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19792

Distr.
LIMITED

IPCT.164(SPEC.)
24 July 1992

ORIGINAL: ENGLISH

UNITED NATIONS
INDUSTRIAL DEVELOPMENT ORGANIZATION

IDDA Expert Group Meeting on
Applications of Biotechnology to
Food Processing in Africa

Ibadan, Nigeria, 16-20 December 1991

APPLICATIONS OF BIOTECHNOLOGY TO FOOD PROCESSING IN AFRICA

Selected Papers*

* This document has not been edited.

V.92-55931

TABLE OF CONTENTS

CHAPTER		PAGE NO.
I.	Introduction	1
II.	Abstracts of Papers	13
III.	Selected Papers	
1.	The Indigenous Fermented Foods and Beverages of Sudan - H. A. Dirar	23
2.	Biotechnology and Food Fermentations - P. E. Cook	41
3.	Production of Wine from Cocoa Juice (Theobroma Cacao L. Kuntze) Using Saccharomyces Species Isolated from Palm Wine and Cashew Juice - H. O. Adesioye	56
4.	Bioconversion of Waste Materials to Food & Useful Products by Fungi - J. A. Buswell & S. T. Chang	61
5.	Industrialization of Lactic Acid Fermentation Technology of Cereals and its Dissemination to Developing Countries - C. H. Lee	76
6.	Soybeans: The answer to Malnutrition: The Impact of Soybean Processing and Utilization in Nigeria - S. M. Osho	84
7.	Uses of Biotechnology for Oil Palm Improvement - L. H. Jones	96
8.	Cytogenetics of Cassava and Related Species - K. V. Bai, S. K. Hahn and R. Asiedu	108
9	Biotechnology's Contribution to Cassava Production and Processing - A. I. Robertson	112
10.	Cacao: New Research Advances and Applications of Biotechnology - M. R. Söndahl	120
11.	Status of Cacao Research, Product and Biotechnological Advances in Nigeria - E. B. Esan	131
12.	Coffee: New Research Advances and Applications of Biotechnology - M. R. Söndahl	147
13.	Root and Tuber Crops Tissue Culture Research Activity at IITA - S. Y. C. Ng	159
14.	An Overview of IITA's Biotechnology Activities for Crop Improvement - G. Thottappilly, D. Vylsteke, S. Y. C. Ng, S. K. Hahn, G. Myers, R. Asiedu, N. Q. Ng, M. Bokanga, M. D. Winslow, K. V. Bai, and R. Terauchi	164

15. Biotechnology in Sub-Saharan Africa: Creating and utilizing endogenous technological capability - J. O. Mugabe 180

16. How to Realize the Potential of Biotechnology for Rural Small-Scale Producers in Developing Countries - J. E. W. Broerse 198

17. The Potential for Biotechnology in Africa - S. N. C. Okonkwo 217

18. Bridging the Gap Between Research and Applications in the Third World - R. Zilinskas 234

I. INTRODUCTION

After the development of microelectronics, the advent of biotechnology is often described as the second scientific and technological revolution of the 20th century. For centuries mankind has been making use of micro-organisms for the preparation of bread, beer, wine and cheese. However, the term biotechnology dates from the forties, when biological processes came to be understood. During the last two decades, biotechnology has taken on a revolutionary character as a result of the development of several fundamentally new biomolecular techniques. The impact of biotechnology is likely to be felt in many sectors, including agriculture and fisheries, human health care, environmental protection and industry. Although the advances in biotechnology and commercialization of their products were started mainly in the industrialized countries, they also offer a wide range of potential solutions to some of the basic food and health problems facing African countries (Okon, 1991; Okonkwo, 1991).

The realization of this potential very much depends on national and international policies towards national development and towards research and development (R&D) methodologies, and on the systematic participation of the end users, producers and beneficiaries in the development of these new technologies (Broerse et al, 1991). A first step is to bring an awareness to African countries about the opportunities and threats of the biotechnology revolution for development. To this end, an Expert Group Meeting on "Applications of Biotechnology to Food Processing in Africa" was organized by the United Nations Industrial Development Organization (UNIDO). It was held at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, from 16-20 December 1991. This article is a report of the Expert Group Meeting and is based on information from papers presented at the Meeting, the plenary discussions and results of the working groups

The Meeting reviewed current research work in improving production and processing of specific African food crops. The review included opportunities for biotechnology in (a) pure culture fermentations, enzyme treatment and food detoxification; and (b) crop production of food crops including cassava, oil palm, coffee and cacao. The Meeting also discussed constraints to the application of biotechnological advances in Africa, suggested solutions, and considered ways to link African institutions and organizations and existing network arrangements in specific fields of biotechnology. The meeting was attended by 44 experts from some 14 African countries, as well as experts from developing countries of Asia and Latin America as well as experts from Europe, the United States of America and IITA.

Opportunities of biotechnology

Technological change can be considered one of the prime carriers of economic development and a crucial factor in the socio-economic development of the Third World, provided that the technology is appropriate. The debate on the appropriateness of technologies for developing countries is a longstanding one. Negative experiences with capital intensive 'high-tech' projects have led many

to believe that Western technology is usually not appropriate for many developing countries. Such technology often competes with non-subsidized local technological activities. and does not make use of local resources (labour. local bioresources and indigenous knowledge). Since biotechnology is rooted in Western technology. its appropriateness for solving problems in developing countries is often questioned

A technology is generally considered appropriate when it both takes into account the prevailing conditions and needs of the target group, makes the best use of locally available resources and has little adverse socio-economic and environmental impact. On the basis of this definition the following points can be made about the appropriateness of biotechnology. In contrast to biotechnology R&D itself, many of its applications are inexpensive, uncomplicated and do not require capital- and energy-intensive inputs. Biotechnology is often flexible in scale and in the type of technology used, facilitating small-scale decentralized application and adaptation to the special circumstances in developing countries, and enabling linkage to indigenous knowledge, existing practices and local initiatives (Broerse et al, 1991). Moreover, biotechnology has great potential of solving several critical problems in Third World agriculture. Major impacts will be the improvement of traditional food processing techniques, introduction of new fermentation technologies, new diagnostics for pests or diseases, rapid micropropagation systems to allow the multiplication of new varieties and disease-free plants, and the development of new plant varieties. In the following paragraphs the current research work in food processing, mushroom cultivation, cassava, oil palm, cacao and coffee is reviewed.

Food processing

Food fermentations have developed in many parts of the world as a low cost method for food preservation. Fermented foods are particularly important in developing countries where canning, refrigeration and freezing may be limited or unavailable (Cook, 1991). Food fermentations usually require little energy and improve the nutritional value of foods by bio-enrichment with microbial protein, amino acids, lipids and vitamins. Fermentation processes can also contribute to the degradation of toxins and antinutritional factors present in many plants. Furthermore, the organoleptic properties of fermented foods often differ from the unfermented substrate; in addition to flavour and aroma, the physical form, texture and colour may change (Cook, 1991). Well-known African fermented foods include Nigerian ogi (a weaning food produced by the fermentation of maize, sorghum and millet), Kenyan uji, Nigerian gari, fufu (fermented cassava), sorghum (Bantu) beer, iru (fermented locust beans, cowpeas, soybeans or benniseed), and m'bannick (a drink obtained by fermentation of whole cow milk). Most of the fermented foods of Africa have not been well documented. An inventory study in Sudan showed that there are at least 60 different fermented foods and beverages in Sudan alone (Dirar, 1991). Fermented food culture in Africa is a deep-rooted, ancient culture and the major sources of information are the rural elderly women. The bulk of the fermented foods has been developed as part of a strategy to cope with chronic food shortage and survival problems (Dirar, 1991). Most of the traditional food fermentation processes can be upgraded, expanded and improved. Current research on food fermentative organisms is mainly undertaken in areas with a large industrial base (beer, bread, dairy, meat, soy sauce) (Cook, 1991). On small-scale, indigenous food fermentations little research has been conducted. Therefore, biotechnological research on fermented foods of Africa should be preceded by a thorough documentation of the foods and their preparation procedures (Dirar, 1991).

Biotechnology R&D can be applied at two levels: process engineering level and microbial starter level. At process engineering level, much progress could be achieved by the use of basic techniques such as the design of simple fermenters both for liquid and solid state fermentations. Especially in solid state fermentation systems improvements in the field of microbial growth, behaviour and product formation are required (Cook, 1991). At microbial starter level, selection techniques can be used to 'improve' strain properties whereas the development of mixed culture systems may offer advantages over the use of single strains. At the moment, little benefit is expected from improving starter cultures using genetic modification techniques because this may offer little advantage over existing naturally selected mixtures of strains (Cook, 1991). The extensive study of traditional fermentation processes and their micro-organisms offers scope for new industrial fermentation processes. Hundreds of micro-organisms are involved in the fermentation of foods in Africa. This is a great microbiological resource, and a diverse range of products can be derived directly from these micro-organisms or produced as a result of their activity. These organisms may be sources of useful components such as enzymes, uncommon lipids, pigments and flavour compounds (Cook, 1991; Dirar, 1991)

Mushroom cultivation

The cultivation of edible mushrooms embodies the conversion of domestic, agricultural, and industrial organic wastes into digestible nutrients, such as proteins and vitamins through solid state fermentation. It is considered one of the most economically viable processes for the bioconversion of lignocellulosic waste materials (Buswell, 1991; Chang and Miles, 1991). Huge quantities of these materials are generated annually and much of it is either burnt, shredded and/or made into compost. The technique for cultivation of mushrooms can be simple and low-tech, as in rural farming of *Volvariella* and *Pleurotus* mushrooms, but it can also be highly sophisticated involving the use of advanced technology and equipment, as in *Agaricus* and *Lentinus* production in urban areas (Chang and Miles, 1991). Currently, edible mushrooms are mainly cultivated in industrialized countries and a few Asian countries. Although hardly practised in Africa , mushroom cultivation offers much scope to enrich the human diet with high quality protein and to increase the economic welfare of the people in this region (Buswell, 1991). Relatively little is required in terms of equipment, facilities, capital and land. The substrate residue, left after mushroom harvesting, can be converted into feedstock for ruminants and/or used as a soil conditioner. The introduction of mushroom cultivation in Africa should start with the selection of mushrooms acceptable to the consumer taste in the region. From these mushrooms, cultures with the genetic capacity to form fruiting bodies under suitable growth conditions could be selected (Buswell, 1991). The next steps involve the development of spawn, the preparation of compost, and mycelial (spawn) running.

Cassava

Cassava (*Manihot esculenta*) is the fourth most important African staple crop. Cassava is mainly grown by women for subsistence purposes, because of its efficient production of food energy, year-round availability, tolerance to extreme stress conditions (e.g. drought and poor soils), and suitability for present farming systems (Hahn, 1989; Robertson, 1991). Cassava tubers are a valuable source of starch and its leaves are a good source of protein and vitamin. Processing of cassava tubers and leaves is essential because they are not palatable in raw form, they contain varying amounts of cyanide which is toxic to humans and animals, and the fresh tubers rot within 3-4 days after harvest

(Hahn, 1989). The future of cassava as a commercial crop will depend much on its suitability for alternative uses such as animal feed and industrial raw material: e.g. the industrial use of cassava starch for the production of adhesives, paint, and ethanol (Robertson, 1991). Improvement of cassava production and processing using biotechnology can increase labour efficiency, productivity, and standard of life of farmers and the urban poor, as well as improve the shelf-life, quality, and marketing opportunities of products (Hahn, 1989). However, not much research is focused on cassava; only CIAT and IITA give it serious attention (Mugabe, 1991)

Tissue culture methodologies, particularly meristem culture, are routinely used to eliminate virus infection from improved clones of cassava and to micropropagate them (Hahn, 1991; Robertson, 1991). So far, large-scale commercial micropropagation of cassava has not been undertaken in Africa. Embryo culture techniques for the germination of immature cassava embryos are being developed (Hahn, 1991). A monoclonal antibody test is available to identify African Cassava Mosaic Virus (ACMV) (Robertson, 1991). Genetic engineering of cassava is constrained by the lack of appropriate regeneration systems and transformation methods (Hahn, 1991; Robertson, 1991). Callus cells have been successfully transformed and transient expression has been demonstrated, but no regeneration has been achieved. Research is being conducted to develop cassava varieties that contain resistance against ACMV by inserting a viral coat protein gene, that contain insect resistance by inserting *Bacillus thuringiensis* toxin gene, that have relatively low levels of cyanide and that have modified protein content (Mugabe, 1991; Robertson, 1991). Restricted Fragment Length Polymorphism (RFLP) is applied to map the cassava genome. Biotechnology could improve traditional cassava processing by use of more efficient strains of micro-organisms associated with fermentation. It could also contribute to optimizing the conditions for large-scale industrial fermentation of cassava starch (Hahn, 1991; Robertson, 1991).

Oil palm

Oil palm, which originated in West Africa, has been cultivated in plantations since the beginning of this century and is an extremely profitable commercial crop. The world production of palm oil is nearly ten million tons and among the major producing countries are Nigeria, Côte d'Ivoire, Cameroon and Zaire. Oil production can be increased by a variety of techniques including biotechnology.

Tissue culture techniques are available for rapid micropropagation of elite oil palm clones. Commercial development of this technique has been seriously delayed by the appearance of abnormal flowers in some clones (Jones, 1991). Intensive work on this problem over the past few years has shown that there has been a semi-heritable change in gene expression relating to flower development which is induced in the culture stages. By changing the culture conditions and by avoiding friable 'fast-growing callus' there will probably be little risk of abnormal development (Jones, 1991). There is optimism that large-scale production of clonal oil palms can soon be resumed. Genetic engineering of oil palm is constrained by the lack of an appropriate regeneration system and transformation method. Although successful formation of calluses from oil palm protoplasts was reported, no regenerant plants were obtained. Transformation of oil palm cells with foreign DNA has not been reported. The RFLP technique is now in routine use for clonal typing and a start has been made in constructing an RFLP map of the oil palm genome (Jones, 1991). The value of genetic engineering as a useful source of new variation for the oil palm breeder is considered doubtful. Since

oil palm is a highly heterozygous outbreeding species there is much variation available within the existing germplasm, which can be most efficiently exploited by using conventional breeding techniques combined with clonal propagation (Jones, 1991).

Cacao

Cacao (*Theobroma cacao*) is the third largest agricultural export commodity in Africa and is a vital foreign exchange earner for several countries, including Kenya, Tanzania, Côte d'Ivoire, Ghana, Nigeria and Cameroon. These countries produce two-thirds of the world output of cacao and cater to about 60% of the export market. A cacao pod has ca. 40 seeds which are utilized to prepare chocolate after defatting, roasting and grinding (Söndahl, 1991a). The modern use of the word 'cocoa' refers to the drink made from its seeds and the word 'cacao' refers to the tree. Cacao cultivation faces numerous problems with diseases and pests and the lack of high yielding clone materials which could be solved with the help of biotechnology.

Biotechnology R&D in cacao is still in its infancy. There have been a limited number of studies on tissue culture of cacao. It has been possible to initiate callus but regeneration has failed so far. Progress has been made to complete germination and plantlet development from cacao somatic embryos. This somatic embryogenesis process opens the door for commercial rapid micro-propagation of elite cacao plants, although the process still needs considerable improvement to allow its large-scale utilization (Söndahl, 1991a). Somatic embryogenesis could also be used in genetic engineering of cacao. Very little, however, is known about the genetics of useful characteristics such as yield factors, vigor and disease resistance in cacao (Söndahl, 1991).

Coffee

Coffee is a beverage prepared from seeds (beans) of *Coffea* species after roasting and grinding. The genus Coffea has approximately 100 species of which only two are of commercial importance: *Coffea arabica* (Arabica coffee) and *Coffea canephora* (Robusta coffee). Coffee is an important foreign exchange earner for more than 50 countries located in tropical regions of Latin America, Africa and Asia. Commercial production of coffee has been greatly improved by plant breeding during the past 50 years, but conventional breeding is limited by the genetic barrier of chromosome number, auto-incompatible alleles and long-term breeding cycles (at least 24 years for the release of a new variety) (Söndahl, 1991b). Biotechnology could provide a valuable contribution to coffee production by counteracting frequent disease and pest outbreaks as well as by improving yields. Additionally, biotechnology can provide ways to introduce specialty coffee brands in response to market niches (Söndahl, 1991b).

In tissue culture, successful regeneration via somatic embryogenesis has been achieved, and embryo rescue has been used for the recovery of interspecific crosses. Regeneration from embryogenic cell suspensions and isolated protoplasts of Arabica and Robusta genotypes has been described. Large-scale micropropagation is now feasible through the use of embryogenic suspensions in Erlenmeyer flasks or bioreactors (Söndahl, 1991b). Genetic transformation using the protoplast uptake method has been reported. This will enable the utilization of genes for coffee improvement on short or medium term. Research on somaclonal variation in coffee breeding indicates that this method is a promising tool for shortening coffee breeding programmes (Söndahl, 1991b).

Constraints

Biotechnology has been developed in industrialized countries in governmental institutions. The private sector, too, has become interested in biotechnology and has attained a dominant position in biotechnology, *inter alia*, based on protection by intellectual property rights (Broerse et al, 1991). In most industrialized countries, governments stimulate and influence development in biotechnology through specific biotechnology programmes which are, by and large, guided by economic considerations of large corporations and governments. Hence, the focus of biotechnological R&D in industrialized countries is on resource-rich producers and large-scale urban industries, and directed towards crops, animals, diseases, etc. that are of (economic) importance in these countries (Broerse et al, 1991; Okonkwo, 1991). It has been estimated that less than 1% of the research in industrialized countries has direct relevance to the Third World and some of it even has a negative impact (Okonkwo, 1991; Zilinskas, 1989). If research priorities in industrialized countries are not redefined, and if developing countries are not able to influence the development of innovations to meet their own needs, biotechnology will not bring its heralded benefits to the Third World. To ensure that the expected benefits of biotechnology actually accrue and that the negative impact is limited as much as possible, African countries should take steps to build research capabilities. Most African countries, however, face some serious constraints in this respect. In the next paragraphs the main constraints will be discussed.

Privatization of knowledge and technology

Industrialized countries have extensive intellectual property protection regimes. On-going discussions in the USA and Europe and on an international level are considering to extend their current patent system to include genetically modified micro-organisms, plants and animals. While the strengthening of intellectual property regimes may have some positive impacts on innovation in general, it limits the ability of African countries to acquire and use biotechnological innovations (Mugabe, 1991).

Lack of funds, trained manpower and deficient infrastructure in Africa

In biotechnology, research and development are closely linked. It is also multi-disciplinary and requires a coherent team of qualified specialists - from molecular biologists to biochemists, process technologists and technicians - in order to ensure the design, development and application of any biotechnological innovation. The majority of developing countries are short of funds, and have a shortage of trained personnel and a deficient infrastructure which makes it very difficult for them to establish a critical mass in biotechnology R&D. Between 1960 and 1980, real expenditure per African researcher has decreased by 25%. In contrast, in developed countries, expenditure per researcher increased by 70% in the same period and from a much higher baseline (Broerse et al, 1991). Manpower with the specialized skills for modern biotechnology is grossly inadequate; many African scientists do not hold postgraduate qualifications and most African institutions are understaffed (Okonkwo, 1991). In terms of general infrastructure, African countries usually lack adequate laboratory space and equipment. Even if equipment is available, spare parts and maintenance personnel are often unavailable. Other problems are the unavailability of rare chemicals like enzymes and radioisotopes, and unreliable water and electric power supplies. Another major drawback is the lack of relevant literature and data base access. Mainly non-sophisticated biotechnologies are being and can be undertaken in Africa at the present time (Okonkwo, 1991; Zilinskas, 1989).

Lack of commitment

African countries continue to treat technical change as an exogenous activity of their socio-economic development. Many governments in African countries are either unaware of or insensitive to the contributions being made by their indigenous scientists in science and technology (Okonkwo, 1991). Though the need to engage in different technological innovations is acknowledged in development plans of several African countries, the actual support for science and technology is meager (Mugabe, 1991). In this context it is not surprising that biotechnology does not receive much attention either. Many policy makers in Africa are not even aware of the potential benefits and threats associated with biotechnology. At the moment none of the African countries have initiated biotechnology policies and programmes geared to national priorities.

Gap between research and application

Many African countries are to some extent engaged in biotechnology research activities, but little of this research is demand-oriented and integrated in national development policies. Nearly all biotechnology-related activities are driven by the interests of individual researchers who have been able to lobby successfully for support. Many researchers are, however, more interested in career advancement than in increasing capabilities in development or industry. Traditionally, little applied research is being undertaken at universities; the role of scientists in universities is to teach and perform basic research (Zilinskas, 1989). Most of the applied research takes place at public research institutes, but few innovations are successfully adopted since the work is often inappropriate or of low quality. There is little association between research units and potential technology end-users; links between research, extension, producers and the market (consumers) are poor in most African countries (Mugabe, 1991; Zilinskas, 1989). Consequently, researchers and policy-makers are often ignorant of the problems and needs of industrial and agricultural producers, and priorities and goals are set through conclusions drawn from own theoretical models and value systems of what ought to be appropriate and not by involving end users directly in the process of problem formulation. As a result some of the most impeccable 'practical' pieces of research end up with disappointingly few adopters. Meanwhile the majority of African producers continue to rely on foreign technology suppliers or rely on their own 'informal' knowledge and research systems (Broerse et al, 1991; Zilinskas, 1989). If the gap between research and application is not successfully bridged, biotechnology research will do little of practical value for African people and nations. Lack of achievements may in the end result in a complete lack of support for biotechnology R&D activities from both governments and donor agencies (Zilinskas, 1989).

In sum, many African countries are hardly capable of establishing a critical mass in biotechnology R&D. Technology transfer from industrialized countries to Africa is constrained by an increasing privatization of biotechnological innovations. Moreover, due to lack of funds, trained manpower and a deficient infrastructure, it is difficult to initiate an endogenous biotechnology R&D. From African governments there is inadequate support for science and technology in general and for biotechnology in particular. Biotechnology R&D that is conducted often has little practical relevance for industrial and agricultural producers due to lack of communication between researchers and technology end users.

S lutions

In this part of the article, the necessary conditions for the successful establishment of a local research capacity in biotechnology will be discussed. These include policy issues, education and training, acquisition and exchange of information, and international support.

