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STATE OF THE ART OF BIOTECHNOLOGY RESEARCH IN ZIMBABWE

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ABSTRACT

The thrust of biotechnology research in Zimbabwe is to promote already developed technologies, especially for commercialised crops such as tobacco, wheat, cotton and sugarcane. This involves tissue culture techniques for disease elimination and rapid propagation, development of transgenic plants (tobacco) and development of biopesticides and biofertilizers. Work in the animal sector focuses on laboratory diagnosis of diseases, vaccine development and vector control. There is also an on-going study on the molecular biology of hepatitis B virus (HBV) and its involvement in the etiology of hepatocellular carcina (HCC).

Key Words: **Biofertilizers, biopesticides, tissue culture, vaccine**

RÉSUMÉ

L'idée maîtresse de la recherche en biotechnologie au Zimbabwe est de promouvoir des technologies déjà développées, surtout pour des cultures commercialisées telles que le tabac, le blé, le coton et la canne à sucre. Ceci implique des techniques de culture tissulaire pour l'élimination de maladies et la propagation rapide, le développement des plantes transgéniques (tabac), de biopesticides et engrais biologiques. Le travail dans le secteur animal se focalise sur le diagnostic, en laboratoire, de maladies, le développement de vaccin et la suppression de vecteur. Il ya aussi une étude en cours sur la biologie moléculaire du virus de l' hépatite B (HBV) et son rôle dans l'étiologie du carcina hepatocellulaire (HCC).

Mots Clés: **Engrais biologiques, biopesticides, culture tissulaire, vaccin**

INTRODUCTION

Biotechnology dates back thousands of years to the time when humankind began using micro-organisms for baking and brewing. The technology continues to be used in Zimbabwe and other countries in traditional food and beverage processing. Breakthroughs in recombinant DNA (rDNA) technology, which allows precise alteration of the genetic make up of a living organism, have revolutionised biotechnology; it now holds the promise of new and additional breakthroughs in the field of agriculture, food products, medicine, environmental clean-up and energy.

In Zimbabwe, biotechnology has the potential to play an important role in a wide range of fields. The use of biotechnology in veterinary diagnostics began as early as 1986, while its use in seeking solutions to crop production constraints gained momentum in the early 1990s. In the crops sub-sector, tissue culture was the pioneering discipline,

perhaps because of its relative ease, low investment level and low technology compared to genetic engineering and other molecular biology techniques. Several private and public sector institutions are engaged in tissue culture for the production of planting material for vegetatively propagated horticultural crops. Fermentation is routinely used in the food and beverage industry and rDNA technology is being applied in human health, veterinary medicine and crop improvement.

The government of Zimbabwe recognises the potential of biotechnology and is presently supporting the establishment of the Biotechnology Research Institute (BRI) of the Scientific and Industrial Research and Development Centre (SIRDC), a technology park mandated with providing research and development services to industry by the Research Council of Zimbabwe. This institute will undertake research and development activities in crop, veterinary, vitamins, vaccines and food biotechnology in response to the national needs within these sectors. The institute is expected to collaborate with both public and private research institutes. A Master of Science course in Biotechnology was launched at the University of Zimbabwe in 1991 in an effort to develop a critical mass of manpower in the field of biotechnology. This course is being offered jointly by the Departments of Biochemistry, Biological Sciences and Crop Science, and has a strong component of collaboration with institutions in developed countries.

While basic research and development underway in biotechnology, the thrust is to target the technologies that are already in existence and to adapt these to the national needs.

BIOTECHNOLOGY IN AGRICULTURE

Biotechnology is regarded as having an important potential to contribute to the national goals of agricultural research, which are to achieve and sustain self-sufficiency in food supply. The importance of agriculture to the Zimbabwean economy cannot be overemphasised. Agriculture contributes 13 - 15% of Gross Domestic Product and it is an important foreign currency earner. In the decade 1981-1991 it contributed, on average, 48% to the total foreign currency earnings (Anon., 1994). It provides food to the rural and urban population, provides jobs for 35% of the employed people, and is a source of 60% of the raw materials used in the manufacturing sector. The main thrust in biotechnology in Zimbabwe has therefore been to harness its potential in the agricultural sector.

