the laboratory a sound basis to be really established. Since then the CSIR-CRI molecular biology laboratory has been locally adjudged the best biotechnology laboratory in Ghana and is yearly conducting training courses for researchers and students both locally and within the West African sub-region. Several research publications have been released and these include the following:

Assessing transferability of Sweetpotato EST SSR primers to cocoyam and micropropagation of nine elite cocoyam varieties in Ghana; Cocoyam, an important staple crop in Ghana, provides edible leafy vegetable and starchy cormels. Due to difficulty in getting primers for cocoyam, sweetpotato EST SSR primers were used to amplify genomic DNA of elite cocoyam lines. Genomic DNA was isolated from 10 sweetpotato and nine cocoyam cultivars. Ten sweetpotato accessions were screened alongside three cocoyam cultivars, using 22 EST Sweetpotato SSR primers, 13 of which could amplify cocoyam sequences and were subsequently used to screen nine cocoyam cultivars. Thirteen random primers were also used for diversity study. Cocoyam cultivars were established *in vitro*. Dendogram generated after screening cocoyams alongside sweetpotato, grouped sweetpotato varieties in two main clusters and cocoyam in one cluster. The random primers and the SSRs grouped the cocoyam into two clusters which corresponds with known morphological classification. The method would be used to screen large cocoyam germplasm (Quain et al., 2010b). Through this study genetic fingerprint of eight elite and one local check cocoyams has been documented. These fingerprinted cocoyam accessions are presently being evaluated on the field to establish their agronomic attributes and select some lines for release to farmers and the Ghanaian public for utilization. This will be the first time ever in Ghana that research output is releasing cocoyam varieties.

Genetic diversity of elite *Musa* cultivars and introduced hybrids in Ghana using SSR markers; in this study, molecular diversity was carried out on 10 *Musa* cultivars using SSR. *Musa* SSRs (49) marker was used for the diversity and NTSYS Data analysis used to establish conclusions on studies. Dendogram and similarity matrix generated, indicated that local false horn and intermediate French plantain are distantly related (16.78%). The closest related cultivars are two false horn (Apantu-Dichotomy and Osoboaso) at 70.32%. Similarity between introduced hybrids and local false horn plantains and local intermediate French plantains was in the range of 20.81–49.67% and 18.85–42.27% respectively. Apem (local intermediate French plantain) was distantly related to all the cultivars screened (16.78–36.84%). The information generated has documented diversity between the introduced hybrids and elite local cultivars and this will aid breeders mine for genes in the local cultivars that are responsible for earliness, peculiar taste and preferred cooking qualities (Quain et al., 2010c).

Genetic relationships between some released and elite Ghanaian cassava cultivars based on distance matrices has also been carried out (Acquah et al., 2010); Eleven (11) released and two local Ghanaian cassava cultivars were fingerprinted to estimate the genetic diversity among them using 35 SSR markers. Genomic DNA of thirteen cassava cultivars (*UCC,IFAD, Agelifiaa, Nyerikobga, Nkabom, Essam Bankye, Akosua Tumtum, Debor, Filindiakong, Afisiafi,Doku Duade, Bankye Hemaa* and *Bankye Botan)* were isolated and used as template for PCR amplification involving 35 SSR markers. The recorded gel bands (163 polymorphic bands) were subjected to NTSYS Version 2.1 software for cluster analysis and development of dendrogram to show the corresponding similarity coefficients. Genetic relationships between *Bankye Hemaa* and *Filindiakoh* and that between *Bankye Hemaa* and *Afisiafi* had similarity coefficients of 1.2%. The local cultivars, *Debor* and *Akosua Tumtum* were related at 52.31% similarity. *Filindiakoh* was found to be the relative to

Akosua Tumtum and *Debor* at 17.9 and 29.1% similarity, respectively. *Bankye Botan* and *Bankye Hemaa,* however, were distantly related to most of the cultivars, including the local varieties. *Bankye Botan* and *Bankye Hemaa* are distant relatives to most of the cultivars, including the local varieties which could however make these cultivars also very useful in breeding. This research work documented molecular information on released cassava varieties for the first time in Ghana. This information will contribute towards variety identification as they are released for utilization by farmers. The various research groups that have released cassava varieties over the years can also track the performance of their lines within the country and the subregion.