Policy issues

A first step to ensure that effective policy measures to create an enhancing environment for the development of appropriate biotechnology will be considered is to create an awareness in policy makers as well as the public in Africa on the potential benefits and threats of biotechnology. At the Meeting the importance for establishing national and regional biotechnology R&D programmes was stressed. It should, however, be ensured that such programmes and their products meet real needs of the countries they serve. This requires a demand-oriented approach to technology development. This implies that the objectives of governments should not be to build a biotechnology capacity at all costs but to target socio-economic development first and then to consider any possible role for biotechnology (Broerse et al, 1991). For example it is not advisable to replace old techniques with modern ones just because they are new and exciting; they should be worthwhile in terms of cost and productivity (Jones, 1991). For many African countries it would be advisable to first get involved in less sophisticated biotechnologies that can yield short-term benefits, and when local research capabilities and infrastructure improve, embark on more advanced and expensive biotechnologies (Okonkwo, 1991). Policy makers in African countries should be aware of not only the benefits of biotechnology but also the risks, especially with respect to genetic engineering and the environmental release of genetically modified organisms (GMOs). Risk assessment of GMOs is still in its infancy. While research to improve risk assessment is being conducted, many industrialized countries have instituted strict regulations on the handling of GMOs in the laboratory and their environmental release. African countries have no legislation on GMOs at the moment. To guide the experiments involving recombinant DNA technology, African countries should take steps to establish regulations and legislation on biosafety (Okon, 1991; Okonkwo, 1991).

Education and training

It is necessary to increase the quantity and quality of scientific personnel in the relevant scientific fields in order to be able to conduct more advanced biotechnology R&D and training activities. African countries should revise the curricula of their national colleges and universities so as to include courses in modern biotechnology. Currently, only the University of Zimbabwe has developed a special Masters of Science degree programme on biotechnology. In addition, it is advisable to organize, on regional or inter-regional basis, intensive training courses on basic and advanced techniques used in biotechnology R&D (Okonkwo, 1991).

Information acquisition and exchange

Since biotechnology is quite knowledge intensive, access to information is extremely important. Efforts should be made to order relevant books, subscribe to international scientific journals, and to establish computer links to acquire access to data bases (Mugabe, 1991; Okonkwo, 1991). Also the exchange of information between scientists is important. To this end, national biotechnology societies should be established. Through scientific activities such as annual

conferences and seminars, the societies will be able to catalyze and build up research strength, provide a forum for information exchange and discussion, collaboration and fast achievement of results without duplication of efforts (Okonkwo, 1991). Networks in biotechnology R&D are beginning to develop in Africa, e.g. the African Plant Biotechnology Network (APBNet) which was established in January 1989 (Okonkwo, 1991). In addition, the exchange of information between different organizations and social groups is crucial. Scientists, expert consultants, donor organizations, policy makers, extension workers, entrepreneurs and farmers often have different perceptions of what the problems and their appropriate solutions are. All these groups have specific relevant expertise, but lack other types of useful knowledge. Without effective exchange of information it is possible neither to plan nor to coordinate the necessary activities, thus strongly hampering effective decision-making. Providing a platform of discussion between these groups will provide a more balanced view of opportunities and threats, and could reduce the gap between research and application (Broerse et al, 1991).

International support

Development through international cooperation offers an important means of realizing the benefits of modern biotechnology. International organizations and donor agencies can provide help to policy makers, support capability building in research (provision of technical information, training opportunities, and funds to purchase major equipment and chemicals), and provide backing for the establishment of (inter)national networks (Zilinskas, 1989). Since international cooperation is required in enforcing biosafety regulations, this is an area in which international organizations and donor agencies also have a role to play. A number of organizations are already involved in promoting and supporting biotechnology activities in developing countries, e.g. Overseas Development Organization (United Kingdom), Directorate General for International Cooperation (The Netherlands), Food and Agriculture Organization (United Nations) and of course UNIDO. In 1982 UNIDO established the International Centre for Genetic Engineering and Biotechnology (ICGEB). ICGEB provides a research and training programme as well as a wide range of services, including access to databases, technical support and advisory services, to its Member States. ICGEB is not yet an autonomous intergovernmental organization. Until ratification by 24 Member States, the development of ICGEB remains under the auspices of UNIDO and is managed as a project (UNIDO/ICGEB, 1990).

Conclusions and recommendations

It can be concluded that African countries stand to benefit from biotechnology by building a local R&D capacity for application of biotechnology to food production and processing. The sustainable development of biotechnology in Africa can, however, not be achieved unless African governments commit themselves to actively support capacity building. Furthermore, the development of capabilities in biotechnology in Africa should be integral to endeavors made by governments to achieve their overall objectives and not be an end in itself. This, in turn, means that policy decisions made by governments on biotechnology issues should be taken to serve the socio-economic objectives of the country in the best way possible. The benefits and risks must be considered and priorities set, starting with simpler biotechnologies that address the most pressing problems and move on to more sophisticated ones when the infrastructure improves (Okonkwo, 1991). Special attention should be paid to reducing the gap between research and application so as to ensure that the results of the biotechnology R&D efforts will contribute to the socio-economic development of African

countries. Recommendations of the Expert Group Meeting were formulated in four working groups: African foods, transfer of technology, networking and training, and biosafety.

African foods

The group stressed the importance of food fermentation as a low-cost food processing method, its improvement in the nutritional value of foods, its ability to convert inedible commodities into palatable foods and its potential to produce value-added materials. The group emphasized the need to survey existing indigenous food fermentation technologies on country and on regional level and to classify the fermented products on the basis of the technologies involved in the substrates. Research is needed to promote simple processes, to achieve the industrialization of major fermented foods, to mechanize labour-intensive processes, to develop or expand the use of starter culture, and to achieve quality standardization.

Technology transfer

The strong development of science in Africa necessitates that the region acquires technologies from industrialized countries and that results and findings from endogenous R&D is effectively transferred to the applied sectors. A number of requirements must be met to effectively transfer science and technology:

- The biotechnological capabilities of local universities and research institutions must be strengthened by governments and international agencies providing, *inter alia*, training opportunities to researchers, adequate research facilities and equipment, and improvements to the overall research infrastructure.

- Basic and applied research needs to be integrated and the strategic role of basic research must be fully recognized by governments and international agencies.

- Professional societies should take on the responsibility of informing and assisting policy makers and legislators on the significance of scientific/technical matters, and on their social and environmental implications, so they will be well prepared to adopt and implement policies and strategies for effective technology transfer.

- To enhance the linkage between the research establishment and the applied sectors, measures must be taken by professional societies, industrial interest groups, farmers' associations and governments to promote direct contacts between researchers and technology end users. African universities should consider setting up technology transfer units whose tasks would include presenting results and findings from university research to technology end users and brokering joint cooperative R&D projects between the two.

- Non-governmental organizations should be strongly encouraged to take on the role of sensitizing government officials, legislators, technology end users and the public to the practical problems and social consequences of technology transfer. If appropriate non-governmental organizations do not exist in a country, their establishment should be supported.

- There is a strong need to set up a regional bioinformatics network,

including E-mail and CD ROM to facilitate African researchers accessing scientific/technical information and communicating with one another.

Networking and training

Since most aspects of biotechnology which are principally associated with African women are less developed and most of the end products of such preparations are not usually properly processed, there is need for a Biotechnology Network involving women in Africa. The aim of the Network should be to stimulate: (i) research activities on relevant food crops; (ii) training activities for both local women, industrialists and scientists in the different areas of biotechnology; (iii) exchange of information through circulation of newsletters within each country and at regional level; and (iv) workshops on annual basis and rotating among regions. The working group identified the following research areas for consideration:

- Food processing: lactic acid fermentation technology, processing of cassava into several food items, processing of locust bean seeds into iru/dawadawa, mushroom cultivation on waste materials, and soybean processing.

- Fermented traditional drinks, e.g. wines from sorghum, pineapple, palm trees, etc.

- Rapid multiplication of important food crops which are resistant to pests and diseases.

- Market feasibility.

Biosafety

The group urged governments of African countries to develop appropriate biosafety policies as a matter of national interest for domestic research, development and utilization of new crops and foods. The group endorsed the distribution, dissemination and adoption of the Voluntary International Code of Conduct for Biotechnology Safety prepared by the UNIDO Secretariat for the UNIDO/UNEP/WHO/FAO Informal Working Group on Biotechnology Safety in 1991 in all appropriate national fora. Institutions involved in biotechnology R&D in African countries should establish institutional biosafety committees. African countries should be strongly encouraged to develop African expertise in all scientific disciplines relevant to biosafety issues. The group recommended that UNIDO coordinates an advisory service network on biosafety issues to provide expert advice, on request, to governments of developing countries lacking regulatory policies. FAO and international donor agencies should support a baseline field study programme on African food crops that are likely to be genetically modified and as such will be candidates for release to the environment.

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II. ABSTRACTS OF PAPERS

THE INDIGENOUS FERMENTED FOODS AND BEVERAGES OF SUDAN

by

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ABSTRACT

Food fermentations have lately captured the attention of nutritionists and food scientists because of the subtle changes that take place in the food as a result of the growth of micro-organisms in it. Positive changes include an increase in vitamin contents, an improvement in protein digestibility, a development of desirable flavours and colours etc.

Little is known about the fermented foods of Africa, knowledge of which can only be found with the African woman. That knowledge has not been documented. The information to be presented in the present paper has been gathered over a period of six years from elderly Sudanese rural women. The material thus gathered, together with that obtained from the literature on African foods, is being compiled in a manuscript for a book.

The bulk of the Sudanese fermented foods is of true sub-Saharan African origin, with only few examples that are of Arab or Middle Eastern root. The most sophisticated of these foods and the ones that are prepared by the most complicated procedures, are those made from sorghum (and pearl millet). Some of these foods are quite ancient, going back to thousands of years, as concrete evidence from Sudan suggests. Further evidence suggests that some of the methods used to prepare sorghum foods travelled from the central Nile Valley to West Africa and possibly also to East Africa. This food processing evidence confirms previous hypotheses advanced by botanists, plant geneticists, as well as by archaeologists that the Sudan is possibly the origin of sorghum.

At least 85 fermented foods exist in the Sudan today. The Sudanese seem to ferment just about anything edible or barely edible. In addition to the conventional raw materials such as cereals, milk, fish, meat, fruit and honey, unorthodox materials such as bones, hides, hooves, caterpillars, locusts, frogs, cow urine, etc., are also fermented.

A number of these fermented foods are sun-dried while others are rendered stable by ripening for years. It is concluded that food fermentations in these parts of the world constitute part of the coping strategies that women developed through the ages to combat problems of food shortage and famine.

BIOTECHNOLOGY AND FOOD FERMENTATIONS

by
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ABSTRACT

Fermentation is one of the oldest forms of biotechnology with evidence of its use dating back over several millennia. Although modern biotechnology is well established in some fermentation industries (e.G. brewing, baking, dairy) there are many indigenous fermentations which have yet to see the benefits of this technology. Much can still be achieved using low cost approaches through the design of basic fermentation equipment and simple selection techniques for the improvement of fermentative micro-organisms.

Solid-substrate fermentations may be particularly suited to developing countries. Possible applications include the utilization of raw starch substrates, microbial detoxification of plant materials and the upgrading of waste products through microbial enrichment.

Microbial metabolites make an important contribution to both the safety and organoleptic properties of fermented foods and there are considerable opportunities for their exploitation. Biotechnology is likely to play an important role in the development of safe, low cost methods of food preservation both in developed and developing countries. Products from food fermentative micro-organisms may also have applications in areas other than fermented foods.

The genetic manipulation of food fermentative micro-organisms using molecular biology techniques will eventually benefit developing countries although much can still be achieved using existing strains, mixed culture fermentations as well as basic selection methods.

PRODUCTION OF WINE FROM COCOA JUICE (*Theobroma cacao* L. *Kuntze*) USING SACCHAROMYCES SPECIES ISOLATED FROM PALM WINE AND CASHEW JUICE

by
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ABSTRACT

Juice obtained from the mucilage of cocoa beans containing 18.60 soluble solids was fermented with strains of yeast isolated from palm wine and cashew juice. The strains from each substrate source belong to the genus Saccharomyces. Saccharomyces species isolated from palm wine yielded 10.2% (v/v) alcohol while that from cashew juice yielded 10.5% (v/v) resulting into a sweet table wine. They both had 3.4 and 3.8 pH, respectively.

BIOCONVERSION OF WASTE MATERIALS TO FOOD AND USEFUL PRODUCTS BY FUNGI

by
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ABSTRACT

One of the most economically viable processes for the bioconversion of agricultural and industrial lignocellulosic wastes is the cultivation of edible mushrooms. Since the protein content of mushrooms is relatively high (19-35% on a dry weight basis), they can serve to enrich the human diet in those regions which suffer from a shortage of high quality protein. Mushroom protein can be produced with greater biological efficiency than proteins from animal sources and relatively little is required in terms of large-scale equipment, facilities, capital and land. Furthermore, the substrate residue which is left after mushroom harvesting can be converted into feedstock for ruminants and/or used as a soil conditioner. In developing countries, properly developed and managed mushroom farms can make important contributions to the nutrition and economic welfare of the people.

INDUSTRIALIZATION OI LACTIC ACID FERMENTATION TECHNOLOGY OF CEREALS AND ITS DISSEMINATION TO DEVELOPING COUNTRIES

by
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ABSTRACT

The content of the UNIDO Project (ROK/89/002) at Korea University is introduced. This project comprises of three major activities: (1) A joint research programme between scientists and engineers of the Department of Food Technology at Korea University, Korea Food Research Institute and the Department of Biotechnology of the Technical University of Denmark. (2) An international training programme in food fermentation technology with special emphasis on cereal based non-alcoholic fermentation. (3) An international workshop on lactic acid fermentation of non-dairy food and beverages.

The joint cooperative researches have been carried out successfully, and the results are used for the training of the eight UNIDO fellows selected from Asia and Africa. An international workshop on the lactic acid fermentation of non-dairy food and beverages will be held in 26-28 June, 1992, in Seoul, Korea.

SOYBEANS: THE ANSWER TO MALNUTRITION: THE IMPACT OF SOYBEAN PROCESSING AND UTILIZATION IN NIGERIA

by

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ABSTRACT

The goals of soybean utilization are to improve human diets. In 1987 through the aid of the International Development Research Center, Canada (IDRC), the International Institute of Tropical Agriculture (IITA) and National Programs collaborated on a soybean utilization project - to document the status of soybean, develop household level and small scale processing of soybeans and to disseminate the technologies developed to extension workers. Training programs were used to strengthen utilization at rural level. About 25,059 people have been trained. When fortified with whole soybean and soymilk residue, the protein content of local foods is increased. Soymilk, soygari, soy vita, and soy iru were made. Partially refined soybean oil is acceptable among low and middle class rural people. Extruded products like soy corn blend, is acceptable as children's food, Soy full-fat flour is preferred for akara, while defatted flour is acceptable for vegetable soup in rural areas. Training of agricultural extension workers results in rapid dissemination of technologies for soybeans to the country.

USE OF BIOTECHNOLOGY FOR OIL PALM IMPROVEMENT

by

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ABSTRACT

Tissue culture methods for clonal propagation of oil palms are now well established, but are slow and require much hand work. Modern developments of embryogenic liquid suspension cultures promise to improve efficiency and reduce costs in the future. There are still problems of abnormal flowering in some clonal material subjected to large-scale production, but these are being resolved by using techniques which avoid the problem. At present there is no understanding of the mechanism of induction of the flowering abnormalities.

Less progress has been made in the use of protoplasts or haploids, and DNA transformations are not yet possible. This should become feasible by combination of embryogenic suspensions and the use of ballistic DNA injection techniques, but as yet there are no clearly identified genes which it would be advantageous to transfer to oil palm.

The most important application of molecular biology will be in the development of genetic maps for use by oil palm breeders, using either RFLP analysis or the more recent PCR reaction. These methods applied to existing oil palm germ-plasm collections, augmented by fresh collections from wild grove palms in Africa must be coupled to active palm breeding programmes, with proper trials,

recording and selection methods, followed by field trials of chosen genotypes in the areas where the palms will be grown.

Propagation of selected genotypes would be best contracted out to established tissue culture laboratories, especially where requirements for small numbers of plants would make it uneconomic to set up new tissue culture facilities. This could be done through established commercial labs or within an International Institute, such as NIFOR acting on behalf of co-operating African countries. A first step would be the limited propagation of elite *dura* and *pisifera* parents which could be used to set up seed nurseries for improved *tenera* hybrids for local use by growers.

CYTOGENETICS OF CASSAVA AND RELATED SPECIES

by
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ABSTRACT

Cassava, *Manihot esculenta* Crantz, is a major food crop in Africa. The genus comprises about 98 species, confined to the American tropics, and IITA has a collection of about 22 species. Normal pairing of chromosomes with 18 bivalents at M-I is recorded for these species. Interspecific hybrids with cassava as well as between wild species using 12 of these species are reported. The cytogenetics of these hybrids is discussed.

Unreduced or 2n gametes were frequent in the hybrids and their role in the origin of sexual tetraploids and triploids of cassava is discussed. Besides sexual tetraploids, spontaneous asexual tetraploids and induced tetraploids are also produced in cassava. From the triploid progenies, two aneuploids were obtained for the first time in cassava and their cytogenetic behaviour is discussed.

CACAO: NEW RESEARCH ADVANCES AND APPLICATIONS OF BIOTECHNOLOGY

by
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ABSTRACT

Cacao has been cultivated for more than 400 years and this crop still faces numerous problems with diseases, pests and the lack of high yielding clone materials. Cellular genetics and molecular biology could play an important role to complement existing germplasm evaluation and breeding efforts.

Recent progress has been made on the recovery of somatic embryos from non-sexual explants (petals and nucellus tissues). This progress is now opening the door for large scale micropropagation methods for cacao. Superior donor plants could be selected in the field and subjected to a cloning process. In addition, progress has been made to complete germination and plantlet development for cacao

somatic embryos. Cacao improvement programs that would rely on transformation methods can now use the new somatic embryogenesis process derived from nucellus or petal tissues and recover intact plants. Reports have also been made on the recovery of shoots derived from axillary buds. This technique can be very useful for multiplying valuable genotypes for clonal orchards or germplasm banks.

Future work on cacao needs to focus on methods for haploid production, embryogenic cell suspensions, protoplast cultures and transformation techniques. At the same time, refinement of the micropropagation methods for non-sexual explants should be completed.

STATUS OF COCOA RESEARCH, PRODUCTION AND BIOTECHNOLOGICAL ADVANCES IN NIGERIA

by
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ABSTRACT

A brief history of the Cocoa Research Institute of Nigeria (CRIN) and a summary of its objectives, achievements, recent advances and immediate future programmes are presented and discussed in relation to some developments that have affected the Nigerian cocoa industry. Proposals are made to alleviate effects of stalemated situations created through intensive research findings by major consumer developed nations, into industrial cocoa utilization, public consumption habits and to international market forces systems.

Finally suggestions are preferred on how to amend some of the major production, processing and utilization problems posed. A modest justification is presented to support the need for the application of modern biotechnological approaches to cocoa research. Relevant major constraints in Nigeria are also highlighted.

COFFEE: NEW RESEARCH ADVANCES AND APPLICATIONS OF BIOTECHNOLOGY

by
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ABSTRACT

Coffee improvement programs can focus on three different areas of application: agronomy, processing industry and consumer benefits. Agronomic characteristics should focus on reducing direct and indirect farming costs. In a modern coffee farm in Brazil, direct costs can reach 63% of the total costs. To reduce coffee farming costs, the new technologies need to address fertilizer efficiency, disease and pest resistance, and crop management aspects that will

reduce total labor hours (herbicide resistance, mechanized cultivation and harvesting). The development of coffee varieties with short (3 months), mid (6 months), and long (9 months) maturation cycles would permit a more even distribution of the harvesting and processing activities at the farm level and thus increasing the efficiency of farm labor utilization. The development of a commercial micropropagation process or a true hybrid system in coffee would be highly desirable to counteract frequent disease and pest outbreaks as well as for maximizing the exploitation of agroecological niches and to increase productivity.

Coffee quality depends primarily on the genotype, but is also highly susceptible to environmental conditions. Plantation management, which affects plant microclimate, nutrition level, and seed processing quality has a major impact on the coffee quality. Superior beverage quality is produced from Arabica cultivars grown at higher elevations. Crosses between Arabica and other coffee species confirm that Arabica genes are responsible for superior beverage quality. Storage and postharvest processing also have a major role in determining final coffee quality.

There are several characteristics of coffee that could be altered, resulting in some benefits to the coffee industry and final consumers: increased total soluble solids, larger and more uniform bean size, bean density and texture, uniform maturation, caffeine content, increased levels of compounds responsible for desirable coffee flavor and aroma.

In coffee tissue culture, there have been reports of successful regeneration via somatic embryogenesis of several wild *Coffea* species, five *C. arabica* cultivars and two interspecific hybrids. Recovery of plants via somatic embryogenesis in such a wide range of genotypes demonstrates the potential of using in vitro methods for coffee improvement. Regeneration from embryogenic cell suspensions and isolated protoplasts of Robusta and Arabica genotypes has also been described. Transient transformation utilizing the protoplast uptake method has now been reported. Considering the repeatability of the protoplast regeneration systems available today, the utilization of useful genes for coffee improvement is now a near- to mid-term possibility with a high degree of success. Embryo rescue and anther culture techniques need further developments. Micropropagation on a large scale is now feasible through the use of embryogenic suspensions in Erlenmeyer flasks or bioreactors. Utilization of this technique will make available segregating individual plants to commercial production. Somaclonal variation as a breeding tool for coffee improvement has now been described for Arabica coffee and a few interspecific hybrids.

Stable prices, superior quality and attention to consumer needs will be the most effective long-term strategies for increasing the coffee market. Biotechnology probably can provide a more efficient way to introduce value-added coffee to the coffee industry. The availability of certain value-added coffee cultivars would open opportunities for market niches for specialty coffee brands. Most agronomic benefits will bring quality and increase net farm profits. Increasing the net return to coffee farmers will contribute to production stability and long-term success of this industry.