The priorities for agricultural research in Zimbabwe are shown in Table 1. Considering the priorities, it is apparent that biotechnology has a role to play in addressing some of the most pressing problems of agricultural production. Of these, drought resistance in maize deserves special attention. Maize is the most important food crop and is one of the crops which is most severely affected by drought, especially in marginal areas where the majority of people who depend on it for food are situated. Pest control is also a major component of the cost of agricultural production in excess of Z$ 250 million being spent annually in the agricultural sector. With the increasing awareness of the potential hazard to the environment paused by persistent chemical pesticides, biotechnology is being viewed as having the potential to contribute to reduced pesticide use.

The ongoing research activities and the potential of several fields of biotechnology is described in relation to the constraints below (Table 1). Crops such as wheat, sugar and tobacco are sometimes not highlighted as part of the national goals for agricultural research. This is because they are produced by the sophisticated commercial sector, which is mostly privately owned, and research in all the problem areas is adequately undertaken by private research institutions which are well supported by the industries. Tobacco is the biggest foreign currency earner in the agricultural sector, and production is well serviced by a research institute (Kutsaga Research Station), funded by the Tobacco Research Board. This institution has since established a functional and well equipped biotechnology laboratory.

CROP PRODUCTION SECTOR

Tissue culture: virus elimination and rapid propagation. Tissue culture has gained a very important position as a technique for commercial production of planting material for high value horticultural crops. Techniques of virus

TABLE 1. Priorities for agricultural research in Zimbabwe

Crop/Livestock	Priorities	Focus of research activities
Crop sector		
Maize	High yield Drought tolerance Insect resistance	Improving yield potential
Small grains	High yield Nutritive factors	Improving yield potential Improvement of nutritive factors in red sorghum for use as feed Bird resistance in white sorghum
Cotton	Production costs High yield	Pest resistance (Bollworms) Yield potential
Groundnut	Quality, high yield	Yield potential High oil content High oil content
Sunflower	Quality, high yield Pest resistance	Yield potential High oil content Resistance to birds
Livestock sector		
Cattle	Diseases	Disease management
Small ruminants	Productivity	Improvement of fertility
Poultry	Productivity, disease	Improvement of fertility
Draught animals	Productivity	Disease management

elimination have been coupled with rapid propagation techniques and are being employed by several institutions in the public and private sector. Research work is ongoing on standardising *in vitro* growth conditions for various crops. Table 2 shows the institutions that are currently involved in tissue culture application and research in the country.

The ongoing work in the private sector is targeted at commercial large scale agriculture. The efforts by the Biotechnology Research Institute and the Horticulture Research Institute are meant to service the small scale sub-sector.

Genome reduction. This technique has been used to develop a cultivar of tobacco which is commercially important. There is a lot of potential for this technique in hybrid production, in view of the importance of hybrids in the production of maize seed (over 95% of the maize produced in Zimbabwe is grown from hybrid seed). Hybrids are also becoming important in sorghum, another important crop in Zimbabwe.

Molecular marker assisted selection. Molecular markers and linkage maps for use in selection in plant breeding programmes have been developed for a number of crops in various international institutions. The potential reduction of time spans required for the production of new crop varieties with incorporated traits can go a long way in increasing productivity. Although there are no ongoing projects in this field, plans are at an advanced stage to use molecular marker assisted selection to improve drought tolerance and insect resistance in maize. This work is a national effort being coordinated by BRI, and all stake holders are involved. This work is to be carried out collaboratively with the Kenya Agricultural Research Institute (KARI) and Centro International de Mejoramiento de Maiz y Trigo (CIMMYT) with the financial support of the