Groundnut is a member of the genus *Arachis* and the crop is divided into two subspecies and six botanical varieties based on morphological characteristics. A groundnut core collection of 831 accessions was developed from a total of 7432 US groundnut accessions based on morphological characteristics. Identification of DNA markers associated with the botanical varieties of groundnut would be useful in genotyping, germplasm management and evolutionary studies. A study was initiated to evaluate 22 groundnut genotypes representing six botanical varieties from a US groundnut core collection to determine their diversity using DNA microsatellites. Cluster analysis located the lines in their assigned specific botanical groups in agreement with available morphological classification for groundnut (Asibuo et al., 2010). Groundnut production and utilization in Ghana is presently very promising and selected groundnut varieties are produced for the confectionary industry. The output of this study will thus enable organizations that produce seed for farmers to cultivate scrutinize the stability and identity of their seeds.

2.3 Cryopreservation

Cryopreservation is a process of cooling cells, tissues or organs at ultralow temperature to preserve them indefinitely. The applied temperature is typically at −196 °C which is the boiling point of liquid nitrogen. Other temperatures applied in cryopreservation include -40°C, -70°C, -80°C, in programmable or ultra-low freezers, or in the vapor phase of liquid nitrogen at -150°C, or at -210°C in nitrogen slash. Technically, at these low temperatures, any biological, metabolic activity as well as biochemical reactions that would lead to the cell losing viability should cease and the preserved cell tissue or organ should be viable when retrieved from cooling. Due to the complex nature of cells, organs and tissues subjected to cryopreservation, other additives including cryoprotectants are used to prevent damage otherwise caused by cooling.

As the biotechnology research advanced, conservation of clonally propagated crops was identified as an important aspect. To facilitate the development of this technological tool, a PhD student worked on Complementary Conservation of Root and Tuber Genetic Resources - *Dioscorea* species and *Solenostemon rotundifolius*. The major focus of this study was the application of cryopreservation techniques to complement *in vitro* slow growth methods, since *in vitro* conservation under slow growth has been used for the conservation of clonally propagated crops. However, it demands periodic subculturing and regular attention and, with interruptions in electric power supply, conserved cultures are in danger of being lost. Presently the existing root and tuber germplasm conservation techniques serve a short to medium-term purpose only. The ultimate means of long-term conservation, which will complement all the existing modes being used and serve the purpose of base collections, is conservation in liquid nitrogen at –196^{o}C (Engelmann, 2000). Storage of biological material at ultra-low temperatures, preferably that of liquid

nitrogen, arrests all metabolic activities: consequently, no genetic changes occur, theoretically, permitting indefinite germplasm storage periods (Panis & Lambardi, 2005). However, in practice, although very long, indefinite (seed) storage may not be attainable (Walters et al., 2005) and this probably applies to other forms of germplasm as well (Benson & Bremner, 2004). The development of protocols tested three cryomodels: being vitrification–based, silica gel dehydration, encapsulation vitrification, as well as encapsulation desiccation. The study revealed that, the successful cryopreservation of *Dioscorea rotundata* which is an important crop producing mealy tubers is possible using a simple vitrification protocol. The procedure incorporates:

- pregrowth of the donor plant on 0.09 M sucrose-supplemented medium for five weeks
- preculture on 0.3 M sucrose supplemented medium for 5 d
- PVS2 solution for 40 min
- Rapid cooling in liquid nitrogen or slow cooling to -80^{o}C

Through this study, *Dioscorea rotundata* accession "Pona" which is an elite variety in Ghana was successfully cryopreserved for the first time. The technique developed was simple, cost-effective and potentially reliable methodology that does not require sophisticated equipment. The procedures can be adapted for germplasm conservation of other species, using limited resources in laboratories in sub-Saharan Africa. It was also brought to the fore that to achieve an optimal recovery of cryopreserved explants, the donor plants should be adequately conditioned. *S. rotundifolius* (Frafra potato) was extremely sensitive to the vitrification based protocol. The nodal explants used in the experiments easily becomes hyperhydric, and are impossible to dehydrate sufficiently for cryopreservation, this provide a sound basis for further attempts to cryopreserve Frafra potato genetic resources. Presently, cryopreservation tools are being developed further for other vegetatively propagated crops in Ghana.