ROOT AND TUBER CROPS TISSUE CULTURE RESEARCH ACTIVITY AT IITA

by
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ABSTRACT

At IITA, tissue culture methodologies such as meristem culture, shoot tip and node cutting cultures are routinely used to eliminate virus infection from improved clones of cassava and yam and micropropagation of cassava, yam, sweet potato and cocoyams respectively. Virus-tested improved clones of cassava, yams and sweet potato have been distributed to national programs for evaluation and testing.

The *in vitro* reduced growth storage method is applied to conserve clonal germplasm of root crops. Plantlets can be kept in the same tube for 1 to 2 years. A total of more than two thousand accessions of root crops germplasm are maintained.

Embryo culture technique for the germination of mature embryos of *Manihot* sp. and yams, and immature embryos of cassava are being developed. Somatic embryos matured to cotyledon stage were obtained from several IITA improved clones using *in vitro* young cassava leaf. Some plantlets were regenerated. Anther and unpollinated ovary culture of cassava aiming at producing haploid plants are under investigation.

In vitro microtuber of yam and minitubers obtained from greenhouse grown virus-free yams are alternatives for international distribution of virus-tested clonal materials. However the dormancy and uniform germination of microtubers will have to be investigated.

AN OVERVIEW OF IITA'S BIOTECHNOLOGY ACTIVITIES FOR CROP IMPROVEMENT

by
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G. Myers, R. Asiedu, N.Q. Ng, M. Bokanga, M.D. Winslow,
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ABSTRACT

Biotechnology brings new tools, new ideas and new approaches to agricultural research. IITA is exploring the possibilities offered by biotechnology to apply nonconventional technologies for the improvement of its mandated crops such as cassava, yams, plantain, cowpea and maize. One example of an application already in use is tissue culture in disease elimination, micropropagation, international distribution and germplasm conservation.

Areas of research in biotechnology include embryo culture and rescue of hybrids, somatic embryogenesis and plant regeneration, developing biochemical (protein and isozyme) and molecular markers (cDNA and RFLP) as screening tools,

phylogenetic studies using RFLP analysis, polyploidization, somaclonal variation, transformation system, haploidization through anther culture, cytogenetic studies and isolation of aneuploids, incorporation/transfer of genes resistant to biotic and abiotic stresses, monoclonal antibodies and cDNA probes for the detection of viruses and their strains, and cryopreservation. Some of these studies are carried out in collaboration with advanced laboratories.

IITA also participates actively in biotechnology networks. Through its collaborative research with advanced laboratories and the training offered to national scientists and technicians, IITA forms a link between the laboratories in the advanced countries and the national agricultural research systems in Africa. Technical assistance in setting up tissue culture facilities is already provided to national agricultural research systems in several African countries.

BIOTECHNOLOGY IN SUB-SAHARAN AFRICA
Creating and utilizing endogenous technological capability

by

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ABSTRACT

African countries are experiencing ecological and economic problems that are disrupting the socio-economic and political systems. While the region's population grows at about 3%, food production is growing at 1.8%. The level of malnutrition has risen in the region. The productivity of the industrial sector is marginal to sustain the economies. The region's debt is increasing while the ability to service debt is increasingly declining. These economic changes are leading to irreversible socio-economic, political and ecological problems; the loss of biological diversity, the loss of soil fertility, and deforestation cause economic problems, at least in the long-run.

While African countries are seeing these unfortunate changes, most other regions of the world are enjoying relatively positive economic change. The economic progress of the industrialized world and newly industrializing countries (NICs) is to a large measure a result of the introduction of new technological knowledge and, new forms of institutions to manage the evolution of technologies. The emergence of biotechnology will, at least in the long-run, enhance the ability of these countries to achieve higher economic growth and sustain their economic systems. For Africa, the biotechnology revolution may not positively contribute to economic renewal unless countries in the region build a critical minimum level of technological capability in those biotechnological techniques that may be applied to increase food production, conserve the ecological base, improve health status of the population and increase the region's industrial productivity. The basic premise of the paper, therefore, is that the ability of African countries to renew their economies largely depends on the level of technological capability they build in the biotechnology regime.

THE POTENTIAL FOR BIOTECHNOLOGY IN AFRICA

by

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ABSTRACT

Africa's current problems, especially food security crisis, will find best solution through deployment of modern biotechnology. Biotechnology will have greatest impact in agriculture, health, and industry. In agriculture, biotechnology through cell and tissue culture will lead to improved plant propagation, fast breeding, better control of pests and diseases, and thus better food security. Besides, improved soil fertility through biofertilizers from biological nitrogen fixation (BNF), improved animal, health and husbandry, appropriate food processing and preservation, will enhance production and availability of food. For human health, modern biotechnology will bring about a healthier population through new and more effective vaccines and drugs against endemic tropical diseases of Africa. Industrial growth and environmental protection also stand to benefit from biotechnology.

Problems which impede the introduction of biotechnology in Africa, include inadequate numbers of trained manpower, low funding of biotechnology R&D, poor infrastructure and information acquisition and exchange.

Strategies for enhancing biotechnology in Africa must therefore include capacity building to provide the facilities and support for work in biotechnology. To this extent, efforts should be exerted to embark on education and training of young Africans to produce a critical mass of highly trained biotechnologists. The programme will be facilitated by provision of adequate information and data base on biotechnology. There must also be appropriate networking, linkages and coordination between centres of activity in biotechnology in Africa and laboratories and scientists in advanced countries. Besides, adequate funding of the programmes by national and international organizations should be provided to support the efforts. Most importantly, in adopting the biotechnology option, African countries need to consider its benefits and risks, order of priorities, start with simpler biotechnologies that address the most pressing problems and ensure immediate and short-term benefits. More sophisticated biotechnologies especially those involving rDNA technology, should be embarked on later, as the infrastructure and other facilities improve, for achieving mid-term and long term benefits. Where certain obvious benefits will accrue in the short-term from genetic engineering of plants and animals e.g. for improved resistance to diseases and pests of crops, deliberate effort should be mounted to establish the facilities to enable the inception of such studies, as a crash programme.

Adequate regulations must be established to govern R&D involving rDNA so as to ensure biosafety in testing and release to the environment of genetically modified organisms.

III. SELECTED PAPERS

The indigenous Fermented Foods and Beverages of Sudan

by

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ABSTRACT

Food fermentations have lately captured the attention of nutritionists and food scientists because of the subtle changes that take place in the food as a result of the growth of micro-organisms in it. Positive changes include an increase in vitamin contents, an improvement in protein digestibility, a development of desirable flavours and colours etc.

Little is known about the fermented foods of Africa, knowledge of which can only be found with the African woman. That knowledge has not been documented. The information to be presented in the present paper has been gathered over a period of six years from elderly Sudanese rural women. The material thus gathered, together with that obtained from the literature on African foods, is being compiled in a manuscript for a book.

The bulk of the Sudanese fermented foods is of true sub-Saharan African origin, with only few examples that are of Arab or Middle Eastern root. The most sophisticated of these foods and the ones that are prepared by the most complicated procedures, are those made from sorghum (and pearl millet). Some of these foods are quite ancient, going back to thousands of years, as concrete evidence from Sudan suggests. Further evidence suggests that some of the methods used to prepare sorghum foods travelled from the central Nile Valley to West Africa and possibly also to East Africa. This food processing evidence confirms previous hypotheses advanced by botanists, plant geneticists, as well as by archaeologists that the Sudan is possibly the origin of sorghum.

At least 85 fermented foods exist in the Sudan today. The Sudanese seem to ferment just about anything edible or barely edible. In addition to the conventional raw materials such as cereals, milk, fish, meat, fruit and honey, unorthodox materials such as bones, hides, hooves, caterpillars, locusts, frogs, cow urine, etc., are also fermented.

A number of these fermented foods are sun-dried while others are rendered stable by ripening for years. It is concluded that food fermentations in these parts of the world constitute part of the coping strategies that women developed through the ages to combat problems of food shortage and famine.

1. INTRODUCTION

If it is true that Africa is the origin of man, as most archaeological and genetic findings suggest (Lamberg-Karlovsky, 1949; Tierney et al., 1988; Lemonick, 1987; Clark, 1978), and since it has been established that tool-making hominids were present in tropical Africa some two million years before their appearance in Europe and Asia, then the first human to consume a fermented food item must have lived in Africa.

The African hunter-gatherer probably consumed, under normal conditions, fresh fruits and meats. But when these products became scarce, for one reason

or another, he or she must have picked up a fermented berry or a rotten piece of meat. The urge to consume fermented food products from the wild must have become maximized at times of acute food shortage or outright famine. Repeated consumption of such foods led to the development of the taste for them and the hunter-gatherer must have discovered very early that fermented products of nature possessed an augmented flavour, were easier to digest, quicker to cook, store longer and perhaps health-promoting.

The discovery of fermented food thus preceded the domestication of agricultural crops and animals. But the development of agriculture must have given a great thrust to the art of food fermentation in general.

It is possible that certain regions of Africa, where agricultural practice developed to a more advanced stage than in other areas, and where the size of sedentary populations grew larger, the art of food fermentation also became more advanced and the products more diversified. Such regions may be legitimately called the origin of fermented foods.

Since warm climates, humid conditions and abundance of plants and animals are basic conditions conducive to fermentation, the Savannah belt of Africa up to the edge of the Sahara Desert is a likely candidate for the early nurture of food fermentation. Studies on the proto-Chadic language of the Afroasiatic group of African languages of Nigeria, Cameroon and Chad, spoken about 6000 years ago, showed words for "cow", "sheep", "goat", "flour", "porridge", "pearl millet" and "sorghum". Likewise, prot-Nilotic, proto-Central Sudanic and Eastern Sudanic languages, spoken mostly in southern Sudan, some 5000 years ago, had words for "weed", "cultivate" and "herd". Linguistic evidence also suggests that the Cushites of Sudan and Ethiopia developed grain cultivation some 7000 years before present (Ehret, 1984). These Savannah African lands are therefore among the earliest food-producing cultures of the world; it is therefore possible that they knew of food fermentation.

At any rate, it is reasonable to assume that the regions which knew fermentation of food the earliest would have today the most diversified set of fermented food products and that this food culture would be well integrated into the fabric of the social, religious as well as the culinary culture of the people. If Africa is the continent where the practice of food fermentation started, then it should have the greatest variety of fermented foods and drinks, compared to other continents. Further, within Africa, the regions with the oldest history of food fermentation would probably have the greatest variety of such products, i.e., those regions would be centres of diversity.

Unfortunately, the foods of Africa have not been all documented and the decision on a possible centre or centres of origin of food fermentation will have to await such an undertaking. We do not know the total number of the indigenous fermented foods of even a single sub-Saharan African country. It has been estimated that Nigeria has about 20 fermented foods (Odunfa, 1981) and that the whole African continent has a little over 30 fermented foods (Odunfa, 1985; Odunfa, 1988).

This author decided six years ago to find out just exactly how many foods of a fermented nature are found in one African country, the Sudan. The project was a personal initiative, with no financial support from any agency. After six years of day and night work the list of foods now contains 85 items and as some of these are closely related it would be safer at this point to say the list contains about 60 different fermented foods. There are certain regions of the country which were not reached due to insecurity and civil strife reasons. But what is left of the information on Sudan's fermented

foods must be a trickle. The major sources of information are the rural elderly women. The information is now piled in a manuscript for a book to be forwarded to publishers within one year, hopefully. In the remainder of this paper an attempt will be made to summarize some of the findings of this quest.

2. SUDAN'S FERMENTED FOOD PRODUCTS

The rural Sudanese traditionally divide the fermented food products (in fact all foods) into 4 categories of which 'miscellaneous' is not one. The foods and drinks are either kissar (singular, Kissra), staples; milhat (singular mulah), sauces and relishes to go with the staples; akil-munasabat, food for special occasions; and marayis (singular, merissa), beers and other alcoholic drinks. This system and possible similar systems of classification used by other Africans have been completely ignored by authors of African food literature. It is suggested that these indigenous classification systems of foods be adopted by African scientists in their endeavour to reveal and document the continents' huge food heritage.

Nevertheless, the foods to be discussed below are grouped more or less on commodity basis for reasons dictated by the need to facilitate discussion of certain points of interest in this paper.

2.1 Sorghum Foods and Beverages

The Sudanese rural populations are traditionally sorghum eaters. Sorghum makes about 80% of their staple food, being followed by pearl millet which makes about 10%. Sorghum fermented foods stand out as the most sophisticated foods, prepared by the most complicated procedures of all. The Sudanese make about 30 different fermented food and drink products from sorghum. These foods and beverages seem to be unique in a number of ways when compared to the sorghum products so far reported for other African countries. First, a number of bread types (about 12) are prepared in rural Sudan from sorghum and this is quite surprising because Africa is not famous for bread forms of food. Second, a number of solid and liquid food and beverage products are made from malted sorghum grain. Sorghum malt in Africa is mainly used in the brewing of beers and rarely to prepare non-alcoholic products such as the West African drink variously called aliha, ahei or ahliho, which is no more than a sweetened beer wort (Campbell-Platt, 1987). In the Sudan 7 solid food products are made from sorghum malt in addition to 3 types of beer: merissa, assaliya, baganiya. The opaque beer, merissa has between 30 and 50 kinds. The procedure leading to the production of the standard merissa type is probably the most complex brewing procedure in Africa (Dirar, 1978). Similarly, that followed in the brewing of the clear beer assaliya (or um-bilbil) is surely the most complex on the continent. Clear beers are not common in Africa (Novellie and Schaepdrijver, 1986) and the procedures followed in the preparation of the known ones such as Otika (Ogundiwin, 1977) and amgba (Chevassus-Agnes et al., 1976) are far less complex.

One of the solid foods made from sorghum malt is hussuwa which is a sweetish product clearly considered a quick source of energy. It is sometimes called laban-el-miskeen (poor man's milk) or rajwat-el-rajil [the goodies awaiting a returning husband from a long, tough, journey].

Another sorghum or millet malt-containing product is khemiss-tweira (five birds) which is a meal made from five ingredients: flour, malt, sesame, salt and sugar. It is food for travellers and is consumed by simply adding water.

Hussuwa is related to the South East Asian products tape-ketan, tape-ketella and brem (Hesseltine, 1965; ko, 1982; Cronk et al., 1977; Yokotsuka,

1982). On the other hand, khemiss-tweira has no relative in the recorded literature as far as one knows, but in its characteristic swelling when mixed with water it is quite related to gari and tapioca, the cassava products that reportedly swell to three- to five-fold their original volumes (Spickett et al., 1955) due to their content of dextrin (Ayres, 1972).

Of the many porridges made from sorghum none is more sophisticated than jiriya. This translucent stiff porridge is prepared from jir or sorghum or millet starch. The procedure followed in the preparation of jir is probably the most complicated procedure of all food processes in Africa, at least within the boundaries of the available literature. The method followed in the preparation of the West African Cornstarch, ogi is much simpler (Bascom, 1951; ;Banigo and Muller, 1970). Jir of Sudan contains 91% starch and only 1.3% crude protein (Khattir, 1990) whereas sorghum ogi contains 84.3% starch and 8.3% crude protein (Akingbala et al., 1981).

Of the sheet-baked sorghum breads it appears that kissra (the staple bread) and the two Ramadan (Muslim fasting month) products abreh and hulu-mur are unique to Sudan. Kissra (1.0 mm thick, baked for 17 seconds) and abreh (0.25 mm thick) are probably the world's thinnest sorghum breads and flakes. The product called abreh, which is easily swept away by a gentle breeze, is almost a see-through flake product, and is no doubt the finest sorghum product of Sudan. Abreh is consumed as a sweetened suspension after mixing with water. It is prepared from an oversoured sorghum dough and it is designed to become slippery when wet as that it slips down the throat without being chewed. Abreh can be envisaged as an acid capsule and it is so designed that large quantities of lactic acid are introduced into the stomach without being detected by the taste buds of the buccal cavity. Abreh is proudly described by women as the "product that embarrassed the guest". The thirsty guest, who was offered a bowl of abreh suspension, decision to drink the water and leave the flakes to demonstrate to his hosts that he was not voracious. But, to his embarrassment, when he put down the bowl it was completely empty - he was not aware of the particulate matter "sneak" down his throat due to the exceedingly smooth nature of the product.

Abreh is considered a thirst-quenching product. In Africa, particularly in the dry countries of the Sudano-Sahel sector, thirst-quenching fermented foods are of particular importance. Many of these products have lactic acid as a common ingredients and it is possible that this acid has an effect on the physiology of thirst. African scientists should address themselves to these very important foods - individuals in Africa do not only die of hunger, they also die of thirst, although admittedly to a lesser extent.

A last example of sorghum breads, which is of a special interest, is kissrat-ker. Five cereals enter into the making of the dough for this bread: sorghum (50%), wheat (12.5%), barley (12.5%), maize (12.5%) and pearl millet (12.5%). The mixture of flours is kneaded for hours and hours in a most tedious manner until, finally, a sweetish, liquefied batter is obtained. This is then baked into sheets to give kissrat-ker. This is an enzymic as well as a mild fermentation process in which use is made of the amylase enzymes of wheat and barley to break up the starch of sorghum. In its enzymic aspect kissrat-ker is related to mahewu of southern Africa (Akinrele, 1970).

2.2 Dairy Products

The roots of the overall contemporary Sudanese culture go to a basic nomadic culture. Milk has always been considered a basic item in adult nourishment as well as the basic weaning food for babies. Today, as always, the bulk of the estimated 3 million tons of milk produced annually is in the hands of nomadic or transhumant tribes.

A good proportion of this milk is fermented into some kind of dairy product. The truly indigenous fermented dairy products of rural Sudan are four: rob, gariss, biruni and mish. In addition, there are two quasi-indigenous products, jibna-beida (white cheese) and zabadi (yoghurt) which have been introduced from the Mediterranean or Middle East through Egypt, perhaps about a century ago. These last products prevail in the cities.

The most important fermented milk product of rural Sudan is rob. It is perhaps appropriate to mention at this point that this product is not produced for its own merit as food, rather it is a by-product of milk fermentation intended to facilitate butter extraction. Rob can therefore be considered a sort of buttermilk that is sometimes wasted away by spillage on the ground or in large water holes. Nevertheless rob is also consumed in various ways either through direct drinking or after converting it into one kind of a number of sauces to go with the staple stiff porridge, aceda. The fact remains, however, that the most valued product of this fermentation is the butter which is boiled to give samin or butteroil which stores well for months.

The remaining three indigenous fermented milks, on the other hand are intentionally fermented to be consumed whole as food. Gariss, for instance, is fermented camel milk prepared and consumed by camel-boys while herding their animals in remote areas. The product which is kept in two large skin bags on camel back (the gariss camel) undergoes a sort of fermentation while the animal goes about its business, grazing, trotting, walking, etc. The milk is thus subjected to various states of rest and unrest. Moreover, the process of fermentation is semi-continuous as the portion retrieved for consumption is replenished by fresh camel's milk. This state of affairs goes on for months and the camel-boys feed themselves solely on this product during that period.

Gariss differs from other Sudanese fermented milks in that it has substantial amounts of ethanol. The product is thus a member of the acido-alcoholic fermented milks which include kefir, koumiss and busa of central Asia (Kosikowski, 1982; Platt, 1955). In particular, gariss is more akin to the camel milk koumiss types called chal and shubat (Bairamov and Gavrichkin, 1983; Belokobylenko, 1982). The fermentation of gariss is brought about by lactic acid bacteria and by yeasts.

Biruni is a fermented milk product of the Nuba Mountains. It is also called laban-gedim (aged milk) as it could sometimes be ripened for up to 10 years. The product becomes thick, brownish and rancid through the years. It is used to prepare sauces for aceda porridge.

Mish is prepared by a number of different ways either using rob or fresh milk as a starting material. Some spices such as black cumin and garlic may be added to the fermenting product, and fermentation could last for up to one month. In some cases fly maggots flourish in the milk and are consumed with avidity, together with the milk.

2.3 Meat Products

Campbell-Platt (1985) mentioned that Europe is the origin of fermented meat products prepared from whole meat or from chopped and comminuted meat. The author also asserted that, by comparison, there were relatively few fermented meat products produced in the world's hotter regions. However, if we take 'meat' to denote every tissue in the slaughtered animal, it would seem very difficult to reconcile the above-mentioned opinions with the fact that in tropical Africa the Sudan alone has more than 10 different fermented meat products.

2.3.1 Shermout

This is of the Western jerky type of dry meat. Two types are found in the Sudan. One of them is prepared by sun-drying very thin, flat strips of lean meat. The other type is made from fat-bearing muscle meat, cut into irregular, thick (1.0 inch) strips which are best stored in a closed room with no windows. The meat undergoes conspicuous fermentation and simultaneous drying. One precaution often voiced by old women is that no light should fall on the fermenting meat - 'not even moon-light'. The product develops a strong smell and even a greenish coloration at the bends caused by the rope on which the strips are hung.

2.3.2 Miriss

This is an extremely white, extremely foul-smelling product prepared by fermentation of the peritoneal screen of fat surrounding the stomach of lambs. The fat is chopped, pounded into a paste, mixed with combu (potash obtained from plant material ash) and fermented in a covered earthenware for 6 days.

2.3.3 Mussran

The small intestine (mussran) is freed of its content, placed into a burma (earthenware) and allowed to undergo proteolytic fermentation for 3 days. It is then mixed with combu, sun-dried and cut into smaller pieces which are wrapped into small bundles to be stored or sold.

2.3.4 Beirta

The lungs, the kidneys, the liver, the heart, the spleen and the visceral fat of the slaughtered he-goat are all chopped and mixed into a burma. Then about 2 kg of muscle meat from the hind quarters are also chopped up and mixed with the contents of the jar. Now, about half a liter of milk is poured into the pot and well mixed with the meat pieces. The whole is fermented for 4 days. At this point the pot is opened and a dash of table salt mixed in. Then another stretch of fermentation amounting to 3 days is allowed to finally obtain the food called beirta.

2.3.5 Um-tibay

The alimentary tract of the animal is removed and evacuated. Then the intestine (colon not included), the heart, the kidneys, the liver, the spleen and the neck bones are all chopped into small bits and pieces and the whole stuffed into the empty rumen. The open ends of the latter are then tied up and the package hung up high on some support and given 3 days to ferment.