TABLE 2. Applications and research in tissue culture in Zimbabwe

Institution	Sector	Activity	Focus	Status
Crop Science Dept. (University of Zimbabwe)	Public	Disease elimination Rapid propagation Regeneration/ transformation	Irish potato, cassava sweet potato strawberry coffee, banana	ongoing
Horticulture Research Centre	Public	Rapid propagation Disease elimination	sweet potato	planning phase
Biotechnology Research Institute	Parastatal	Rapid propagation Disease elimination	sweet potato cassava, Irish potato strawberry	advanced planning
Tobacco Research Board	Private	Regeneration/ transformation Rapid propagation	tobacco other crops, (collaborative)	ongoing planning
Tissue Culture	Private	Disease elimination Rapid propagation	sweet potato banana Irish potato carnation strawberry	ongoing

Netherlands DGIS Biotechnology Special Programme.

Transgenic Crops. The absence of substantive guidelines on biosafety presents a hindrance to progress in the area of transgenics.

Cassava. The Department of Crop Science at the University of Zimbabwe is undertaking research on regeneration and transformation of cassava. To date, a system of regeneration of somatic embryos has been developed and multiple shoots have been induced from meristems. Histology work has been carried out to establish the actual cells from which these multiple shoots originate. The next stage is to devise a way of delivering DNA to these cells. Several possibilities to achieve this are being considered.

The ongoing work on cassava transformation is being carried out in collaboration with ILTAB. Transformation experiments involve particle bombardment using the Du Pont PDS 1000 He Biolistics system. The main targets for bombardment are leaf lobes and somatic embryos. Studies have also been initiated on the use of meristems as targets for both biolistics and *Agrobacterium*. Histology work has shed some light on where the DNA should be delivered.

Cotton. Plans are in the pipeline for the Cotton Research Institute to import *Bacillus thuringiensis* (*Bt*) transformed cotton lines for use in backcross breeding programmes. Initially, some lines were sent to the USA for studies on regeneration ability, due to capacity limitations in tissue culture at this institute. Results indicated that the lines were not easily amenable to *in vitro* manipulation, hence the need to import transgenic lines. The institute is trying to achieve this with the aid of ISAAA. In cotton, the issue of concern is the long term effectiveness of *Bt* resistance and its effect on pest biology. There is also need for monitoring resistance in this crop.

Cowpea. The University of Zimbabwe's Biochemistry Department is involved in the characterisation , sequencing and cloning of the cowpea aphid-borne mosaic potyvirus (CAbMV). The objective of this work is to finally confer resistance to CAbMV using the coat protein approach and transdominant lethal mutations in the viral replicase. This work is being carried out

with the aid of reverse transcriptase polymerase chain reaction (RT-PCR) techniques. Work is also ongoing in conferring atrazine-resistance into Zimbabwean crops using site directed mutations in the *psbA* gene.

Sweet potato. Research being undertaken on sweet potato is focusing on the identification and molecular characterisation of viruses which affect sweet potato in Zimbabwe. Progressive yield deterioration over the seasons is partly attributed to the effects of virus infection. Once the full repertoire of Zimbabwean sweet potato viruses is established, the main thrust of the project will be the development of virus disease control mechanisms based on conferring the resistance phenotype using genetic engineering techniques. Work on virus elimination is also ongoing.

Tobacco. The Tobacco Research Board has since acquired transgenic tobacco lines with the tabtoxin (*ttr*) self-protection gene cloned from *Pseudomonas syringae pv. tabaci,* and these are currently being used in greenhouse trials.

Epidemiology. Epidemiology work is ongoing in barley, an important crop in beer production. Reduced yields have been a problem in recent years and have been attributed to a seedborne fungus, *Pyrenophora teres.* Studies are being undertaken on the infection of seed, the prevalence of net blotch (as the disease on leaves is called), and to characterise the forms of *P. teres* occurring in Zimbabwe by molecular biology techniques. PRC methods are being used to amplify the DNA and RAPDs are being used to separate out strains. Use of PRC in diagnostics in other crops is to be initiated by the Plant Pathology Laboratory in the Crop Science Department, University of Zimbabwe .