2.4 Marker assisted breeding

Marker assisted selection (MAS) is indirect selection process where a trait of interest is selected, not based on the trait itself, but on a marker linked to it (Ribaut and Hosington, 1998). During selection for tolerance to an abiotic or biotic stress by means of MAS, the plants not on basis of quantified but rather a marker allelomorph (allele) which is linked with the particular trait (disease) is used to determine the presence of the trait (disease). The assumption being that linked allele associates with the gene and/or quantitative trait locus (QTL) of interest. MAS can be useful for traits that are difficult to measure, exhibit low heritability, as well as those that are expressed late in development.

Over the years most breeding activities in the CSIR-CRI have been mainly through selection, based on agronomic traits. However, since 2005, due to training obtained from The International Centre for Tropical Agriculture (CIAT), Cali Columbia, the cassava breeding group has initiated development of crosses to select varieties. Some of the breeding efforts are towards pyramiding genes responsible for tolerance/resistance to cassava mosaic disease using wild relatives from the centre of origin in South America into local Ghanaian accessions through mechanical hybridization. Other similar crop development to select varieties that can withstand biotic and abiotic stresses include drought tolerance in maize, pod shattering in soybean and disease resistance in peanuts. All these processes are being hastened by the utilization of molecular markers to shorten the number of years used in breeding from 10 – 15 years to about four to six years.

2.5 Plant disease diagnostics

Effective crop management involves the early and accurate diagnosis of plant disease. Early introduction of effective control measures in plant development can facilitate plant diseases management. Reliance on symptoms is usually not satisfactory in this regard. This is because the disease may be well underway when symptoms first appear, and symptom expression can be highly variable. Biological techniques for disease diagnosis and pathogen detection are usually highly accurate but too slow and not amenable to large-scale application. Recent advances in molecular biology and biotechnology are being applied to the development of rapid, specific, and sensitive tools for the detection of plant pathogens. (Miller & Martin, 1988). Some Immunological and nucleic-acid hybridization-based methods available for pathogen detection in crop systems are listed below:

- Immunoassay Technology
  - Enzyme Linked Immunoabsorbent Assay (ELISA)
  - Colloidal Gold
  - Immunofluorescence Assay (IFA)
  - Radio Immunoassy (RIA)
- Nucleic – Acid hybridization – Based Pathogen detection
  - Nucleic Acid-Based Detection Technologies
    - Dot – Blot Assay
    - Nonradioactive Labels
    - Restriction Fragment Length Polymorphisms (RFLPS)
  - Nucleic Acid Probes
    - Uncloned Probes
    - Synthetic Probes
    - Cloned Probes and RFLPS
    - Viruses and Viroids
    - Mycoplasma – like organisms and bacteria
    - Fungi
    - Nematode

Molecular biology tools that have been explored so far in our Laboratory include the application of molecular markers to screen for occurrence of disease in crops. This is being applied for African Cassava Mosaic Virus (ACMV) in cassava, Tomato Yellow Leaf Curl Virus (TYLCV) and Root Knot diseases in tomatoes, yellow mottle virus in rice and Sweet Potato Virus Disease (SPVD) in sweetpotato is still at the developmental stages. In a recent study on cassava, disease observations in the field were confirmed with laboratory diagnostics using the polymerase chain reaction assay. African cassava mosaic virus (ACMV) and East African cassava mosaic virus were detected on all the cultivars either as single infections or as mixtures. The detection of EACMV on cassava at Fumesua and Ejura is the first to be reported in Ashanti region in Ghana. This study recommended that, with the advent and spread of the EACMV serotype of the mosaic virus in important cassava growing eco-zones and the emergence of some severe strains of the African cassava mosaic in the pathosystem highly resistant planting materials should be used for ratooning of mother plants as one of the methods to increase the production of clean planting materials for farmers. It was also indicated that, there is also the need to conduct an extensive survey in all the cassava growing areas in Ghana to determine the incidence and spread of emerging species of the cassava mosaic begomoviruses virus in order to develop better strategies to reduce the menace of the cassava mosaic disease in Ghana and sub-region as a whole (Lamptey et al., 2011).