Next, a big fire is built on a sandy soil and when all the wood has been burnt to coals and ashes, the package of strong smelling meat is buried into the hot sands and embers overnight. The product may then be consumed in various ways or sun-dried and stored. Unfortunately, the best um-tibay is made from gazelle meat.

2.3.6 Shin

This is a traditional fermented sausage similar to the semi-dry fermented sausages, such as summer sausage and Lebanon bologna of Western cultures (Deibel et al., 1961; Dierick et al., 1974; Lucke, 1985). To prepare shin, the large intestine often called kebir-akhwanu (big brother - of the intestines) is emptied and tied at one end. In the meantime, the visceral fat, alone or mixed with the cleaned small intestine, is chopped, mixed with combu and stuffed into the large intestine prepared as above. Now, the other

end of the distended intestine is tied up tightly. The sausage so prepared is hung up high away from cats and dogs to undergo slow fermentation and drying for at least one month.

2.3.7 Twini-digla

To make twini-digla (intestine ball), the alimentary tract, from the rumen to the rectum, is emptied of its contents by hand pressing and squeezing. Then without washing, it is cut into long strips that are sun dried. Similarly, long strips are made from other internal organs, such as the liver, the heart, the lungs, the kidneys and the spleen, and are likewise sun-dried. Now, the whole collection of dried internal organs is pounded to give a mixed meat meal. Some combu or naturn, a little salt and some water are added and a paste is prepared. This is then moulded into balls, the size of a tennis ball, which are then slightly flattened to give lens-shaped discs. These are then again dried out in the sun for 8 days. The final product has a rancid and proteolytic flavour, particularly pronounced at the centre of the disc.

2.3.8 Dodery

This is a bone paste of foul odour. The best dodery is that made from those not so solid bone ends such as the ones making the balls of the ball-and-socket joints which are impregnated with large quantities of fatty substances and marrow material. The bones are first broken down into smaller pieces which are placed in a burma and submerged with cold water and allowed to ferment for 3 days. At the end of this period, the bone pieces, with their attendant fat, marrow, tendons, and meat scrapings are crushed into a paste, mixed with combu (5%) and put back into the burma to ferment as a solid substrate for up to 5 days. Dodery is often referred to as mulah-el-sebit, i.e., Sabbath or Saturday sauce, a name which at first impulse is suggestive of Judaic roots. The people of Darfur, however, where this product abounds, explain the name by saying that during Friday, the Muslim weekend, individuals visit relatives and so eat much and mix foods. On Saturdays they need a food that is lighter and easier to digest and that food is dodery. The product is also referred to as akl-el-muluk, or food of the kings! However, the visual and, particularly, the olfactory qualities of the product can hardly merit its elation to majestic order. But people do not just give names without good reasons.

2.3.9 Kaidu-digla

Here, the vertebrae of the slaughtered animal are first stripped of their meat and then chopped with an axe while still threaded with the spinal cord. The chopped bones are then sun-dried for about 8 days. They are then crushed into a coarse meal, using stones. Some water and naturn are mixed in and the paste thus formed is moulded into balls, the size of a fist. The balls or lens-shaped discs, which look like those of twini-digla (see above) are next dried in the sun for 9 more days. The dry balls of kaidu-digla (bone balls) can be stored for months and when soup or sauce is to be made from them they may need to be smashed with a hammer.

2.3.10 Other Fermented Meat Products

Jerbi-jerbi is prepared by fermenting skinned whole wild rabbit for 3 days in a burma. The frail, fermented carcass is next boiled in water, pounded thoroughly and made into a thick, meaty sauce for aceda.

Lahmat-nimir (leopard's meat) is not meat obtained from leopard's flesh as the name might suggest, but it is a kind of fermented meat prepared by a

method simulating the meat-eating habit of the leopard. Beef is cut into large chunks which are then fermented in a closed container for two days before consuming it.

A number of small game animals such as the porcupine, the ant-eater and the kaikaw (a mountain rodent) are fermented before consumption with the aim of tenderizing the meat.

2.4 Fish Products

Whenever fermented fish products are mentioned, South East Asia jumps to mind. According to Platt (1964) fermented fish products are almost confined to that part of the world. The literature dealing with the fish products of that oceanic region is simply overwhelming (Van Veen, 1953; Van Veen, 1965; Mackie et al., 1971; Adams et al., 1985; Beddows, 1985).

Africa is not portrayed in the literature as a continent with important fermented fish products. Generally speaking, fish in Africa is mostly smoked or sun-dried. Nevertheless some fermented or partially fermented fish products are found in some African countries. In Sudan, for instance, all the types of fermented fish products of S.E. Asia is to be found: sauces, pastes, whole fish, dried fish, etc. There are four major fermented fish products in Sudan: fessiekh, terkin, mindeshi and kejeik. In addition, there is a limited amount of fermented fish eggs (or roe) called batarikh which must be the same as botarque of Italy and Greece which came to Sudan through Egypt.

2.4.1 Fessiekh

Two medium-sized, red-finned Nile fish, Alestes spp. and Hydrocynus spp., are used to make fessiekh which is whole, salted, semi-dry fermented fish. The fish are salted (10-25% salt) one by one with more salt being applied to the gills. They are then stacked in layers separated by salt layers on palm leaf mats. The pile of fish is next covered and wrapped with another set of mats and left to ferment for 3-7 days, depending on ambient temperatures. Following this period, the fish are packed in petrol cans, fermented for another stretch of time equal to the preceding one and then the cans are sealed off by welding. Fessiekh may be eaten even raw within 9 days only. The product is however, mostly exported to Egypt.

2.4.2 Terkin

This is both a fish sauce and a fish paste of very strong odour. It is mainly prepared in Nubia in the north and seems to bear a tag of antiquity. Various types and ages of Nile fish can be turned into terkin and in a number of ways. Basically, however, the fish is salted, packed into an earthenware jar and allowed to ferment for up to 15 days. The jar is then placed in the hot sands outdoors and the contents poked at and stirred for 4 days. The final product is a completely liquefied slurry or paste with an uninviting darkish colour but quite appetizing to those who use it on a regular basis. Sometimes the separated juice obtained during terkin preparation is collected and bottled as a sauce called meluha.

2.4.3 Mindeshi

In the far south-western part of Sudan huge numbers of fish fry accumulate in a certain season of the year and people catch them and pound them into pastes which are then packaged in wickerwork baskets and exported to neighbouring Darfur Region. In some methods the sticky sap of a certain creeper plant is mixed with the paste which then undergoes some kind of a

pickling or ensilaging. Mindeshi, which is mainly prepared from Mormyrus cashive fry, later dries up to give a prickly mass of fish meat and bone.

2.4.4 Kejeik

This is a typical African fish product. It is simply sun-dried, non-salted large fish. The individual fish is split longitudinally in a non-symmetrical fashion and then simply spread out on rocks, tree branches, etc., to dry up. But as no salt is added, a degree of fermentation takes place. Kejeik is a very important protein source for farm workers manning the large mechanized sorghum-producing agricultural schemes of the country. In the past, the product, which is produced in the South, used to be exported to the Belgian Congo (Zaire).

2.5 Vegetable Products

There are at least 10 fermented vegetable products the most important of which are the meat substitutes and the sour milk substitutes.

2.5.1 Kawal

This is prepared from the green leaves of the wild legume Cassia obtusifolia. The green leaves are pounded using the common mortar-and-pestle arrangement and the green paste packed in a burma and buried in the ground. The paste undergoes fermentation for about two weeks to finally give a strong-smelling product that is moulded into small irregular balls and sun-dried. The product is used as a flavouring material for the relatively affluent and as a meat substitute for the very poor (Dirar, 1984). Kawal has about 30% protein of a very high quality and is extremely rich in calcium. The organisms involved in the fermentation are mainly Bacillus subtilis and Propionibacterium sp. Although substantial quantities of acetic and butyric acids are produced during fermentation, the pH value remains around neutrality due to the high buffering capacity of the leaves (Dirar, et al., 1985).

2.5.2 Sigda

This is a meat substitute derived by fermentation of sesame seed presscake. The best sigda is produced from sesame seed paste prepared by hand-kneading to remove most of the oil. In the process, combu slurry is added to facilitate separation of oil from solid matter. The paste is then placed in an earthenware jar and fermented spontaneously for 14 days. After this period a strong odour would have developed. The paste is hand-mixed and fermented for 2 more days before it is ready to be used as sauce ingredient. The product contains between 33.7 and 44% crude protein. Samples collected from western Sudan contained B. subtilis. The fermentation of sigda is basically proteolytic but some sigda types undergo a lactic sour fermentation in which case Streptococcus sp. predominate (El Faki et al., in press). Sigda is probably related to the West African Ogiri-sara which is made from sesame seed (Campbell-Platt, 1987; Aidoo, 1986).

2.5.3 Furundu

This meat substitute is obtained by fermentation of roselle seeds (Hibiscuss sabdariffa). The seeds are first roasted and then reduced to flour. Then combu slurry is added to the flour and the whole mixed well to give a stiff paste which is fermented in a burma for 10 days. It is then mixed again and fermented for two more days before it is moulded into balls and dried in the sun as usual. Some Sudanese of recent Nigerian origin call furundu dawadawa. Incidentally, a trade of dawadawa reportedly occurs across

the Sudan, from Nigeria, through the Sudanese border town Geneina, all the way to Saudi Arabia under the name of Kawal.

Furundu falls in the family of W. African, strong smelling flavours such as the oil bean ugba (Achinewhu, 1983; Odunfa and Oyeyiola, 1985 , the castor bean ogili-isi (Raymond, 1961) but the product is more akin to the locust bean dawadawa (iru) (Odunfa, 1986; Odunfa and Adesemoju, 1985; Aykroyd and Doughty, 1964). Furundu has 27% crude protein of an excellent quality (chemical score 80) and like kawal and sigda it is very rich in sulfur amino acids.

2.5.4 Rob-ful

As the name suggests, this is a substitute for sour milk (rob) prepared from groundnut (ful) paste or slurry. The paste prepared from roasted beans is placed in a covered container and allowed to undergo a souring fermentation out in the sun for 12-24 hours. The fermented paste is then made into a kind of sauce very similar both in colour and taste to sauce made from sour milk or rob.

2.5.5 Rob-heb

This product is similar to the preceding one, only it is made from the seeds (heb) of the water melon (*Citrullus vulgaris*).

The use of oil seeds to produce dairy product analogues is well known. Peanut butter milk, yoghurt and soy cheese are good examples (Andres, 1978; Chandrasekhara, 1974).

2.6 Non-Conventional Products

Some of the fermented products discussed above, such as bones, are non-conventional enough but the category of foods to be included in this section is truly exotic.

2.6.1 Beiga

This is fermented caterpillars. Like many Africans, some Sudanese tribes eat a number of caterpillar types but in most cases these are consumed unfermented. However, in the Nuba Mountains caterpillars collected in the rainy season from the arad tree (*Albizia amara*) are placed live in a burma and allowed to die and undergo fermentation. The tiny animals are then sun-dried and stored for later use either as a delicacy after roasting or for the preparation of soups.

2.6.2 Duga

In Darfur, eggs carrying, fat locusts are collected and placed into a burma to die and ferment within three days. They are then sun-dried and pounded to give a powder called duga used in sauces as a protein source.

2.6.3 Kesherneh

In western Darfur, near the borders with Chad, people ferment frogs to give the product called kesherneh. A particular kind of slender, jumping frogs, found in the damp sands of seasonal streams, is used for the purpose. The frogs are first boiled in water until they expand permanently. They are then dried in the sun, pounded into powder, fermented and subsequently sun-dried to give kesherneh.

2.6.4 Itaga

The gall bladder of cattle is removed from the carcass, carefully opened to avoid spillage, and then sorghum or millet flour is added to the juice to bind it. The bladder is then tied up and hung in a safe place to undergo drying and possibly fermentation for a few weeks. It is then reduced to fine powder called *itaga* which is used as a flavouring substance for fat meat dishes. The condiment is applied at the time of eating and not during cooking the goulash.

2.6.5 Okah

We descend now from the gall bladder to the renal bladder. *Okah* is fermented and ripened outright cow urine. The product can be ripened for up to 10 years, that is why in Arabic it is called *bol-gedim* (ripened or aged urine). Not any urine may be fermented though; only the urine from young heifers that are no longer suckling but which have not yet reached the age of puberty. Urine of young bulls is not suitable. Not only this, but the heifers so defined must be fed on wild green pasture. In the process of preparing *okah*, the housewife places a large earthenware jar, called *zeer*, in the corral where the cowboys fill it up with the correct cow urine and every morning add more urine to make up for evaporation and leakages losses. This is continued for three months of the rainy season (July-September). Following this, the housewife would take the *zeer* into her hut and keep it there for at least 9 months more before part of the now dark-brown and thicker product can be used to prepare sauce. *Okah* is so relished by people that the woman who possesses it has to stash it in a secret place or it will be begged away from her.

2.6.6 Fermented Mushrooms

Much more information needs to be gleaned on this product. It has been reported that the Bongo of Bahr El Ghazal eat many mushrooms which are first kept till on the verge of decay after which they are sun-dried, pounded and used as a condiment (Corkill, 1939; Evans-Pritchard, 1929). It has also been alleged that the Berta of the Blue Nile ferment mushrooms by first chewing them then spitting them into a *burma* where they undergo fermentation before they are consumed. Chewing of material suggests a contribution of the saliva amylases to the process of fermentation. In South America, tribes of the Amazon chew *cassava* and then spit it in a large bowl to undergo fermentation leading to the *cassava* beer called *masato* (Woolfe and Woolfe, 1984).

2.6.7 Hides and Hoofs

Fresh hide is sometimes cut into small strips which are charred on the fire to burn away the hair and then pounded and consumed during *merissa* drinking sittings. However, more often the skins, hides and hoofs are buried in mud or moist ash before they are consumed. During the period when they are buried these animal parts undergo fermentation. Later they usually are sun-dried and made into powder before use in sauces.

2.7 Wines and Meads

Palm wine, the one that is made from the sap of *Elaeis guineensis*, *Raphia vinifera* or *R. hookeri* in West Africa, is not known in the Sudan, neither is *lagmi*, produced from the sap of the date palm (*Pheonix dactylifera*) in northern Africa (Bassir, 1968; Okafor, 1966).

2.7.1 Wines in Sudan are all made from the date fruits. The wine land of Sudan is therefore the Northern Province where the bulk of the country's date

crop is produced. There are at least 10 wine types in Sudan but perhaps dakkai, nebit, sherbot, arizona and gemzout are the most important. Only the first three of these have had any luck with research (Ali and Dirar, 1984). Some of these are made from date syrup others from whole date fruits. Some have spices or sorghum malt as part of the mix while others have only dates. Yeasts, lactic acid bacteria and acetic acid bacteria make the microbial association of concern during the fermentation process.

2.7.2 The most important mead of Sudan is duma produced mainly in Equatorial Region of the southern Sudan. The fermentation of honey to make mead, such as tej of Ethiopia and mbote of Zambia, is well known to be a very slow process taking days and weeks (Steinkraus, 1983; Campbell-Platt, 1987). The fermentation is generally spontaneous and at best has a portion of a previous batch as a starter. The duma process is quite unique in a number of ways. The process takes only hours (usually 12 hours for commercial purposes), a special starter culture is used and the organisms involved are either thermotolerant or thermophilic. The key link in the process is the starter culture which is called iyal-duma (duma grains or seeds). Every family brewing duma for sale keeps its starter as a secret transferred vertically from grandmother to mother to grandchild. The starter is originally raised by enrichment technique from the roots of certain trees through a painstaking process. Once the starter has been obtained it is then produced in a large quantity of biomass as a paste of grains readily discerned by the naked eye. When diluted honey is added to the mass of duma grains fermentation takes place immediately as witnessed by the numerous bubbles evolved. It is probably using the resting cells as an enzyme source to bring about instant fermentation. Duma grains can be washed thoroughly with water, sun-dried and kept for years. There are two types of grain: white ones like rice and reddish or brownish ones which are the size of lentil seeds.

The grain consists of an aggregate of a capsulated bacterium and two kinds of yeast, Saccharomyces sp. and Schizosaccharomyces sp. Duma grains are reminiscent of kefir grains and tibi grains of Asia and Europe (Hesselfine, 1965).

3. SUDAN'S FERMENTED FOODS AND COPING STRATEGIES

Archaeological excavations carried out by Arkell (1949) in the closing years of World War I uncovered the remains of men who settled in the site of present-day Khartoum some 9000 years ago. These people lived mainly on wild grasses and on Nile fish. Three thousand years later these early Khartoum or Mesolithic Khartoum communities of negroid stock were replaced by cattle-rearing Neolithic populations of the same stock (Arkell, 1953, krzyzaniak, 1984).

Following that era, a number of kingdoms rose to power in Ancient Sudan. These included the kingdoms of Yam, Kerma, (2600-1500 B.C.), Cush (Kush) (1500-690 B.C.), and Meroe (690 B.C. - 323 A.D.). They all flourished in northern and central Sudan and had their capital cities on the Nile banks. The people of these kingdoms were negroes, hamites and mixtures of the two races.

The best known of these kingdoms is that of Meroe of which Shinnie (1967) wrote, "Meroe was an African civilization, firmly based on African soil, and developed by an African population. It lasted nearly a thousand years, as an urban, civilized and literate state, deep in the African continent". Some of the kings of Meroe such as Pi, Ankhy and Taharqa ruled over Sudan and the whole of Egypt up to the Mediterranean and even tried to conquer Syria and Palestine. These rulers figured in the Egyptian history as

the 25th Dynasty and Taharqa the Great is shown as a Negro on the stela of the Assyrian King Esarhaddon at Sinjirli.

The people of Meroe had an advanced food culture to the extent that their land became famous for the presence of what was called Table of the Sun of which Herodotus (430 B.C.) wrote about in his book The Histories. The Table of the Sun was allegedly a table full of all kinds of foods found in the green meadows on the outskirts of the city of Meroe. The table was said to be replenished by the magistrates of the kingdom every night and who ever passed by during the day may help themselves with the free food.

The Table of the Sun should be considered a symbol of Africa's food abundance and a reminder that advanced food culture is not new to the continent. The Meroites knew food fermentations. They left us a very impressive drawing of two men, under a thatch, drinking merissa from an earthenware container placed between them, a scene that could have been picked out of today's African life. The Meroites also left for us a number of wine presses and wine jars. An inscription has been found at Axum of present-day Ethiopia in which the Axumite King Aezanes described his campaign and the ransacking of the city of Meroe in about 350 A.D. The inscription reads in part, "... and my people seized their corn (sorghum) and their bronze and dried meat". The dried meat must have been the same as shermout of today. The Greek writer Strabo (63 B.C. - 23 A.D.) who visited Egypt in the years 25-19 B.C., wrote about Meroe in his Geography (7 B.C.) mentioning that the Meroites lived, among other things, on sorghum, meat, milk and cheese - a fermented product.

The discussion given above should serve to demonstrate a crucial point and that the fermented food culture of Africa is not the newborn of the centuries but the legitimate child of the millennia, a deep-rooted, ancient culture.

Further, a look at the fermented food items of Sudan discussed above strongly suggests that the bulk of them have been developed as part of a strategy to cope with chronic food shortage and survival problems. Out of the 85 or so food items 33 are both fermented and sun-dried while 17 are either ripened foods or intermediate-moisture foods. The processing strategy here aims at long storage which suggests fear from food shortage. Second, about 20 of these foods are prepared during the very short rainy season of 3 months when many of the raw materials for fermentation are present. These are collected, fermented, dried and stored for use during the remaining 9 lean months of the calendar year. Third, the fermentation of such marginal food items like bones, hides, hooves, pure fat, frogs, locusts, urine, etc., is strong proof that a major aim behind food fermentation in the Sudan (and most likely in the Sahel countries) is to cope with food shortage. It is therefore suggested here that the totality of fermented foods of the region be considered as famine foods.

A point of great implications is that there is a strong similarity between the fermented foods of Sudan, a country which could be considered part of East Africa, and those of West Africa. The connections between West Africa and the central Nile extend to times immemorial. The peoples of the flat expanse of land (Arabic sahl) that extends from the Nile and the western parts of the continent must have been intermingling for thousands of years. A carbonized fragment of the shell of the W. African oilpalm fruit E. guineensis has been found in one of the hearths of a Neolithic Khartoum archaeological sites right at the confluence of the Blue and White Niles, thousands of miles away from its natural habitat in W. Africa (Arkell, 1953). Even today the Arabic names of fermented and other foods of Sudan such as medida, agoud, moss, ajin, shaya, aish, um-bilbil and dawa find their parallel in W. Africa

as _medidi_, _agidi_, _mosa_, _ogi_ (also _uji_ of E. Africa), _suya_, _aish_, _bilbil_ and _dawa-dawa_ (literally, spice of spices in Arabic).

The above-mentioned discussion suggests that the study of fermented foods of the savannah and sahel regions of Africa should always be tackled within the framework of history, famine strategies and regionality. In other words, the food scientists of the region need to join forces for a more rewarding effort. It is very easy to take a strange food from a famished rural woman, study it in the laboratory, publish it in a 'reputable journal' and get promoted. In the meantime this poor woman would have probably died of hunger without even her invention being acknowledged by the male scientist.

4. FERMENTED FOODS AND BIOTECHNOLOGY

Any attempt to modernize the production of fermented foods of any country should first be preceded by the formulation of a clear strategy as to the targeted groups for whom the modernization is intended. If the beneficiary is the rural population then a simple, intermediate technology needs to be developed and such technology should aim at strengthening the role of these foods in the struggle against malnutrition, food shortage and famine.

Fermentation of foods is part of the traditional biotechnology or industrial and applied microbiology. But unlike other traditional biotechnologies this field of human knowledge has been left out of the arena of modern biotechnology in which recombinant DNA techniques and other modern genetic tools have made important contributions. The reasons for this negligence partly include the fact that research and development funds for research in biotechnology have been largely made available by multi-national giants running after profits, and why should these fund research on poor man's food?