PRC is also being used to detect *Pseudomonas solanacearum*, causal agent of bacterial wilt in a number of important crops including tobacco, tomatoes, and Irish potatoes. This technology is well developed and researchers from various private and public institutes are being trained to use this kit with the assistance of the Natural Resources Institute (NRI), UK.

Biopesticides. The Cotton Research Institute has been involved in research on the effectiveness of *Bt* proteins on local biotypes of *Heliothis* bollworms. Several *Bt* proteins have been used in greenhouse trials to control insect pests in cotton. *Bt* protein obtained from Monsanto was administered as a spray. Importation of transgenic coker lines is being actively pursued as follow up to this work.

Bayer Zimbabwe, a private company, is involved in the field testing of Xentari and Dipel (*Bt* products) for the control of *Heliothis* bollworms in tomatoes.

The Tobacco Research Board has carried out research on the use of *Trichoderma harzium* (strain 177) to control *Rhizoctonia,* which causes damping off of seedlings in several crops. This technology has been patented and its commercial use is being pursued by a local agro-chemical company.

Work has also been initiated on *Bt* genes producing proteins specific for mosquito and tsetse control. Bacterial expression has since been obtained and the projection are to get the genes to be expressed in yeast. These proteins will be insecticidal.

Biofertilisers. The Legume Inoculant Factory and its supportive microbiology laboratory have been operational since 1962. This is a public institution providing service to farmers in Zimbabwe as well as carrying out research on developing effective *Rhizobium* strains for the legume crops. The centre has collected and classified over 700 different strains of *Rhizobium* from the local legumes and from some other centres. It is producing legume inoculant for field use on soybean, groundnut, lucerne and Phaseolus beans. Research is ongoing on the appropriate inoculant strains for peas, sunhemp, velvet bean, clover and lupin.

The Soil Science Department at the University of Zimbabwe is also involved in *Rhizobium* research work.

Fermentation. Work on malting barley is being carried out in close cooperation with the breeders, with the aim of producing varieties with good malting qualities. Similar work is being conducted

on sorghum. Research is also being undertaken to investigate the possible use of some varieties of sweet sorghum to produce ethanol through fermentation.

BIOTECHNOLOGY IN ANIMAL HEALTH AND PRODUCTION

Biotechnology can contribute to improved animal health and production, improving meat and milk production through breed improvement, disease diagnostics and control. On going work on disease diagnostics and vaccine development in Zimbabwe is summarised in Table 3.

Laboratory diagnostics of diseases. In Zimbabwe biotechnology applications are widely used in disease detection, ranging from field applications in microscopy and cow-side mastitis testing in commercial dairy operations to more specialised laboratory testing operations such as microbial culture, serological testing for antibodies and antigens for a wide variety of microbial diseases, and histopathology.

Serological testing, being the broadest of these applications, includes such techniques as neutralisation, precipitation, fluorescent antibody testing and Enzyme Linked Immunosobent Assays (ELISA) of different types.

Vaccine development. A few vaccines, mainly those for tick-borne diseases, are being produced locally such as Bolvac for cattle theileriosis and the Babesia - Anaplasma vaccines for cattle. The production of these vaccines is based on biological production of stabilates in ticks for Bolvac, and as a whole blood preparation for the other two. While laboratory and field trials have shown the efficacy of these preparations, there remain problems of their safety and impact in the field. Concern about their field impact would arise from problems in the differentiation of the vaccine strains from a large mixture of other field strains, some of which are not pathogenic. Techniques are also still required to identify and characterise pathogenic strains which may differ from those already known, and to pick up carrier animals which are important in control. Concern about safety is related to the fact that these vaccines are live products with a potential to become virulent, and therefore require close monitoring.

Further development is necessary to produce subunit vaccines which do not carry a safety risk, and which could also be improved by being made polyvalent through such techniques as rDNA, while being made more stable for field use, as well as obviating the need to have multiple vaccination programmes. Presently, collaborative research into subunit vaccine is in progress for Heartwater and Anaplasmosis of ruminants.