2.6 Genetic engineering

Genetic transformation, also referred to as Modern Biotechnology, is the application of the techniques of molecular biology and/or recombinant DNA technology, or *in vitro* gene transfer, to develop products or impart specific capabilities to organisms. Although the various breeding programs in Ghana are still using conventional breeding tools, efforts are being made to improve plants through an additional technique known as genetic engineering or recombinant DNA (rDNA) science. This method does not rely on the pollination of flowers: it allows individual genes with desire traits to be moved directly from one organism into another living DNA of the same or different species. This technique is very vital for the improvement of most of our local staple crops which are vegetatively propagated. Genetic engineering was first accomplished in the laboratory in 1973 (Paarlberg, 2008). However, as of 2011, laboratories in Ghana have not started applying rDNA in crop research activities. In 2008, a biosafety legislative instrument was passed in the Ghanaian parliament to permit confined field trials and contained laboratory experiment by research scientists. In 2009, a project to Strengthen Capacity for the Safe Management of Biotechnology in Sub-Sahara Africa (SABIMA), was initiated by the Forum for Agricultural Research in Africa (FARA) with sponsored by the Syngenta Foundation for Sustainable Agriculture (SFSA). This project identified personalities who can champion the advancement of Biotechnology in Ghana (Champions) and also focal persons to run the project. Following advocacy and awareness creation workshops conducted by champions in the SABIMA project, with focus on policy makers since 2010, the policy makers were incited to pay critical attention to the biosafety bill that has been before parliament. The members of parliament subsequently, took the biosafety bill through consideration in parliament, in February 2011 and it was passed into law in June 2011. In Ghana, there already exist a National biosafety committee (NBC) which has started receiving applications for conducting confined field trials (CFT). The three proposals in the pipeline for consideration by the NBC are:

1. Introduction of BT-Cowpea in Ghana
2. Protein quality improvement in sweetpotato
3. Nitrogen use efficiency and salt tolerance in rice

Other research efforts have been initiated in collaboration with advanced laboratories using crops of local importance, and these include studies on the transgenic potential of *Dioscorea rotundata*, using agrobacterium-mediated genetic transformation (Quain et al., 2011). This study considered *D. rotundata* (yam) which is one of the important staples and sources of carbohydrate in the diet in Sub-Saharan African sub-region. The crop has several post harvest problems including poor storage of the tuber, availability of the edible planting materials and high cost of labour for cropping. The crop therefore needs to be improved using modern biotechnology methods. Studies were conducted to induce shoot regeneration in *D. rotundata* leaf petiole as explants. Explants (0.5 cm long) were cultured on MS medium supplemented with 0.2 mg L-1 2,4-D for 3 days, and transferred to MS medium containing TDZ alone or in combination with 2iP. Shoot regeneration was observed within 21 days on all media; however, the highest shoot regeneration, 7–9 shoots developing per explant were obtained on media supplemented with TDZ and 2iP. The shoots grew vigorously when transferred to MS medium supplemented with GA3. When petiole explants were subjected to *Agrobacterium*-mediated transformation using strain C58 and EHA101 harbouring a binary plasmid containing the β glucuronidase (*gusA* or *uidA*) intron gene under the transcriptional control of CaMV35S promoter, very high efficiency of transformation (25–65%) was obtained. This successful organogenesis and transformation protocol could be

optimized and adapted in engineering of local yam quality and productivity for enhanced protein content, and longer shelf-life.

2.7 Advancements achieved in Ghana

To facilitate research in the application of biotechnology tools, Ghanaian researchers have partnered with regional, subregional and international programs. These include projects with financial and technical support from International Atomic Energy Agency (IAEA), Generation Challenge Program (GCP), United States Agency International Development (USAID), CORAF/WECARD, Syngenta foundation for Sustainable Agriculture, United States Department of Agriculture/Food and Agricultural Science (USDA/FAS), African Agricultural Technology Foundation (AATF), United Nations University – Institute for Natural Resources in Africa (UNU/INRA), World Bank, just to name a few. Collaboration with CGIAR centers as well as universities (Tuskegee University Alabama, USA, and Cornell University) has contributed immensely to recent research outputs, training human capacity, as well as technical and infrastructural capacities.