Biotechnological research on fermented foods of Africa should first begin with traditional research which in turn should be preceded by a thorough documentation of the foods and the procedures followed in their preparation. An aspect of great importance but one that has often been neglected by laboratory researchers is the socio-economic aspect of these foods. The 'scientific' knowledge of the women who invented and preserved these foods for us should not be underestimated or belittled. All information concerning the particular food should be gathered and stored. We are talking nowadays of genebanks to preserve the germplasm of many plants from an imminent and irremediable loss. Let me suggest here that we also need to develop and establish 'food banks' to preserve food processing methods that are disappearing under the cultural pressure of the urbanized communities on the rural communities. The 'food bank' should concern itself with all famine foods: fermented foods, non-fermented foods, wild fruits, vegetables, seeds, insects, roots, etc., of course together with non-famine foods.

Modern biotechnology may be applied to R&D of fermented foods at three levels:

i) _Raw material level_. As these foods are either of plant or animal origin, the applications of biotechnology in the field of general agriculture will all help secure the raw materials from which these foods are made. Only there are certain plants and animals in addition to the so-called 'lost crops of Africa' that need special attention. The biotechnology techniques of cell, tissue and organ cultures, including meristem and anther cultures and protoplast fusion may be made use of. The use of vaccines to combat disease and monoclonals to diagnose disease agents as well as superovulation and embryo transfer techniques could also make very useful tools in this area.

ii) Process engineering level. Here, the various bioreactor designs so far developed and the knowledge gained in this area can of course be extrapolated to include the area of fermented foods and beverages. Since many of these foods are produced by a solid substrate fermentation process, fermenters designed to suit this situation need to be developed.

iii) Microbial starter level. Hundreds of micro-organisms are involved in the fermentation of foods in Africa. This is a great microbiological resource. These micro-organisms must have a wide range of biosynthetic and degradative abilities which may be harnessed in many areas of modern-biotechnology. For instance, the yeasts of the duma grains have already proven vigorous fermenters of cane molasses.

These organisms need to be isolated, purified, characterized and preserved. The enzymic capability of each one of them must be fully studied. The collective germplasm thus obtained may be used to genetically engineer starter micro-organisms which perhaps release larger quantities of vitamins and amino acids in the food to be fermented, thus helping improve the nutritional worth of it for rural population.

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Biotechnology and food fermentations

by

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ABSTRACT

Fermentation is one of the oldest forms of biotechnology with evidence of its use dating back over several millennia. Although modern biotechnology is well established in some fermentation industries (e.G. brewing, baking, dairy) there are many indigenous fermentations which have yet to see the benefits of this technology. Much can still be achieved using low cost approaches through the design of basic fermentation equipment and simple selection techniques for the improvement of fermentative micro-organisms.

Solid-substrate fermentations may be particularly suited to developing countries. Possible applications include the utilization of raw starch substrates, microbial detoxification of plant materials and the upgrading of waste products through microbial enrichment.

Microbial metabolites make an important contribution to both the safety and organoleptic properties of fermented foods and there are considerable opportunities for their exploitation. Biotechnology is likely to play an important role in the development of safe, low cost methods of food preservation both in developed and developing countries. Products from food fermentative micro-organisms may also have applications in areas other than fermented foods.

The genetic manipulation of food fermentative micro-organisms using molecular biology techniques will eventually benefit developing countries although much can still be achieved using existing strains, mixed culture fermentations as well as basic selection methods.

INTRODUCTION

Drying, salting, smoking and fermentation have been used by man for many centuries as methods of food preservation. Fermentation is probably the oldest form of biotechnology and fermented foods make a significant contribution to the human diet in many regions of the world.

Food fermentations have developed in many parts of the world because they have definite advantages over other preservation methods. The low-cost of food preservation by fermentation is particularly important in developing countries where canning, refrigeration and freezing may be limited or unavailable. Food fermentations usually have lower energy requirements than other processing methods and this may be particularly important where fuel is limited.

Fermentation often leads to an improvement in the nutritional value of foods by bio-enrichment with microbial protein, amino acids, lipids and vitamins. Platt (1964) has introduced the term biological ennoblement to describe foods where a significant nutritional improvement occurs through the action of fermentation. Fermentation processes also have a role in improving nutritional value and acceptability by contributing to the degradation of toxins and antinutritional factors present in many plant foods (Reddy _et al_. 1986).

The organoleptic properties of fermented foods often differ from the unfermented substrate. For example, yoghurt cannot be produced simply by

adding lactic acid to milk. New aroma and flavour components may be produced by fermentation including acids, carbonyl compounds, esters, alcohols and pyrazines. In addition to flavour and aroma, the physical form, texture and colour may change. Solid foods may become liquefied (eg. soy sauce from soybean) or liquid substrates converted into gels or solids (eg. milk into yoghurt and cheese).

Fermented foods comprise a diverse range of products from many regions of the world. Campbell-Platt (1987) has listed some 250 categories of fermented foods with more than 3500 individual products. Table 1 shows the geographical distribution of fermented foods based on the substrates used. Those fermented foods based on starch crops, legumes and various mixtures of fish, shellfish and crustaceans are produced mostly in developing countries where they make an important contribution to the diet.

Fermentation processes usually involve either an alkaline (proteolytic), lactic acid, acetic acid or alcoholic fermentation or a combination of these processes. In some South East Asian fish fermented foods autolytic enzymes such as proteases play an important role in the fermentation with minimal bacterial involvement. Ingredients other than the primary substrate are often added to fermentations and may be important sources of microorganisms, enzymes, catalysts, nutrients and selective inhibitors. For example, in Zambia, amylases in the roots of *Rhynchosia* spp. (Leguminosae) reduce the viscosity of maize meal and release reducing sugars which are fermented in the production of munkoyo beverage. In Mexico various plant ingredients appear to act as catalysts which accelerate the fermentation of maize to produce the beverage tesguino (Lappe & Ulloa 1983).

Application of molecular techniques to the improvement of food fermentative organisms is already under way but only in areas (beer, bread, dairy, meat, soy sauce) where a large industrial base already exists. For small scale, indigenous, non- industrialized food fermentations the transition to large scale industrial production may be a necessary prerequisite to the application of recombinant DNA technology. Much progress could be achieved by the use of basic biotechnological techniques such as the design of simple fermenters both for liquid and solid-state fermentations. Simple selection techniques can be used to 'improve' strain properties and even the development of mixed culture systems may offer advantages over the use of single strains.

FERMENTATION TECHNOLOGY

Although the majority of Western fermentation technology utilizes aseptic liquid fermentations solid substrate systems are widely used in food fermentations and have been employed in the Orient for many centuries for the production of tempe (moulded legume cake) and koji for soy sauce and sake production. Solid substrate fermentations have advantages over liquid fermentations particularly with the current interest in 'clean technologies' which generates minimal waste disposal problems.

Advantages of solid-substrate fermentations are:

1. The capital investment in fermentation technology is lower.

2. Energy and water requirements are less.

3. Fermentation is usually non aseptic.

4. Fewer problems with downstream processing and disposal of large volumes of liquid waste.

5. In some food fermentations there is a high affinity between the microorganism(s) and the substrate as developed through domestication.

Despite the economic and environmental attractiveness of solid-substrate processes they have not been widely exploited in biotechnology principally because of difficulties in a) modelling heat and mass transfer, b) quantifying microbial biomass and c) scaling up production. There have been few advances in the provision of instrumentation such as pH, temperature, O_2, a_w and ion selective probes for monitoring solid-substrate systems. Consequently, there are many challenges for food technologists, microbiologists and chemical engineers to model growth, behaviour and product formation in these systems. Some progress has been made in a few areas and we (Cook *et al*. 1991a) have developed a relatively simple low cost method of quantifying fungal growth in fermented rice. Similar approaches may have value in quantifying and modelling fungal growth in other solid substrate food fermentations.

Despite the drawbacks of solid substrate systems the Japanese have very successfully exploited the solid-substrate koji fermentation on a large scale. Opportunities must exist for the application of similar approaches to other fermented foods. Glenn & Rogers (1989) have provided a more detailed discussion of the advantages and disadvantages of solid-substrate fermentations.

Raw starch hydrolysis

Most starchy substrates are cooked before they can effectively be hydrolysed by amylolytic enzymes and considerable energy inputs may be required for this purpose. A number of fungi are known to produce amylases which are capable of acting on raw non gelatinized forms of starch and interest has recently focused on selecting strains of *Bacillus* capable of raw starch hydrolysis. Examples of raw starch utilization occur in a number of fermented foods and include the ragi starter cake fermentation which is used as a traditional sources of amylolytic organisms for alcoholic fermentations in Asia. In Africa a dark moulded cassava flour is made using uncooked cassava (Essers & Nout 1989) and there are probably many other traditional fermentations which use raw starchy substrates.

The ability to hydrolyse raw starch at acid pH has been reported from relatively few organisms but includes species of *Rhizopus* (Yamazaki & Ueda 1951) *Aspergillus* (Ueda *et al*. 1984) and the filamentous yeast *Saccharomycopsis fibuligera* (Ueda & Saha 1983).

One of the attractive features of using raw starch substrates is the low energy input required. In the case of ethanol production from cooked sweet potato, some 30-40% of the energy input comes from cooking the starch prior to hydrolysis. Dalmia & Nikolov (1991) have shown that starch binding domains on *Aspergillus niger* glucoamylase I are responsible for the specific interaction with starch granules. If these could be characterized then this might enable the development of increased specificity for raw starch and the application of molecular techniques to open up much wider exploitation. Clearly if fermentation processes could be developed which utilized raw rather than cooked starches then there would be a significant saving in energy costs for producing food.

Upgrading of waste products

Several indigenous food fermentations use waste products from other industries. In Indonesia for example, press-cakes from groundnut oil extraction or tofu manufacture are fermented using the moulds Neurospora intermedia o- Rhizopus oligosporus to produce oncom a meat substitute. A press-cake residue from coconut oil extraction is also fermented by Rhizopus oligosporus to produce tempe bonkrek. Many of these processes have developed following the transfer of technology from similar food fermentations such as tempe fermentation of soybeans.

The Scandinavian Symba process was developed to convert starchy wastes to an edible product consisting principally of Candida utilis biomass. As Candida utilis is unable to grow on starchy substrates a mixed culture fermentation is used and the amylolytic yeast Saccharomycopsis fibuligera hydrolyses starch to a mixture of sugars which are used by Candida utilis for growth. Fellows and Worgan (1987) have shown that a Symba type process can also be produced using pectic materials or starch pectin combinations. Saccharomycopsis fibuligera is also used in the production of Indonesian tapé using cassava or glutinous rice and to produce Thumba an alcoholic beverage from Bangladesh. Clearly such organisms may have considerable flexibility both for producing traditional fermented foods and for the upgrading of waste products to foods or feeds. Novel fermentations should always be assessed for safety since there may be altered metabolism of the fermentative organisms or the new system may favour growth of food borne pathogens or toxigenic microorgMwisms as in the case of Pseudomonas cocovenenans in coconut presscake.

ROLE OF FOOD FERMENTATIONS IN THE DETOXIFICATION OF FOODS

Plant components

Plant materials such as cereals, green vegetables, legumes and root crops are widely used in food fermentations although many of these contain significant levels of antinutritional and toxic components (Table 2). A number of these components can reduce the nutritional value of foods by interfering with the digestibility of proteins and carbohydrates (eg. lectins, tannins) or reducing the biological availability of certain minerals (eg. phytate). Stages in the preparation of foods prior to fermentation such as washing, soaking, boiling, steaming, roasting and grinding can result in a significant reduction in the levels of some of these components. In some cases fermentation is an important stage in degrading some of the toxic and antinutritional components in foods (Reddy et al. 1986).

Many food fermentative organisms have enzymes capable of degrading toxic and antinutritional factors (Campbell-Platt & Cook 1991) and strain selection or recombinant DNA technology could open up a powerful route to detoxifying plant substrates using microorganisms.

Mycotoxins

In the field and during storage, cereals and legumes may frequently become contaminated with aflatoxins and other mycotoxins. The highly toxic and carcinogenic aflatoxins produced by Aspergillus flavus and A.parasiticus are usually resistant to degradation except by strong oxidizing and alkali

treatments. Certain cereals and legumes used in fermented foods production (eg. maize, ground nuts) are particularly prone to aflatoxin contamination. Although aflatoxins are heat stable, some cooking treatments such nixtamalisation of maize (boiling with lime) for Mexican pozol (fermented maize dough) does reduce levels of aflatoxins (Ulloa & Herrera 1970). Although washing, soaking grinding and steaming probably have a only minimal effects on aflatoxin levels in foods there is evidence that biotransformation of aflatoxins can occur in some traditional food fermentations (Campbell-Platt & Cook 1991). Nakazato et al. (1990) have shown that Rhizopus arrhizus and RhiMwpus oligosporus are capable of reducing aflatoxin B_1 and recently, Bol and Smith (1989) have demonstrated that certain Rhizopus spp. are capable of degrading more than 80% of aflatoxin B_1 to non-fluorescent compounds, although these are of unknown toxicity. In some lactic acid fermentations the low pH may contribute to the conversion of aflatoxin B_1 to the less toxic aflatoxin B_{2a} (Nout 1991). Enzymic processes may be involved in aflatoxin degradation and characterization of these processes may reveal strategies for a more widespread decontamination of food commodities.

FERMENTED FOODS AND FOOD SAFETY

Many fermentation products such as organic acids, alcohols, diacetyl, acetoin and esters kill or suppress the growth of food- borne pathogenic bacteria. This may be particularly important in developing countries and outweigh any nutritional benefits from consuming these foods. Fermentations involving lactic acid bacteria probably have potential for more widespread applications, particularly for the preservation of cereals, root crops and legumes to provide safe, low-cost weaning foods. Lactic acid bacteria found in fermented foods are a rich source of antimicrobial compounds including organic acids, diacetyl, acetoin, hydrogen peroxide and bacteriocins (Klaenhammer 1988; Daeschel 1989). Bacteriocins are relatively narrow spectrum antimicrobials and many are only active against related bacteria. Table 3 presents a selection of bacteriocins which have been characterized from lactic acid bacteria. Nisin a bacteriocin produced by Lactococcus lactis is active against Gram +ve bacteria, is relatively stable to cooking and low pH, has received GRAS (Generally Regarded As Safe) status and is now approved for use as a food preservative in more than 40 countries (Delves-Broughton 1990). With increasing interest in natural minimally processed foods other bacteriocins produced by lactic acid bacteria may eventually get approval for use as preservatives both in fermented and non fermented foods. Although many fermented foods are regarded as safe products, under certain circumstances food borne pathogens may be capable of growing. For example, Bacillus cereus is able to grow and survive during Indonesian tapé fermentation (Cook et al. 1991b) and during Indonesian tempe production (Ashenafi & Busse 1991). Bacteriocins such as nisin may have applications for controlling undesirable organisms such as Bacillus cereus in fermented products although the stability of bacteriocins in the fermentation environment is unknown.

A relatively new area of antimicrobials is that of enzymes produced by bacteria which can be used as food preservatives. Lotz et al. (1990) have described a process for preserving raw meat using a bacterial lysing enzyme from Streptomyces. Nielson (1991) has described some novel bacteria lysing enzymes and Proctor and Cunningham (1988) have reviewed the chemistry of lysozyme and its use as a food preservative. Bacteria or fungal lysing enzymes have not been reported from fermentations although it seems likely that such interactions probably occur between some bacteria in fermented foods.

BIOTECHNOLOGICAL RESOURCES

Cook (1991a b) has drawn attention to the diverse range of products which can be derived directly from food fermentative microorganisms or produced as a result of their activity. These organisms may be sources of useful components such as enzymes (eg. amylases, proteases and lipases) uncommon lipids, pigments and flavour compounds (eg. pyrazines). Gamma linolenic acid is traditionally obtained from plant sources but is now being commercially produced from food fermentative fungi (Campbell- Platt & Cook 1989). Those food fermentations which use mucoraceous moulds (eg. tempe, tapé, sufu) may also be valuable 'natural'sources of this polyunsaturated fatty acid.

Pigments of Monascus spp. have traditionally been used as natural food colorants for wines, soybean curd, fish and candy. Monascus pigments are regarded as safe natural alternatives to many coal tar dyes and between 1971 and 1[illegible]0 some 39 patents were filed on food colorant applications using Monascus.

Chitosan a natural polymer of n-acetyl glucosamine is abundant in mucoraceous fungi used in food fermentations and offers an alternative source to that produced by alkaline hydrolysis of crustacean chitin. Sandford (1989) gives examples of the diverse range of applications for the chitosan polymer.

Fermented foods are potentially a rich source of antimicrobial agents both from fungi and bacteria. The bacteriocins produced by lactic acid bacteria (Table 3) have already been mentioned. Bacillus spp. also occur in a number of fermented foods (Iru/Dawadawa in West Africa, Natto in Japan, Kinima in India and Nepal and Thu-nao in Thailand) and the genus is well known as a source of more than 30 broad and narrow spectrum antimicrobials. Clearly more work is need to establish potential applications for antimicrobials produced by food fermentative microorganisms.

SELECTION AND GENETIC MANIPULATION OF FOOD FERMENTATIVE ORGANISMS

Traditional mutation and selection methods and genetic manipulations using recombinant DNA technology are now being used to optimise food fermentative bacteria and fungi. These approaches should eventually enable the selection of desirable starter organisms which are non-toxigenic and have desirable enzymic profiles for food production. Other characteristics which may be suitable for genetic manipulation include increased protein production, the ability to detoxify plant and microbial toxins, resistance to bacteriophages and the stable expression of bacteriocins in bacterial starter cultures for fermented foods. The controlled production of enzymes such as amylases, lipases and proteases should enable more predictable fermentations and the development of novel organoleptic properties.

Currently, there is considerable interest in the extracellular enzymes of fungi and both protoplast fusion techniques and DNA transformation systems using drug resistance markers are being developed for a range of species (Table 4). It is only a matter of time before this technology is applied for the improvement of food fermentative fungi such as the mucoraceous moulds used in tempe and tapé production. Glenn & Rogers (1989) have draw attention to the potential of strain selection for the development of temperature sensitive and asporogenous mutants. In the case of fungi these would be particularly valuable since it is usually undesirable for a fungus to sporulate on a fermented product.

Although there is considerable interest in the use of bacteriocins from lactic acid bacteria, applications have been hampered by the instability of bacteriocin production in pure cultures. The application of genetics to lactic acid bacteria is relatively recent and most interest has focused on dairy starters particularly Lactococcus lactis with genera such as Lactobacillus and Leuconostoc being relatively little studied. Because many bacteriocins appear to be plasmid encoded their production can be readily lost by a particular strain. Molecular techniques are being used to characterize production of bacteriocins and will hopefully lead to more stable expression by transfer of genes to stable chromosomes, both in producer strains and other fermentative bacteria.

Although there is considerable appeal in improving starter cultures using molecular techniques it must be remembered that many fermentation processes developed over many centuries through a process of selection by domestication. In many instances organisms which are 'improved' by genetic modification may offer little advantage over existing naturally selected mixtures of strains. There are several reasons for this including:

1) Bacterial cultures of a single strain are more prone to bacteriophage infection which can seriously affect food production through product loss, spoilage and wasted operating costs. This problem has most often been seen in the dairy industry where bacteriophages survive the pasteurisation of milk.

   As shown in Table 5 bacteriophage infection is probably not restricted to the dairy industry. In some fermentations, food safety might be compromised by bacteriophage infections, since in non-aseptic processes, reduced acid production could enable the growth of food-borne pathogens. The incidence of bacteriophage infections in non industrialized fermented foods is unknown but it may be important factors in the often reported variability of fermented products.

   There is currently considerable interest in the use of recombinant DNA techniques with the aim of developing bacteriophage resistant strains of lactic-acid bacteria for the dairy industry. Molecular techniques are being used to establish the mechanism of naturally occurring bacteriophage resistance including the inhibition of bacteriophage development and its adsorption to the host cell.

2) Genetic manipulation may lead to a loss of competitive ability. Unless fermentations with genetically modified organisms are carried out under aseptic conditions, genetically modified organisms may show poor competitive ability compared to 'wild' type strains. Genes for the over-production of large molecular weight metabolically expensive components such as enzymes and bacteriocins might render such strains competitively inferior to wild type or domesticated strains. Any benefit from the over-production of metabolites must be balanced against the ability of genetically modified organisms to support increased uptake of fermentation products so that growth rate and production of other metabolites is not affected.

3) Genetically modified organisms must offer economic advantages over the use of existing pure or undefined cultures of microorganisms. The costs of

using genetically modified strains must not exceed the economic advantages gained such as faster processing times and reduced product variability, energy and manpower costs.

4) Mixed culture systems may offer a simpler, low cost alternative to the use of genetically engineered strains. Mixed culture systems have not been widely used in biotechnology although there are a number of industrial processes employing more than one organism including yoghurt production and the Symba process. Whereas single strains under aseptic conditions are used in the production of pharmaceuticals, organic acids and enzymes many traditional fermented food processes involve mixtures of microorganisms (consortia) under non-aseptic conditions. Simplification of a fermentation process by using single strains modified by selection or recombinant DNA techniques may remove the ability of fermentations to sustain environmental (temperature, pH, gases) and biological (competitors, bacteriophages, food borne pathogens) purtebations. Such factors can only be controlled effectively in closed aseptic systems which may not be economic for the production of many traditional fermented foods.

FUTURE OUTLOOK

Fermented foods have been used by man over several millennia and are likely to remain an important part of our food supply. Interest in food fermentations is likely to increase, particularly with the attractions of minimal processing and efficient use of food and fuel resources. Modern biotechnology has an important role to play in the development of new starter cultures for the efficient large scale production of fermented foods. However, increasing the efficiency of many food fermentation processes should not be at the expense of 'simple' low cost technology which will continue to have an important role to play in food production.

It is important that scientists in developing countries are kept in touch with developments in modern biotechnology and this is probably best achieved through the formation of a network of interested workers in different countries. Only by regular contact through newsletters, workshops conferences and exchange programmes will the benefits of modern biotechnology be effectively applied to food fermentations in developing countries.

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Table 1. The major producing regions for fermented foods based on substrate categories.