Vector control. Principles of biotechnology have been successfully applied in vector control. This is now demonstrable in the control of tsetse fly, largely based on ox-odour attractants to targets impregnated with effective insecticide. Similar developments have also occurred in tick control, in particular the control of the amblyomma tick vector of heartwater.

Pheromone attractants have been successfully demonstrated to aggregate ticks in the field facilitating acaricidal control. However the problem of acaricide/insecticide resistance still requires a solution. Current research is attempting to show whether resistance is genetically programmed such that this could be used in strategising vector control.

Livestock breeding and production. Biotechnology applications in this area have been understood for longer than in many other areas. It has been used and continues to be used in artificial insemination, based on selected animals, and to assist in economic translocation of selected breeds over long distances, thus obviating the more difficult quarantine procedures for grown animals.

The assay of hormones that assist in determination of breeding cycles to aid breeding is another application of biotechnology with a potential of farmer use. This, however, remains a laboratory based technology. Other areas presently needing attention are the determination of genetic basis of disease resistance and certain kinds of hereditary and congenital diseases.

Phenotypic evidence of production traits of local breeds need further substantiation through studies of genomes. Knowledge about the genetic basis of productivity traits such as milk production,

TABLE 3. Summary of ongoing work in disease diagnostics and vaccine development in Zimbabwe

Disease	Research Activities
Bovine Babesiosis	Improved diagnostic tests Production of whole blood vaccines
Theileriosis	Development of diagnostic test using PRC Characterisation of parasites Production of monoclonal antibodies
Heartwater Project	Diagnostic tests using PRC, among other methods Development of effective vaccines
Anaplasmosis	Development of effective subunit vaccine and diagnostic tests

growth rates and feed conversion could facilitate breeding programmes to assist improved production.

Animal nutrition. Animal nutrition is dependent on microbial breakdown of ingesta in the stomach. This aspect is amenable to biotechnological innovations.

In Zimbabwe there is need to continually assess various animal feed and to improve their potential. In the search for cheaper alternative feed sources, biotechnology applications to feed digestion and digestibility studies are important. Although such studies have been on going, the potential for the introduction of new feed or pasture varieties would necessitate further studies into such nutrition studies.

Ecological studies in fisheries. Research is being undertaken to characterise phyllogenetic relatedness among the *Tilapia* species of fish using southern blot techniques. Because of the inadequacy of markers/probes from abroad, the Biological Sciences Department has developed its own probes which are giving more definitive results.

BIOTECHNOLOGY IN THE FOOD INDUSTRY

Apart from the routine fermentation that is involved in food processing, a salmonella probe specific to the genus *Salmonella* has been developed. The probe allows detection and diagnosis of salmonella in food and drink and in clinical and veterinary cases. Commercialisation and marketing of the probe is the next step, and a Zimbabwe Patent No 29/88 has been granted. The African Regional Intellectual Property Organisation (ARIPO) has also granted Patent No 98.

MEDICAL BIOTECHNOLOGY

Research is being conducted on the molecular biology of hepatitis B virus (HBV) and its involvement in the etiology of hepatocellular carcinoma (HCC). The project is attempting the characterisation of the HBV-DNA. This has been pursued by cloning DNA in the plasmid PBR 322 and propagating in *Escherichia coli.* While there might be other activities in the medical field, this work is most prominent and the capacity developed can be applied to service other sectors.

ENVIRONMENTAL APPLICATIONS

Research is going on in the development of immunoassays for the detection and quantification of pesticides in the environment. ELISAs are currently being developed for atrazine, DDT and methoprene. This work has been initiated by the Tobacco Research Board's Biotechnology Department.

It is apparent that biotechnology has an important role to play in the agriculture and other industries of Zimbabwe. With the rapid pace of development in the use of transgenics, the need for the urgent

development of risk assessment frameworks and biosafety regulations cannot be over emphasised. Zimbabwe is developing her own biosafety guidelines based on the experience of other countries such as Mexico (Alvares-Morales, 1995), the European Union (Dale, 1995; Frederick, 1995; Zannoni, 1995), and USA (Sivramiah Shantharam, 1995).

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