Presently, Ghanaian agricultural research organizations have graduated to the status of organizing national and regional training courses which hitherto were organized at Consultative Group (CG) centers in the subregion. This activity re-enforces the fact that both the human and infrastructural capacities have been developed to identify and solve regional problems.

Development of functional and sustainable Biotechnology systems needs to take into account `proper stewardship principle. Ghana is presently a participating country in the project to Strengthen Capacity for Safe Application of Biotechnology (SABIMA). This is an Excellence through stewardship based project ensuring the utilization of modern biotechnology tools is practiced in a responsible manner. Under this project, through stewardship training workshops, researchers are being trained to develop and properly document standard operating procedures as well as critical control points along the life cycle of product development. Biotechnology application policies are also being developed by the various institutes for implementation at the management level in the organizations.

Over the years, Ghanaian Universities had been training plant and animal breeders and researchers in related fields of applied and basic sciences, with limited or no knowledge in molecular biology. There is therefore a generation of Breeders and Research Scientists with little or no knowledge of molecular biology, tissue culture and modern biotechnology. However with the establishment of the West Africa Centre for Crop Improvement (WACCI), Alliance for Green Revolution in Africa (AGRA), Postgraduate Program at the University of Ghana at Legon, and Kwame Nkrumah University of Science and Technology (KNUST) respectively, a new generation of plant breeders with knowledge on the use of biotechnology in crop improvement are being turned out. This new generation of plant breeders is challenged to help solve African's problems on African's soil by developing crop varieties tolerant/resistant to biotic and abiotic stresses, and thus alleviate poverty in order to achieve the Millennium Challenge Goals. The Government of Ghana also assisted by providing funds for the establishment of Biotechnology laboratories in 2006, the CSIR – Crops Research institute and CSIR – Animal Research Institute. As a result, a new breed of lecturers, researchers and students are using conventional and biotechnological tools for crop and animal improvement. To us in the developing countries, some of the outmoded technological tools in the advanced countries are novel ideas. The use of laborious and

conventional breeding methods with its attendant long duration have given way to evaluation and selection using marker assisted breeding, mutation breeding, use of tissue culture to select somaclonal variants and disease elimination, production of clean planting materials, and mass production of clonal planting material by means of tissue culture.

3. Conclusion

The application of modern biotechnology in developing countries especially in Africa, has great prospects. All the necessary efforts have to be employed in the form of financing, policies, technologies, collaboration etc. These will help us to realize the inherent potentials and immense contributions to the scientific advancement worldwide. All stakeholders are needed to play their various roles to ensure the responsible application of biotechnology in developing countries. The case of Ghana with respect to the CSIR-CRI alone stated above gives the clear indication that, consistency, great leadership, team work, human and infrastructural capacity building, good networking and collaboration are keys to establishing a sustainable system. Presently, under the West African Agriculture Productivity Program (WAAPP), with sponsorship from the World Bank, a multipurpose biotechnology facility is being constructed to facilitate root and tuber research activities in the sub-region. It is hopeful that the impetus will keep building up, and more innovative strategies will be put in place to harness utilization of biotechnology tools in the sub-region.

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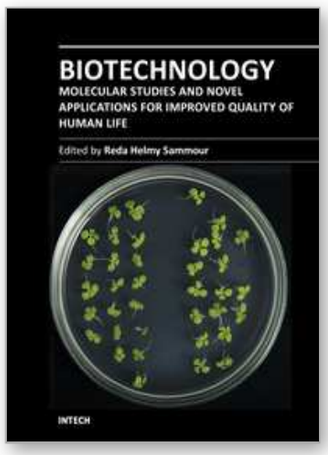

Biotechnology - Molecular Studies and Novel Applications for Improved Quality of Human Life

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This book deals with the importance of application of molecular biology as an approach of biotechnology for improvement of the quality of human life. One of the interesting topics in this field, is the identification of the organisms that produce bioactive secondary metabolites. It also discusses how to structure a plan for use and preservation of those species that represent a potential source for new drug development, especially those obtained from bacteria. The book also introduces some novel applications of biotechnology, such as therapeutic applications of electroporation, improving quality and microbial safety of fresh-cut vegetables, producing synthetic PEG hydro gels to be used as an extra cellular matrix mimics for tissue engineering applications, and other interesting applications.

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