Fermented products	Major producing regions
Meats	Europe, North & South America
Seafoods (fish, shellfish, crustacea)	East & South East Asia
Dairy	Europe, North & South America, Middle East
Cereals	Worldwide
Root crops	Africa
Legumes	East & South East Asia, Indian subcontinent
Vegetables & Fruits	Europe, North America, East Asia

Adapted from Campbell-Platt (1987)

Table 2. **Antinutritional and toxic components present in plant and animal foods used for fermentation**

Component	Principal occurrence
Plant origin 1. cyanogenic glucosides	cassava, some legumes
2. enzyme inhibitors	legumes
3. glucosinolates	_Brassica_ vegetables
4. lectins (haemoglutanins)	legumes
5. oligosaccharides (eg. raffinose, stachyose)	cereals, legumes
6. phytate	cereals and legumes
7. oxalates	cereals, legumes, root, crops, vegetables
8. tannins and other polyphenolics	cereals, legumes, tea
9. saponins	cereals, legumes
Microbial toxins 1. aflatoxins	cereals, legumes
2. ochratoxins	meats
3. other mycotoxins	cereals, legumes, root, crops
4. bacterial toxins	various
Miscellaneous 1. biogenic amines	fish

Sources: Reddy _et al._ (1986); Campbell-Platt (1987)

Table 3. Bacteriocins produced by lactic acid bacteria

Name	Producer organism	Food use status
Acidolin	Lactobacillus acidophilus	
Acidophilin	Lactobacillus acidophilus	
Bulgaricin	Lactobacillus delbrueckii subsp. bulgaricus	
Brevicin	Lactobacillus brevis	
Caseicin	Lactobacillus casei	
Diplococcin	Lactococcus lactis subsp. cremoris	
Helveticin	Lactobacillus helveticus	
Lactacin	Lactobacillus acicophilus	
Lactocin	Lactobacillus sake	
Nisin	Lactococcus lactis	GRAS status, Used in foods
Pediocin	Pediococcus acidilactici Pediococcus pentosaceus	
Reutericin 6	Lactobacillus reuteri	
Sakacin	Lactobacillus sake	
Non peptides Reuterin	Lactobacillus reuteri	

Table 4. Examples where biotechnology is being used to improve fungi for food use or product formation.

Organism	Approach used	Reference
Penicillium caseicolum	protoplast fusion	Reymond *et al*.(1986)
Penicillium nalgiovense	DNA transformation	Geisen & Leistner (1989)
Penicillium roquefortii	DNA transformation	Durand *et al*. (1991)
Rhizomucor spp.	protoplast fusion	Goto-Hamamoto *et al*. (1986)
Rhizopus niveus	protoplast fusion & DNA transformation	Yanai *et al.* (1990 & 1991)
Saccharomyces diastaticus	protoplast fusion	Sakai *et al*. (1986)

Table 5. Examples of fermented foods where bacteriophage infections have been reported.

Fermented food	Infected microorganism	Reference
Dairy Cheese	*Lactococcus lactis* *Lactococcus lactis* subsp. *cremoris*	Whitehead & Cox (1936)
Yakult	*Lactobacillus casei*	Shimizu-Kadcta & Sakurai (1982)
Yoghurt	*Lactobacillus delbrueckii* subsp. *bulgaricus* *Streptococcus thermophilus*	Peake & Stanley (1978) Accolas & Spillmann (1979)
Legume Natto	*Bacillus subtilis* var. *natto*	Yoshimoto *et al*. (1970)
Alcoholic Sake	*Leuconostoc mesenteroides*	Kaneko *et al*. (1955)
Australian wine	*Leuconostoc oenos*	Davis *et al*. (1985)

Adapted from Sanders (1987)

PRODUCTION OF WINE FROM COCOA JUICE (Theobroma cacao L. Kuntze) USING SACCHAROMYCES SPECIES ISOLATED FROM PALM WINE AND CASHEW JUICE

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ABSTRACT

Juice obtained from the mucilage of cocoa beans containing 18.60 soluble solids was fermented with strains of yeast isolated from palm wine and cashew juice. The strains from each substrate source belong to the genus Saccharomyces. Saccharomyces species isolated from palm wine yielded 10.2% (v/v) alcohol while that from cashew juice yielded 10.5% (v/v) resulting into a sweet table wine. They both had 3.4 and 3.8 pH. respectively.

INTRODUCTION

In the tropics, cocoa juice is a processing waste. This is because it is only the beans that is commercialized while the cocoa juice and the cocoa pod husk have not been given any serious attention. In Nigeria, an estimated 30 million litres of cocoa mucilage or sweating goes into waste annually. One of the methods of processing and preserving the cocoa juice is to ferment it into wine. This publication reports the fermentation of cocoa juice into wine using locally- isolated Saccharomyces species from palm wine and cashew juice.

MATERIALS AND METHODS

Yeast isolation
Cashew juice

Eight yellow apples obtained from Cocoa Research Institute of Nigeria (CRIN) cashew plots were used. The apple pomace containing the juice was collected in a sterile plastic container and covered. 50ml of the juice was pasteurized at 65°C for 20 minutes and allowed to stand until spontaneous fermentation started. The pomace exposed to laboratory and atmospheric air for 2hrs. was covered and allowed to stand for 24hrs under laboratory conditions. Inocula were taken from the fermenting juice and chaff by streak and serial dilution methods; then plated on Potato Dextrose Agar (Oxoid) and Malt Extract Agar (Oxoid). Growth, observations and subculturing followed after 48hrs of incubation at 30°C. Isolate was subcultured 5 times to achieve satisfactory colony characteristics showing that the pure culture has emerged. Morphological characteristics were studied by examination while cultural characteristics were studied on the Yeast Extract Dextrose Agar (YEPDA) and potato Dextrose Agar (PDA). Cultural and biochemical studies was according to the methods of Benitez et al., 1983.

Palm Sap

Palm sap was obtained from a freshly tapped palm tree on the Institute's plantation. Under strict aseptic conditions, the tapper collected the sweet colourless juice and transferred it into a sterile dry plastic container which was covered and brought into the laboratory within 1 hour of collection. Using sterile pipette, several dilutions of 1ml of the fresh palm wine was made up to ten folds. 1ml from each tube was added into the media (PDA and MEA). The plates were rotated to enhance the spread of the inoculum homogeneously. Incubation took place at 30°C for 48hrs. The procedure for identification and purification are the same as for cashew juice yeast isolate.

Collection and treatment of cocoa juice

At the CRIN fermentary, cocoa juice was collected in cleaned and sterilized containers earlier washed with sodium metabisulphite solution (2g/L). The collected juice was filtered through a muslin cloth and then treated with 250ppm metabisulphite solution. The juice was pasteurized at 70°C for 10 minutes to prevent oxidative reactions, and elimination of bacteria and wild yeast growth. The moisture content was determined at 100°C using a vacuum oven. The pH was determined using Pye Unicam model 291 pH meter. The ash content, crude protein and total acidity were determined by the methods described by Amerine and Ough (1980). The total soluble solids was measured with Bausch and Lamb Abbe refractometer (Hart and Fisher, 1971).

Fermentation of cocoa juice

The cocoa juice was diluted with water (1:1). Depectinized (2g/L. pectolase enzyme) was put into two separate 20L fermentation vessels. Each vessel was ameliorated with sugar (sucrose) and water up to 25% soluble solids such that the fermentation vessel was 2/3 full thereby allowing for headspace. Proportional levels of citric acid (1.5g/L) and yeast nutrient (1g/L) were added into each 20L vessel. Already propagated yeast strains inoculated into PDA broth was added into the diluted and ameliorated substrate. The vessels were closed with rubber stoppers fitted with airlocks containing 250ppm sodium metabisulphite solution. Fermentation was conducted anaerobically for 21 days at 28° + 2°C. Specific gravity, total soluble solids, total acidity and pH were monitored while fermentation was in progress. The fermented juice were racked at the end of fermentation indicated by diminished gas (CO_2) evolution. The wines were clarified with bentonite clay (0.5g/L) after yeast flocculation and racked. They were aged at controlled temperature (well ventilated cool room).

Organoleptic evaluation of the wines fermented by the yeast isolated were conducted by a panel of eight and compared with a sweet table wine brought from a wine and liquor store in Ibadan.

RESULTS AND DISCUSSION

The morphological and cultural characteristics of the yeast isolates is presented in Table 1. Strains YP and YC formed 4 ascospores per ascus and multiplied by fission (multilateral and bilateral budding). YP showed a distinct pseudomycelium. The colony is white with entire adges, moist surface and low convex elevation. YC showed white colonies with serrated edges, dry surface and low convex. The pseudomycelium is well formed with bilateral budding. The morphological and cultural characteristics of YP and YC strains in terms of number of ascospores, pseudomycelium formation, mode of budding and appearance of the colonies are in agreement with Fowell (1969) and Barnett et al., 1983. Chemical analysis of the cocoa juice is presented in Table 2. Total soluble solids was 18.60% while the total reducing sugar was 17.52%. The high percentage soluble solids indicate its potential as a substrate source for the fermentation industry and the suitability for wine making. Total acidity of the juice (expressed as citric) is 0.78% while protein and ash content were 0.50% and 0.45% respectively. The values obtained for the chemical analysis of the cocoa juice were comparable with those reported for tropical fruits (Maldonado et al., 1975).

The fermentation profiles of the yeast strains in the fermenting cocoa juice are presented in figures 1 and 2. Fermentation with YC yielded a decrease in total sugar from 19.3% (v/v) to 8.8% (v/v) in the first 10 days and 4.2% (w/v) in the next 21 days. Fermentation with YP showed a decrease in total sugar from 21% (w/v) to 11% (w/v) in 10 days and 5% (w/v) in the next

18 days. The yeast strains YP and YC belong to the genus Saccharomyces (Barnett et al., 1983).

Table 3. represents the chemical analyses of the wines. Total acidity increased as the fermentation progressed with a fall in pH of the substrate. Total acidity in wine from YP (0.6%) is higher than that of wine from YC (0.4% and both were within the range of 0.55 and 0.85% (Amerine and Joslyn, 1970). The pH 3.4 and 3.8 for wines from YP and YC were within the range of 3.1 and 3.9 as recommended by Amerine and Joslyn (1970) for table wines. The ash content of the wine produced by YC was 0.11% and that of YP was 0.13%. The values decreased from 0.45% total ash obtained for cocoa juice and this indicated that the yeast strains utilized some of the minerals. The volatile acidity of the wines 0.14% and 0.17% were within the range of 0.11 and 0.25% as reported by Amerine and Ough (1980).

Organoleptic evaluation of the wines based on colour, odour, taste and flavour using the ranking method of Larmond (1982) as presented in Table 4 suggested that wines produced from YC and YP yeast strains were good for human consumption. Comparison of the wines based on the organoleptic quality attributes showed that YC wine was much acceptable however there were no significant differences between the YC and YP wines at 5% level of probability.

ACKNOWLEDGEMENT

The author wishes to thank the Director, Cocoa Research Institute of Nigeria, Ibadan for nominating him to represent the Institute at the UNIDO Expert Group Meeting on the Application of Biotechnology to Food Processing in Africa and also for his permission to present this paper.

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TABLE 1: MORPHOLOGICAL AND CULTURAL CHARACTERISTICS OF YEAST ISOLATES

YEAST	MORPHOLOGY OF COLONY YEPDA	PSEDOMYCELIUM FORMATION	ASCOSPOROUS PERASCUS	DIAMETER OF COLONY
YC	white colonies with entire edges & moist surface, low convex	distinct & well formed	4	2mm colonies ellipsoidal arranged singly with multilateral budding
YP	White colonies with serrated edges and dry surface low convex	well formed	4	3mm colonies arranged in twos with bilateral budding

TABLE 2: CHEMICAL ANALYSIS OF COCOA JUICE

	%
Total soluble solids	18.60
Moisture content	84.50
pH	4.20
Ash content	0.45
Total Acidity (citric)	0.78
Protein (crude)	0.50

TABLE 3: WINE ANALYSIS

	YC	YP
Total alcohol %	10.5	10.2
Alcohol yield	0.54	0.50
Sugar (%)	4.4	4.8
Total soluble solid (%)	8.0	8.8
Total acidity (%)	0.6	0.4
Ash	0.11	0.13
Volatile acidity (%)	0.14	0.17
pH	3.8	3.4
Specific gravity	0.988	0.990

TABLE 4: SENSORY EVALUATION

	Number of juice	YP	YC
Colour	8	47	53
Odour	8	46	55
Taste	8	50	54
Flavour	8	45	51

BIOCONVERSION OF WASTE MATERIALS TO FOOD AND USEFUL PRODUCTS BY FUNGI

by

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ABSTRACT

One of the most economically viable processes for the bioconversion of agricultural and industrial lignocellulosic wastes is the cultivation of edible mushrooms. Since the protein content of mushrooms is relatively high (19-35% on a dry weight basis), they can serve to enrich the human diet in those regions which suffer from a shortage of high quality protein. Mushroom protein can be produced with greater biological efficiency than proteins from animal sources and relatively little is required in terms of large-scale equipment, facilities, capital and land. Furthermore, the substrate residue which is left after mushroom harvesting can be converted into feedstock for ruminants and/or used as a soil conditioner. In developing countries, properly developed and managed mushroom farms can make important contributions to the nutrition and economic welfare of the people.

Bioconversion of Lignocellulosic Wastes into Edible Mushrooms

Huge quantities of lignocellulosic waste materials are generated annually through the activities of the agricultural, forest and food processing industries. Much of this material is either burnt, shredded and/or composted for landfill or improvement of soil quality (1) even though these wastes constitute a potentially valuable resource. Although physical and chemical technologies may, in some cases, play important associated roles, biotechnical approaches are essential for the emergence of practical conversion processes which can be applied to situations where large-scale capital-intensive operations are inappropriate.

One of the most economically viable processes for the bioconversion of lignocellulosic wastes is the cultivation of edible mushrooms. The nutritional value and desirable organoleptic properties of mushrooms are now widely recognized. Of particular significance, especially to regions with populations whose diet is commonly deficient in protein, is the protein content of mushrooms. This is relatively high (19-35% on a dry weight basis) as compared with 7.3% in rice, 13.2% in wheat, 39.1% in soybean and 25.2% in milk. Therefore, although mushrooms rank below most animal meats in crude protein content, they compare very favourably with most other foods (Table 1). With respect to essential amino acid indices, amino acid scores and nutritional indexes, the overall nutritive value of high grade mushrooms almost equals that of milk (Table 1). Furthermore, the proteins of commonly cultivated mushrooms contain all the essential amino acids and are especially rich in lysine and leucine which are lacking in most staple cereal foods (Table 2). Mushrooms also contain many vitamins and have a low fat content (Table 3).

Although mushrooms rank below most animal meats in crude protein content, an overriding advantage of mushroom protein is that it can be produced with greater biological efficiency than proteins from animal sources. It is true that, in some highly industrialized countries, cultivation of the Agaricus mushroom may be a highly sophisticated operation requiring a sizeable capital outlay for controlled environment facilities. However, production normally requires relatively little in terms of large-scale equipment, facilities, capital and land, and the mushrooms themselves often have less complicated demands in terms of processing. The straw mushroom, Volvariella

volvacea, is commonly grown in southeast Asian countries on small, family-type farms. Perhaps the most compelling consideration is that mushroomlxcan be cultivated on a wide variety of inexpensive substrate wastes including such diverse materials as cereal straws, bagasse, ba ia leaves, coffee grounds, sawdust and cotton wastes from textile factories. This is extremely important in rural areas where often large quantities of waste is available which is ideally suited for growing some types of edible mushrooms. The major comMwnents of these wastes, cellulose, hemicellulose and lignin, are relatively resistant to biological degradation. However, mushrooms possess the enzyme complexes which enable them to attack and degrade these industrial and agricultural by-products thereby resulting in a highly valued food protein suitable for direct consumption. Of further value is the spent compost, the substrate residue left after mushroom harvesting, which can be converted into feedstock for ruminants and/or used as a soil conditioner.

Although simple in concept, there are various intricacies associated with the process of mushroom cultivation which must be understood for the enterprise to be successful. In every case, the ultimate aim is to obtain the maximum yield from a given surface area per period of time by the use of high yielding strains, by shortening the cropping period, or by increasing the number of high yielding flushes. An achievement of maximum yield requires an understanding of substrate materials and their preparation, appropriate control of physical, chemical and biological parameters (e.g. moisture content, pH, temperature, competitive microflora), and proper management of mushroom beds, including mushroom pest and disease control.

The major phases of mushroom cultivation are shown in Table 4 and are: (a) selection of an acceptable mushroom, (b) requirement for and selection of a fruiting culture, (c) development of spawn, (d) preparation of compost, (e) mycelial (spawn) running, and (f) mushroom development.

(a) before any decision to cultivate a particular mushroom is made, it is important to determine if that species possesses organoleptic qualities acceptable to the indigenous population, if suitable substrates for cultivation are plentiful, and if environmental requirements for growth and fruiting can be met without excessively costly systems of mechanical control.

(b) a "fruiting culture" is defined as a culture with the genetic capacity to form fruiting bodies under suitable growth conditions. The stock culture which is selected should be acceptable in terms of yield, flavour, texture, fruiting time, etc.

(c) a medium through which the mycelium of a fruiting culture has grown and which serves as the inoculum or "seed" for the substrate in mushroom cultivation is called the mushroom spawn. Failure to achieve a satisfactory harvest may often be traced to unsatisfactory spawn. The potential of the spawn is ultimately set by the genetic constitution of the fruiting culture used in its manufacture. Consideration must also be given to the nature of the spawn material since this influences rapidity of growth in the spawn medium as well as the rate of mycelial growth and filling of the beds following inoculation (spawn running). Some of the substrates used in spawn production include various grains (rye, wheat, sorghum), rice straw cuttings, cotton waste, rice hulls and cotton seed hulls.

(d) while a sterile substrate free from all competitive microorganisms is the ideal medium for cultivating edible mushrooms, systems involving such strict hygiene are generally too costly and impractical to operate on a large scale. However, substrates for cultivating edible mushrooms

normally require varying degrees of pretreatment in order to promote growth of the mushroom mycelium to the practical exclusion of other microorganisms (Table 5). To accomplish this, certain chemical and physical qualities must be built into the substrate. Some edible species (Lentinus edodes, Pholiota, Tremella) can utilise lignocellulosic wastes, e.g. wood, with little or no pretreatment, others (Volvariella volvacea, Pleurotus spp) can colonize plant material, e.g. cereal straws, after some composting, physical and/or chemical pretreatment, while Agaricus bisporus requires a lengthy controlled composting of wheat straw with manures or other nitrogen rich additives (Table 5). For Flammulina and some Pleurotus spp., sterile substrates are prepared by autoclaving sawdust/rice bran mixtures or straw. The substrate must be rich in essential nutrients in forms which are readily available to the mushroom, and be free of toxic substances which inhibit growth of the spawn. Moisture content, pH and good gaseous exchange between the substrate and the surrounding environment are important physical factors to consider.

(e) following composting, the substrate is placed in beds where it is generally pasteurized by steam to kill off potential competitive microorganisms. After the compost has cooled, the spawn may be broadcast over the bed surface and then pressed down firmly against the substrate to ensure good contact, or inserted 2-2.5 cm deep into the substrate. Spawn running (mycelial running) is the phase during which mycelium grows from the spawn and permeates the substrate. Good mycelial growth is essential for mushroom production and will depend on proper maintenance of the beds and mushroom house in terms of temperature, moisture content, humidity and aeration.

(f) under suitable environmental conditions, which may differ from those adopted for spawn running (Table 5), primordia formatiolxoccurs followed by the production of fruiting bodies. The appearance of mushrooms normally occurs in rhythmic cycles called "flushes". Harvesting is carried out at different maturation stages depending upon the species and upon consumer preference and market value.

World production of cultivated edible mushrooms is reported as 2,176 thousand tons and 3,194 thousand tons in 1986 and 1989/90 respectively, a 74.4% increase in just three years. Based on a figure of 88.8 US cents per pound, reported to be the average price received by growers in the United States in 1990-91, the value of the total world mushroom crop in the 1989/90 financial year was almost US$7,500 million. A comparison of production levels for different mushrooms between 1986 and 1989/90 reveals that production yields of all cultivated mushrooms increased during the period (Table 6). Increases ranged from 16.3% for Volvariella upto 437.9% for Pleurotus species (Table 6). The second biggest increase of 236.1% was in the production of Auricularia (Table 6). While Agaricus remains predominant, other species of mushroom (Pleurotus, Auricularia, Volvariella, Flammulina, Tremella) have enjoyed a major upsurge in interest especially in China and other Far Eastern countries. Thus, in terms of percentage of total world output, figures for Agaricus and Lentinus decreased as a consequence of the increase in production of other cultivated mushroom species, in particular Pleurotus spp (Fig. 1).

The upward trend in world production of cultivated edible mushrooms is clearly seen in Fig. 2. This shows a particularly sharp increase over the past five years, a trend that is expected to continue in the future due to advances both in our basic knowledge of mushroom biology and in the practical technology associated with mushroom cultivation.

As well as being consumed as a source of vegetable protein, many edible

mushrooms are receiving additional recognition for other qualities. For example, recent pharmacological and clinical evaluations have demonstrated significant biological properties, particularly immunopotentiation, anti-tumour and hypocholesterolemic effects. A variety of proprietary products derived from edible mushrooms, including health drinks, foods, flavourants, and even pharmaceuticals are already available and the market for such materials is expected to increase.

Upgrading of Lignocellulosics for Animal Feed Production

Edible mushroom production also represents an attractive method of improving the nutritional quality of lignocellulosic wastes for use as an animal feedstock. Agricultural and forest industry byproducts and wood which is unsuitable for pulping are carbohydrate-rich residues that represent a potential source of dietary energy for ruminants. However, the feed value is limited by the low polysaccharide degradation achieved during digestion within the rumen. This restricted digestibility is due to the presence of lignin which acts as a barrier depriving the cellulolytic and hemicellulolytic enzymes access to the polysaccharide components.

Given the annual production of the various lignocellulosic byproducts, considerable effort is underway to develop systems for upgrading their nutritive value. Chemical and physical delignification methods have been used extensively but, along with a better understanding of the microbial physiology and biochemistry of lignin biodegradation, more attention is being focused on delignification treatments based on lignin-degrading fungi, including several edible species (Table 7). Relatively higher lignin degradation rates and consequent increases in digestibility are obtainable using cereal straws as a substrate as compared with wood, and several white-rot fungi exhibit a high capacity to increase the in vitro digestibility of wheat straw (Table 8). Even so, biological delignification of wood preparations may also offer possibilities for the production of ruminant feedstuff. In southern Chile, fungal delignification of wood has been observed under natural conditions. The product of delignification, known as 'palo podrido' is a white decomposed wood which is used as an animal feed. In vitro digestibility of the wood is increased from 3% to 77% in some cases although the process is long and slow. More effective treatments for enhancing the digestibility of wood, straws and other lignocellulosic byproducts using ligninolytic fungi are dependent on further co-ordinated research aimed at optimization of the solid state fermentation processes involved.

In conclusion, as a result of technical advances achieved during recent years, the commercial cultivation of edible mushrooms has spread to many countries throughout the world. Since cultivated mushrooms can be grown on agricultural and industrial wastes, they provide a solution to many problems of global importance including protein shortages, resource recovery and re-use, and environmental management.

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TABLE 1. Comparison of Nutritive Value of Mushrooms with Various Foods

Essential amino acid indexes		Amino acid scores		Nutritional indexes	
100	pork; chicken; beef	100	pork	59	chicken
99	milk	98	chicken; beef	43	beef
98	mushrooms (high)	91	milk	35	pork
96	*V. diplasia*	89	mushrooms (high)	31	pork
91	potatoes; kidney beans	71	*V. diplasia*	28	mushroom (high)
	P. ostreatus	63	cabbage	27	*V. diplasia*
88	corn	59	potatoes	26	spinach
87	*A. bisporus*		*P. ostreatus*	25	milk
86	cucumbers	53	peanuts	22	*A. bisporus*
79	peanuts	50	corn	21	kidney beans
76	spinach; soybeans	46	kidney beans	20	peanuts
74	*L. edodes*	42	cucumbers	17	cabbage
72	mushrooms	40	*L. edodes*	15	*P. ostreatus*
69	turnips	33	turnips	14	cucumbers
53	carrots	32	mushrooms (low)	11	corn
44	tomatoes	31	carrots	10	turnips
		28	spinach	9	potatoes
		23	soybeans	8	potatoes
		18	tomatoes	6	carrots
				5	mushrooms (low)

Source: Refs. 2, 3

Note: Values for mushrooms represent the mean of the three highest values (high) and the three lowest values (low).

TABLE 2. Comparison of Amino Acid Composition of Edible Mushrooms

(g/100 g protein)

Amino acids	*A. bisporus*	*L. edodes*	*V. volvacea*	*Pleurotus*
Essential				
Isoleucine	4.3	4.4	4.2	4.0
Leucine	7.2	7.0	5.5	7.6
Lysine	10.0	3.5	9.8	5.0
Methionine	Trace	1.8	1.6	1.7
Phenylalanine	4.4	5.3	4.1	4.2
Threonine	4.9	5.2	4.7	5.1
Valine	5.3	5.2	6.5	5.9
Tyrosine	2.2	3.5	5.7	3.5
Tryptophan	ND	ND	1.8	1.4
Total	38.3	35.9	43.9	39.3
Nonessential				
Alanine	9.6	6.1	6.3	8.0
Arginine	5.5	7.0	5.3	6.0
Aspartic acid	10.7	7.9	8.5	10.5
Cystine	Trace	ND	ND	0.6
Glutamic acid	17.2	27.2	17.6	18.0
Glycine	5.1	4.4	4.5	5.2
Histidine	2.2	1.8	4.1	1.8
Proline	6.1	4.4	5.5	5.2
Serine	5.2	5.2	4.3	5.4
Total	61.6	64.0	56.1	60.7

Source: Ref. 4.　　ND: Not Determined

TABLE 3. Nutritional Values of Five Popular Edible Mushrooms

	Agaricus bisporus	*Lentinus edodes*	*Volvariella volvacea*	*Pleurotus ostreatus*	*Flammulina velutipes*
Protein, % (dry weight)	23.9-34.8	13.4-17.5	29.5	10.55-30.4	17.6
Calories, K Cal/ 100 g (dry weight)	328-368	387-392	276	345-367	378
Vitamins, mg/100 g (dry weight)					
Thiamin	1.0-8.9	7.8	1.2	4.8	6.1
Riboflavine	3.7-5.0	4.9	3.3	4.7	5.2
Niacin	42.5-57.0	54.9	91.9	108.7	106.5
Ascorbic acid	26.5-81.9	0	20.2	0	46.3
Ascorbic acid mg/100 g (fresh weight)	1.8	9.4	1.4	7.4 (*P. sajor-caju*)	
Fat, % (dry weight)	1.7-8.0	4.9-8.0	2.4	1.6-2.2	1.9

Source: Ref. 5.

Table 4. Major Phases of Mushroom Cultivation

Major Phases	Main points to consider
Selection of an acceptable mushroom	Location Climate Raw materials Acceptability
↓	
Selection of a fruiting culture	Tissue culture Spore culture (a) without mating for homothallic sp. (b) mating with compatible isolates for heterothallic species Mixed culture Preservation
↓	
Development of spawn	Substrate Vigorous growth Free of contamination Avoid use of senescent and degenerate spawn Good survival of storage
↓ Spawning	
Spawn running → Fructification (mushroom development)	Establishment of mycelium Environmental requirements (a) temperature (b) light (c) aeration (O_2, CO_2) (d) pH (e) moisture Casing Watering and care
↑ Composting	
Preparation of compost	Concept of composting Microbial activity Softening of substrate for ease of colonization Physical characteristics Chemical components Aeration Water content

Source: Ref. 6

TABLE 5. Temperature Range, Substrate Type, Production-Cycle Time, and Approximate Yield of Edible Mushrooms from Nonaxenic Culture Methods

Species	Substrate	Temperature range: Mycelial growth	Temperature range: Fruiting	Production-cycle time	Yield[a]
Little or no pretreatment					
Lentinus edodes	Wood logs (outdoors, sometimes protected)	5-35 (24)[b]	6-25 (15) autumn (10) winter (20) spring	3-6 yr spring/autumn	40
Auricularia auricula	Wood logs (outdoors, sometimes protected)	15-34 (28)	15-28 (22-25)	2-5 yr spring/autumn	2-12
Auricularia polytricha	Wood logs (outdoors, sometimes protected)	10-36 (20-34)	15-28 (24-27)	1-2 yr	20-40
Tremella fuciformis	Wood logs	5-38 (25)	20-28 (20-24)	3-6 yr 7 months/yr	10-30
Some pretreatment					
Volvariella volvacea	(1) Rice straw (outdoor) (2) Cotton waste, rice Straw (indoor)	15-45 (32-35) 15-45 (32-35)	22-38 (28-32) 22-38 (28-32)	4-6 weeks 2-3 weeks	6-10 30-45
Pleurotus sajor-caju	(1) Pasteurized cereal straw (indoor)	14-32 (25-27)	10-26 (19-21)	4-10 weeks	80-100 or more
	(2) Fermented cereal	14-32 (25-27)	10-26 (19-21)	4-10 weeks	80-100 or more
Long composting process					
Agaricus bisporus	Composted cereal straw/ animal manure mixtures	3-32 (22-25)	9-22 (15-17)	14-16 weeks	65-80
Agaricus bitorquis	As above	3-35 (18-30)	18-25 (22-24)	14-16 weeks	40-65

a Kg fresh weight/kg d.m.
b Figures within parentheses are optimal values

Source: Ref. 7.

TABLE 6. Comparison of 1986 and 1989/90 world production of cultivated edible mushrooms

Unit: (metric ton x 1000)

Species	Common Name	1986 Fresh wt.	1986 %	1989/90 Fresh wt.	1989/90 %	% increase
Agaricus bisporus bitorquis	Button mushroom	1,215	55.8	1,446	38.1	19.0
Lentinus edodes	Shiitake or oak mushroom	320	14.7	402	10.6	25.6
Volvariella volvacea	Straw mushroom or Chinese mushroom	178	8.2	207	5.5	16.3
Pleurotus spp.	Oyster mushrooms	169	7.8	909	24.0	437.9
Auricularia spp.	Wood-ear	119	5.5	400	10.5	236.1
Flammulina velutipes	Winter mushroom	100	4.6	143	3.8	43.0
Tremella fuciformis	White Jelly fungus/ or "Silver Ear"	40	1.8	105	2.8	162.5
Pholiota nameko	"Nameko" or Viscid mushroom	25	1.1	53	1.4	112.0
Hericium erinaceus	Monkey head mushroom or Hedgehog fungus	-	-	90	2.4	
Hypsizigus marmoreus	Shimeji	-	-	22	0.6	
Grifola frondosus	Sitting-hen mushroom or Limuo, Maitaka	-	-	7	0.2	
Others		10	0.5	10	0.3	
Total		2,176	100.0	3,794	100.2	74.4

Source: Chang & Miles (1991)

Source: Ref. 9.

TABLE 7. Effect of white-rot fungi on *in vitro* digestibility of lignocelluloses

Fungus	Substrate	Time (days)	Total weight loss (%)	In vitro digestibility (%)[a]	
				After decay[b]	Before decay
Pleurotus sp. Florida	Beech wood	60	17	35	6
Pleurotus sp. Florida	Reed straw	60	30	45	30
Pleurouts sp. Florida	Sunflower stalks	60	27	62	41
Fomes ulmarius (Sox. ex. Fr.) Gill	Aspen wood	77	13	64	46
Polyporus berkeleyi Fr.	Birch wood	44	8	54	20
Lentinus edodes (Berk.) Sing.	Wheat straw	60	13	77	40
Lentinus edodes (Berk.) Sing.	Birch wood	69	25	60	20

a Rumen fluid method
b Values expressed as % of decayed sample.

Source: Ref. 15.

TABLE 8. White rot fungi with high capacity to increase *in vitro* digestibility of wheat straw

Fungus	Temp. °C	Organic Matter loss (%)	Lignin Loss (% OM)	Change in digest-ibility	Process effici-ency
Abortiporus biennis	25	24.2	7.1	+23.4	+0.97
Ganoderma lucidum	25	25.2	10.8	+25.7	+1.02
Hymenochaete tabacina	22	10.5	5.6	+23.8	+2.27
Lentinus edodes	25	17.4	3.0	+24.6	+1.41
Pleurotus eryngii	30	14.3	ND	+23.2	+1.62
P. sajor caju	30	18.7	2.7	+22.0	+1.18
P. ostreatus	22	15.4	7.0	+22.4	+1.45
P. serotinus	25	27.1	13.8	+32.1	+1.18
Polyporus arcularis	22	21.2	12.4	+24.4	+1.15
P. galactinus	30	14.8	11.0	+28.4	+1.92
Poria expansa	25	25.2	6.1	+21.1	+0.84
Sporotrichum pulverulentum	22	55.8	22.0	+15.9	+0.28
Trametes hirsuta	25	24.8	16.8	+23.2	+0.94

Process efficiency = Change in *in vitro* digestibility divided by dry matter loss during fermentation.

Source: Ref.12

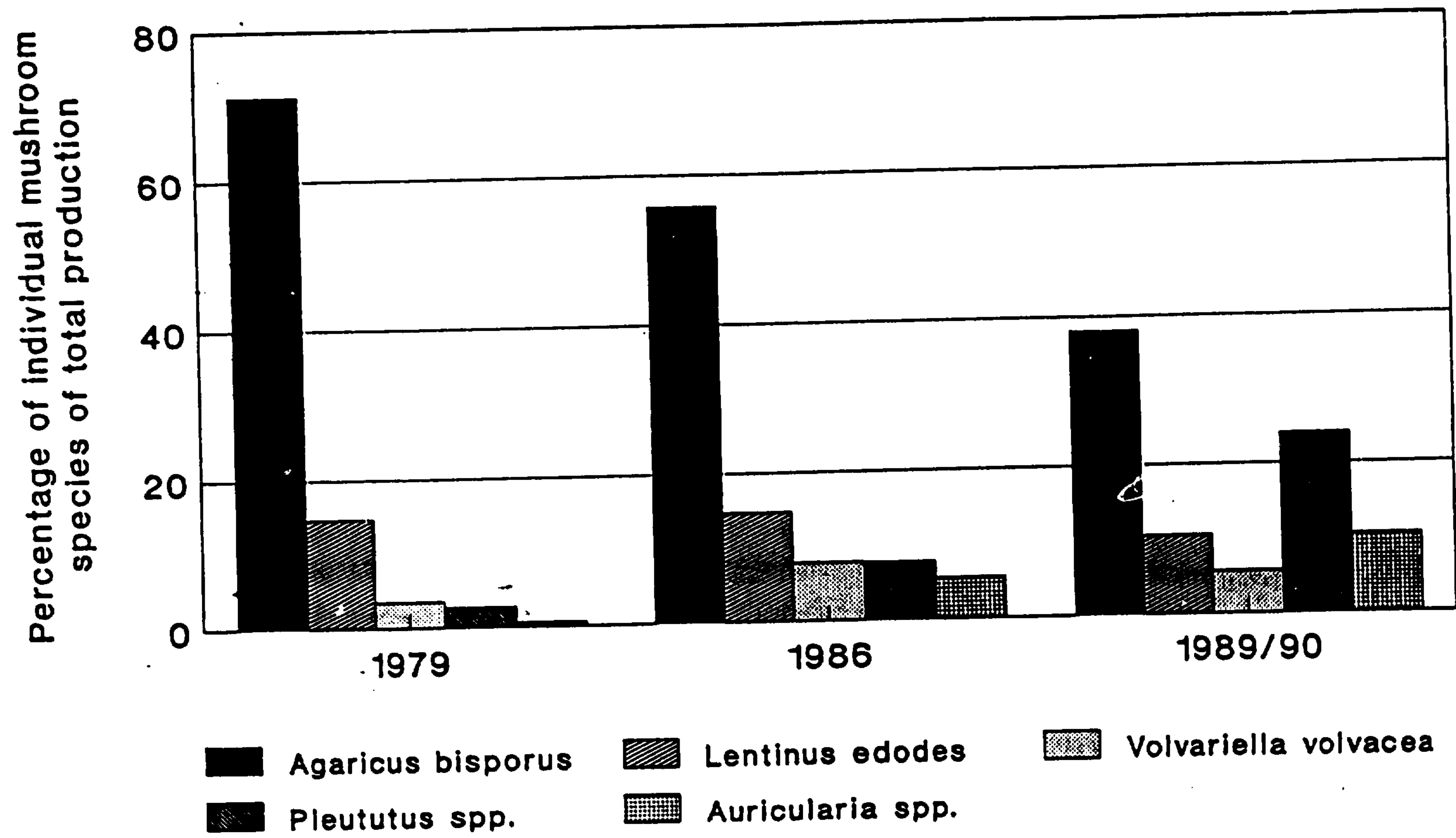

Fig. 1. Percentage of world production of five major mushrooms in 1979, 1986 and 1989/90

Source: Ref. 9.

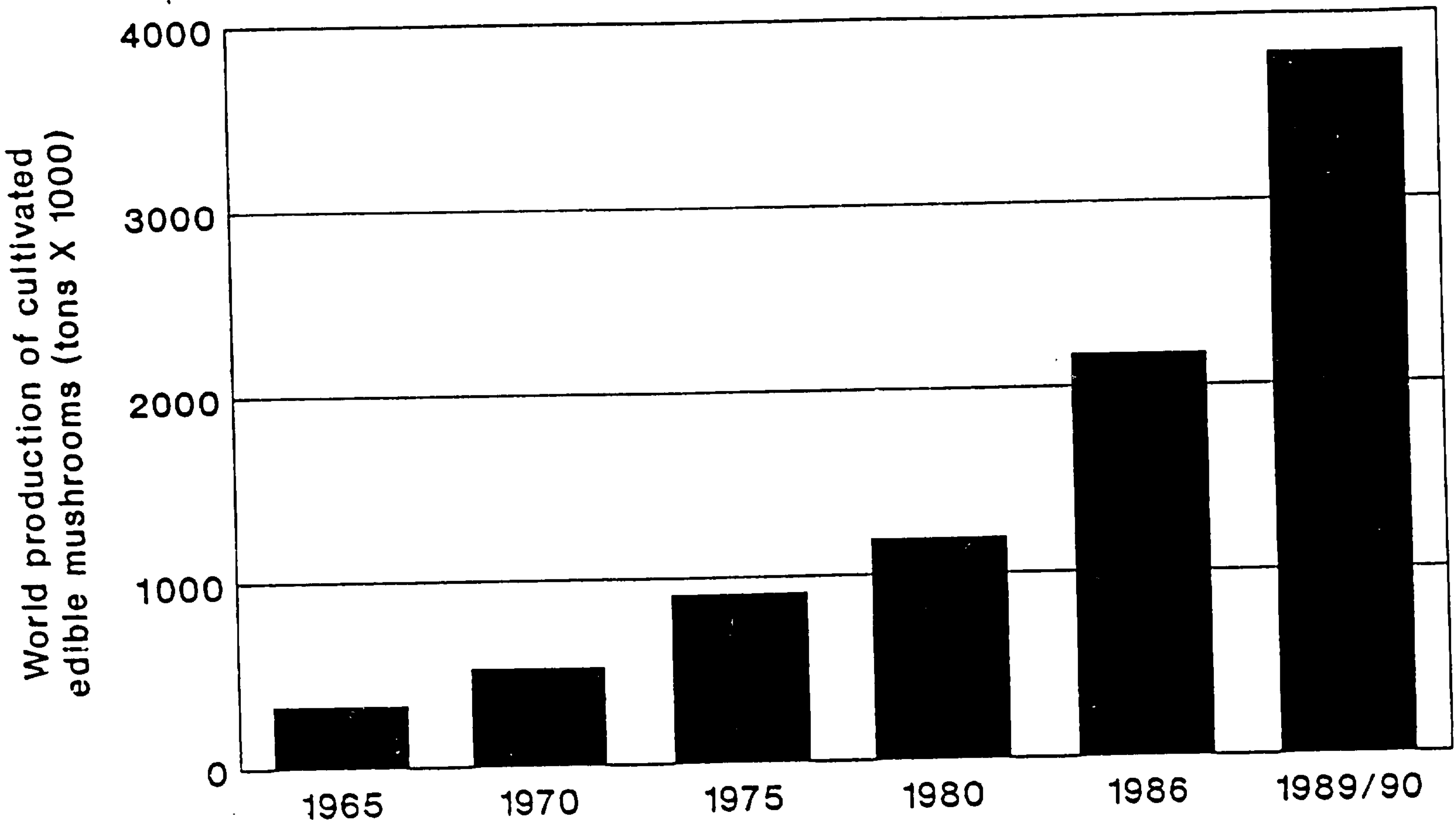

Fig. 2. Annual world production of cultivated edible mushrooms

Source: Ref. 9.

Industrialization of lactic acid fermentation technology of cereals and its dissemination to developing countries

by

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ABSTRACT

The content of the UNIDO Project (ROK/89/002) at Korea University is introduced. This project comprises of three major activities: (1) A joint research programme between scientists and engineers of the Department of Food Technology at Korea University, Korea Food Research Institute and the Department of Biotechnology of the Technical University of Denmark. (2) An international training programme in food fermentation technology with special emphasis on cereal based non-alcoholic fermentation. (3) An international workshop on lactic acid fermentation of non-dairy food and beverages.

The joint cooperative researches have been carried out successfully, and the results are used for the training of the eight UNIDO fellows selected from Asia and Africa. An international workshop on the lactic acid fermentation of non-dairy food and beverages will be held in 26-28 June, 1992, in Seoul, Korea.

INTRODUCTION

Lactic acid fermentation of plant materials has been widely used for the processing and preparation of traditional foods in many countries in Asia and Africa. The acid production in cooked cereals enhances the keeping quality of many staple dishes in Asia and Africa, for example, Couscous in Senegal, Ogi in Nigeria, Idli in India, Nasha in Sudan, Kishk in Egypt and Erera in Ethiopia. The acid production from cereals and vegetables has been utilized as a means for perishable food preservation, like fish and vegetables. Good examples are Korean Kimchi and lactic acid fermented fish products in East Asia. Lactic acid bacteria also exert an important role in the preparation of traditional alcoholic and non-filtered cereal mashes, which have contributed greatly to the nutritional supplement of the diet for the poor labour groups. In spite of the importance of lactic acid fermentation of plant materials for the food processing in the Third World, little scientific studies have been conducted on this aspect, compared to the vast array of researches conducted for fermented dairy foods, such as cheese and yogurt.

A UNIDO-sponsored joint research project to develop high protein content lactic beverages from vegetables has been carried out since January 1987 at Korea University in Seoul and MIT in Cambridge, MA, USA. The prime objectives of the Phase I research project were to establish optimum pretreatment conditions of cereal for lactic acid fermentation and to select and improve the microbial strains. In this study, the beneficial effects of prefermentation and extrusion-cooking of rice prior to lactic fermentation were demonstrated. The prefermentation of rice with Bacillus laevolacticus, which was isolated from Sikhae, a traditional Korean fermented fish product, and a yeast Saccharomyces cerevisiae, and a subsequent extrusion cooking improved the solubilization of rice-soymilk mixture during digestion. However, substantial amounts of proteins were lost as residue after centrifugation of the fermented broth. The original plan of the research project was to solve this problem by manipulating the enzyme activity of lactic acid bacteria. However, this was proved to be extremely difficult and costly, and was unable to be completed in time. Another solution to this

problem was to employ enzymatic hydrolysis of proteins to the point where they were soluble at their isoelectric points but did not produce a bitter flavour.

The Department of Biotechnology at the Technical University of Denmark, DTH, has expertise and extensive experience in the field of flavour control during fermentation especially with hydrolyzed protein products. Therefore, in Phase II of the project cooperative work was carried out with the Department of Biotechnology at DTH.

The second phase of UNIDO project comprises three major activities:

1. A joint research programme between scientists and engineers from the Department of Food Technology at Korea University, Korea Food Research Institute, and the Department of Biotechnology at the Technical University of Denmark.

2. An international training programme in food fermentation technology with special emphasis on cereal based non-alcoholic fermentation.

3. An international workshop on lactic acid fermentation of non-dairy food and beverages.

Joint Research Programme

The major obstacles encountered in developing high-protein content lactic beverages from cereals were (1) selection of micro-organisms which are able to produce an acceptable flavour, (2) pretreatment of high molecular weight plant materials to solubilize and to make fermentable materials for lactic acid bacterial and (3) solubilization of proteins in acidic condition in order to increase the protein content of the product. In the previous studies we were able to select a strain of Leuconostoc mesenteroides separated from a Korean traditional fermented food, Sikhae, to produce acceptable apple juice-like flavour from rice-soymilk substrate. The solid-state prefermentation of rice with Bacillus laevolacticus and yeast Saccharomyces cerevisiae and a subsequent cooking and malt digestion could improve the solubilization of rice, and up to 70% of the total solid matter could be recovered as a clear juice of rice-soymilk lactic beverage (Fig. 1).

However, a substantial amount of proteins was wasted as residue after centrifugation of fermented broth. In order to solve this problem we used enzymatic hydrolysis of soymilk prior to lactic fermentation. But enzymatic partial hydrolysis of protein produced bitter peptides, and so, a debittering process was required. Reducing the bitterness of enzymatic hydrolysis of soymilk would be possible by the following methods: (1) control of the degree of protein hydrolysis, (2) selective destruction of bitter peptides by exopeptidases from malt or lactic bacteria, (3) selective separation of bitter peptides by pH adjustment, (4) masking of bitterness by organic acids and other flavour substances. Fig. 2 shows the strategy of producing high-protein content lactic beverage from cereals. In order to realize the industrial production of cereal lactic beverages, it is necessary to improve and stabilize the flavour of these products to an acceptable level.

Presently we are conducting the following researches:

1. The debittering effect of a controlled degree of hydrolysis (DH) in enzymic hydrolysate of soymilk.

2. The debittering effect of lactic acid bacteria.

3. The debittering effect of malt exopeptidases.

4. The effect of extrusion-cooking of cereals on lactic acid fermentation of bacteria.

5. Conditions for the maximum production of apple juice-like flavour by Leuconostoc mesenteroides.

6. Pilot plant scale production of cereal lactic beverage.

We have found that different types of proteolytic enzymes produce different levels of bitterness in soymilk hydrolysate, and some strains of lactic acid bacteria, for example, Leuconostoc mesenteroides, could reduce the level of bitter peptides in fermented broth. Extrusion-cooking of cereals improved the physical stability and sensory acceptability of lactic acid fermented cereal products.

The research team of Korea Food Research Institute has developed a process of Risogurt production, a curd type yogurt analog made from rice and soybean protein, and has applied for its Korean paten right. A pilot-plant scale production test has been completed and the product has shown good acceptability in consumer tests.

International Training Programme

The UNIDO International Course for Food Fermentation Technology at the Graduate School of Natural Resources, Korea University was launched on 1 October, 1991 in Seoul, Korea. It is a 10-month course comprising lectures and laboratory practices in food fermentation technology and genetic engineering at Korea University, a two-month pilot-plant practices on cereal lactic acid fermentation at the Korea Food Research Institute and cooperative researches by the individual participants with faculty members of the College of Agriculture at Korea University. Table 1 shows the curriculum of the lecture courses.

The information on the International Course was distributed world-wide through UNIDO in October 1990 and a total of 18 applications from 11 countries was received. Out of the 18 applicants, 10 fellows were selected. However, the candidates from China and Ethiopia failed to receive approval from their government for attendance. The list of participants and their profiles are given in Table 2.

Korea University provides teaching facilities and dormitory rooms for the oversea's fellows. The government of Republic of Korea supports the educational expenses costing USD 100,000, and the United Nations Development Programme in Seoul pays the travel expenses and stipends costing USD 130,000. A total of 8 professors of the College of Agriculture at Korea University are participating in the lectures and research supervision, and an additional 8 professors and researches of other institutes have been invited as special lecturers.

International Workshop

A UNIDO International Workshop on the Lactic Acid Fermentation of Non-dairy Food and Beverages is planned for June 26-28, 1992 at the Korea Food Research Institute. The aim of the workshop is to exchange ideas and to collect available knowledge on lactic acid fermentation, especially of under-utilized plant food materials, and to elucidated the potential of applying emerging biotechnological concepts for the improvement and industrialization of these traditional methods.

Experts in this field of research (Prof. A. J. Sinskey of MIT, USA, Prof. J. Adler-Nissen of the Technical University of Denmark, Prof. G. Bärwald of Technical University of Berlin, Dr. M. Souane of Senegalian Food Research Institute and Dr. T. W. Kwon of Korea Food Research Institute) will be invited as special lecturers. The participants of the UNIDO International Course at Korea University will present country papers at the workshop. Contributed papers throughout the world via UNIDO will also be invited for presentation.

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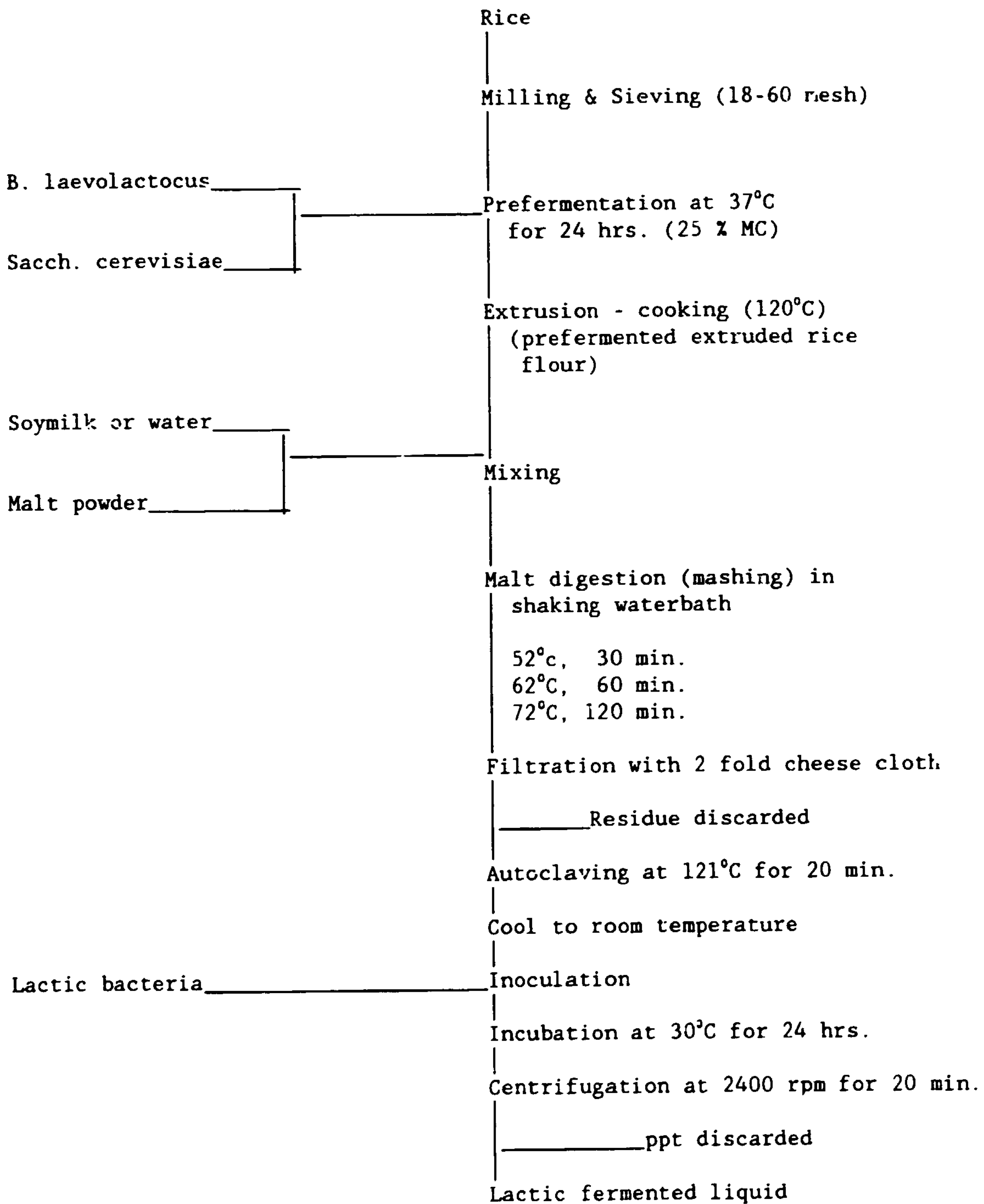

Fig. 1. Flow diagram for the preparation of lactic beverages from rice and soymilk

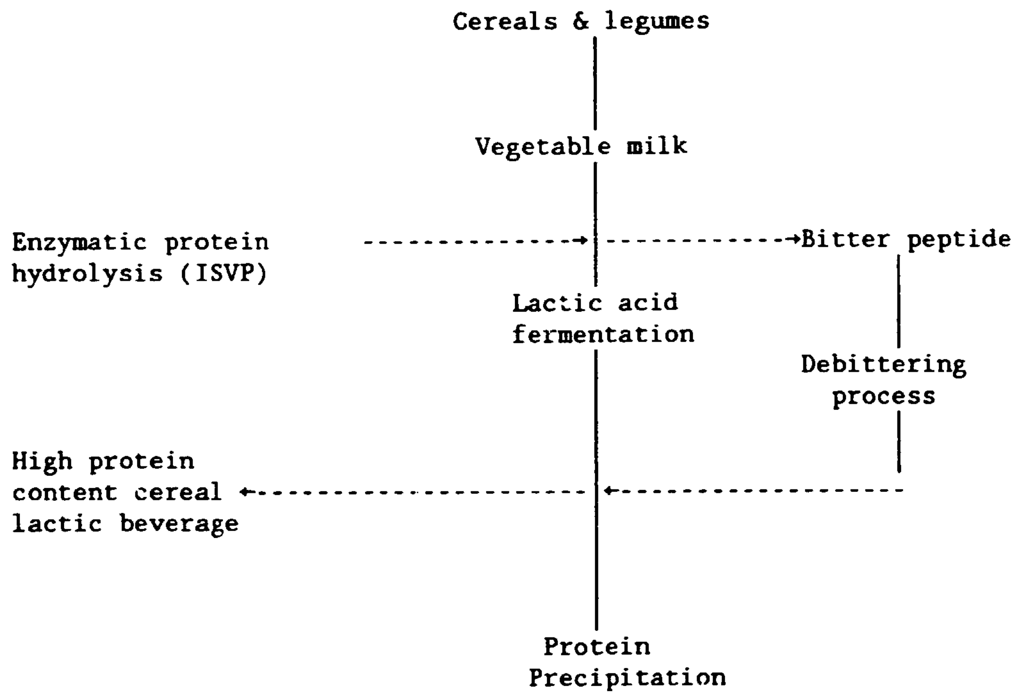

Figure 2. Strategy of producing high protein content lactic beverages from cereals

Table 1. Time schedule and curriculum of the UNIDO International Course for Food Fermentation Technology of Korea University

Time Schedule of the Course

Year	Period	Activity
1991	Oct. 1 - Oct. 5	Arrival, Orientation
	Oct. 7 - Dec.21	First Semester
1992	Jan. 5 - Feb.28	Pilot Plant Practice (KFRI)
	Mar. 2 - Jun.30	2nd Semester
	Jun.26 - JUn.28	International Workshop
	Jul.20 - Jul.30	Return to Home Country

Course Subjects

Subjects	Lecture time
101 Traditional Fermentation Technology	42 hrs.
102 Cereal Lactic Fermentation Technology	24 hrs.
103 Biotechnology and Genetic Engineering	42 hrs.
104 Korean Language	26 hrs.
201 Extrusion Cooking Technology	42 hrs.
202 Starter Culture Technology	24 hrs.
203 Enzymic Process Technology	24 hrs.
204 Korean Dietary Culture	24 hrs.
301 Pilot - Plant Practice	2 months

Table 2. List of participants and their advisors of 1991 UNIDO international course for Food Fermentation Technology, Korea University

Country	Name	Age	Degree	Affiliation	Advisor
Nigeria	Mr. Tajudeen	31	Ms. Biochem.	Fed. Inst. of	Dr. Se Young Lee
Sudan	Miss Hanan Elsoufi	28	B. Sc. Family Sci.	Food Res. Centre, Agri. Res.Cooop.	Dr. Yong Jin Choi Prof. of Microbial Genetics
Philippines	Miss Lilia S. Collado	35	Ms. Food Sci.	Inst.Food Sci. Tech.of Philippines	Dr. Won Mok Park Prof. of Plant Pathology
Sri Lanka	Mr. Mohamed F. Moheedin	31	Ms. Microbiol	Nat. Aquatic Resource Agency	Dr. Kyu Man Chee Prof.of Animal Nutrition
Thailand	Ms Kanchanich Vachanavinichi	40	Ms. Pharmacy	Inst.Food Res.& Product Devel., Kaset Sart Univ.	Dr. Yong In Park Prof.of Molecular Biology
Vietnam	Mr. Nguen Van Viet	40	B. Sc. Chemistry	Food Industry Research Inst.	Dr. Hyo Ill Chang Prof.of Biochemistry
India	Mr. Manchar Balaraman	30	Ms. Chem. Eng.	Central Food Technological Res. Inst., Mysore	Dr. Chul Rhee Prof.of Food Engineering
Egypt	Mr. Mohamed A. Abdel-Naby	36	Ph. D. Biochem.	National Research Centre	Dr. Cherl Ho Lee Prof.of Food Engineering

Soybeans: The Answer to Malnutrition: The Impact of Soybean Processing and Utilization in Nigeria

by
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ABSTRACT

The goals of soybean utilization are to improve human diets. In 1987 through the aid of the International Development Research Center, Canada (IDRC), the International Institute of Tropical Agriculture (IITA) and National Programs collaborated on a soybean utilization project - to document the status of soybean, develop household level and small scale processing of soybeans and to disseminate the technologies developed to extension workers. Training programs were used to strengthen utilization at the rural level. About 25,059 people have been trained. When fortified with whole soybean and soymilk residue, the protein content of local foods is increased. The soybean-products - soymilk, soygari, soy vita, and soy iru were made. Partially refined soybean oil is acceptable among low and middle class rural people. Extruded products like soy corn blend, is acceptable as children's food, Soy full-fat flour is preferred for akara, while defatted flour is acceptable for vegetable soup in rural areas. Training of agricultural extension workers results in rapid dissemination of technologies for soybeans to the country.

INTRODUCTION

Nigeria is facing a serious food crisis. Protein intake for children is inadequate. The low protein intake has been attributed to the increasingly high cost of traditional sources of animal protein (Ogundipe and Weingartner 1987). The search for alternative sources of inexpensive protein has led to increased soybean utilization for household consumption, and industrial processing.

Soybean is a source of high quality and inexpensive protein about 40% while the oil is high in essential fatty acids and devoid of cholesterol. The relevance of soybean for solving Nigeria's food problem has been established (Osho 1988). Soybeans greatest potential involves its incorporation into local Nigerian diets.

Soybean, *Glycine max* (L. merill) is the world's most valuable oil seed legume. Originating from China about two thousand years before the birth of Christ; it is now internationally acclaimed as the miracle crop, the cow of China, the Cinderella crop of West and the Pearls of the Orient, all because of its versatility (Ogundipe and Osho, 1989).

Soybean production and utilization have been on the increase in the world. China, Indonesia, Japan, Korea, East and South East Asia and the United States of America (USA) are the world's major producers of the crop. It became an important commercial crop in the USA in the 1930s.

Soybean production and utilization have been on the increase in Nigeria. This has been made possible by the successful development of improved soybean varieties that can grow well in Nigeria by IITA and other Nigerian Institutions.

Other catalytic factors responsible for an increase in soybean production and utilization include government policies that banned importation of vegetable oils and the economic policies of government which has made imported protein-rich foods like milk, frozen fish, baby foods and milk become expensive to many Nigerians.

Past and present uses of soybean

a) Soybean is reported to have been introduced in Nigeria about 1908, in Benue State. In 1982 and 1983, some villages in Kaduna State; Kafanchan, used soybeans to make "Dawadawa" also known as "Iru" or "Ogiri" in Yorubaland, a soup condiment. Soybean was used as a substitute of locust bean (Parkia filicoides), because the parkia tree grows wild and it takes several years to mature (Oyeleke, 1987).

b) Soy Ogi which was first commercialized by FIIRO is a traditional weanin^ and breakfast food made from fermented maize paste. Akinrele and his colleagues at FIRRO incorporated soybeans into maize (ogi) making a product called soy ogi.

c) Kelsey Children's Home in Ogbomosho has been running a Clinic to treat malnourished children. Since 1984 the Clinic has been using soymilk and local foods fortified with soybean.

d) In order to promote the production and utilization of soybean in the diet of Nigerians the International Institute of Tropical Agriculture (IITA) is collaborating with four national programs in Nigeria; University of Nigeria, (UNN) Nsukka, Institute of Agricultural Research and Training (IAR&T) Ibadan, National Cereals Research Institute (NCRI) Badeggi, and National Agricultural Research and Liaison Services (NAERLS) Zaria; through the aid of the International Development Research Centre (IDRC) Canada. Since 1987 objective of the research work is to develop household and small scale processing technologies for soybean so as to improve the nutritional status of our people.

(e) Soybean fortified products like Nutrend, Golden morn, Nutrimax, Soy custard, etc. are on Nigerian markets. Other soybean products retailed on the markets are soybean flour, soy iru, soy oil etc. As of 1990, there is over 20 commercial products of soybean found on Nigerian markets.

Chemical composition and nutritive value of soybeans

Soybeans occupy a unique position among leguminous crops in having 40% protein and 20% oil in mature seeds. Hence, it occupies an intermediate position between legumes and oil seeds in having more protein (about 40%) than most of the oil seeds. The mature soybean seed has three major components, the seed coat (hull), the cotyledon and the embryo axis (hypocotyl). As such it can be rightly considered as a protein concentrate even without defatting (Table 1). The unique physio-chemical characteristics of the constituents of soybean offers tremendous possibilities of their use in food, feed and industrial applications.

(a) Protein

The protein content of soyfoods is quite high ranging from 40% in full fat soyflour to 90% in isolate. The protein content is considerably higher than in meat or dairy products, fish, eggs, etc., as shown in Table 2. Besides the quantity of soyprotein, it is important to know the quality of soyprotein and the quality of the foods to which soy protein is to be added. The quality of soyprotein can be discussed in terms of amino acid composition as shown in Table 3.

Soyprotein is somewhat deficient in methionine and cystine which is one of

the eight essential amino acid i.e. the sulphur containing amino acid, but they are generally high in lysine content, which is the limiting amino acid in cereals. The fortification of soybean with cereals at the rate of 25% soy to 75% cereal will supplement each other and constitute a well balanced amino acid content (Table 3). Soyprotein can also be used to upgrade the protein and consequently the nutritive values of starchy crops like rice, yam, cassava and others.

(b) *Oil*

The average oil content of soybean is 20%. The composition is similar to other vegetable oils such as sunflower and groundnut. It is highly digestible, high in poly-unsaturated fatty acids and contains no cholesterol. It is believed that the use of poly-unsaturated fats in the diets reduces the level of cholesterol in the blood and thus the reduction of susceptibility to cardio-vascular disease. Soybean oil is composed of about 85% unsaturated and 15% saturated fatty acids. About 60% of soybean oil is composed of essential fatty acids. The term essential fatty acids refers to three fatty acids: linoleic, linolenic, and arachidonic acids which are essential because they have multiple functions in the body. The amount of linoleic acid required in a normal diet is about 5-6 gramms a day. Soy oil contains over 50% linoleic acid. Soy oil can be used for processing margarine, mayonnaise and salad dressing.

(c) *Carbohydrates*

Soybeans contain about 35% carbohydrate (sugars). The insoluble carbohydrates consist of cellulose and hemicellulose. The soluble carbohydrates (sugars) consist of sucrose, and the oligosaccharides starchyose and raffinose. The fermentation of oligosoicharides so as to be absorbed by the intestine, produces flatulence, composed of carbon dioxide, hydrogen and small amount of methane. The elimination or reduction of flatus from soybean foods is needed to increase the acceptance of soy.

Soybean can be processed so that flatus is reduced by removing the oligosaccharides. At low level boiling with tap water or 0.5% sodium bicarbonate solution will remove some of the oligosaccharides.

(d) *Minerals/Vitamins*

Whole soybean contains 1.6% potassium, 0.3% calcium, 0.3% magnesium, 110 ppm iron, 50 ppm zinc, and 20 ppm copper. The minerals present in soy products can contribute to the overall requirement especially for children and pregnant women. Besides the quantity, the availability of minerals must be considered. Phytic acid present in whole soybeans can chelate divalent ions such as calcium, iron, and zinc and lower their availability.

The vitamins present in high amounts in soybean include; thiamine (11.0-17.5 μg/g), riboflavin (3.4-3.6 μg/g), niacin (21.4 μg/g), pantothenic acid (13.0-21.5 μg/g). Vitamins are unstable and they are very sensitive to processing conditions. Generally home cooking leads to a great loss of vitamins and minerals. Operations such as dehulling, grinding, blanching, etc. make significant contribution to mineral and vitamin losses.

Antinutritional factors in soybeans

These are referred to as "biologically active substances" or antinutritional factors. These substances are not "toxic", however, it may inhibit the availability of desired substances that are otherwise useful to the body. Some of these substances are trypsin inhibitor, hemagglutinin, phytic acid, goitrogens, and urease.

(a) Trypsin inhibitor

Soybean trypsin inhibitors are present in raw soybeans at 1.5%. Trypsin inhibitor (TI) depresses growth and causes hypertrophy of the pancreas in animals. It reduces digestibility of protein, thereby lowering the nutritive value by increasing the sulfur amino acid requirement. Trypsin inhibitor is inactivated by heat. Moist heat is more effective then dry heat. Boiling for 20 mins will inactivate most of the trypsin inhibitor. The rate of denaturation increases with rising temperature. About 100°C atmospheric steam for 15 minutes will inactivate 95% of the TI activity.

(b) Hemagglutinins

Soybeans contain a protein which agglutinates red blood cells and thus called Hemagglutinins. Some hemagglutinins are toxic to animals, and in soybeans it makes up to 1-3% of the protein. It is easily destroyed by heat. Soybean hemagglutinin does not affect the nutritional quality of soybeans protein.

(c) Phytic acid

Soybeans contain about 2% phytic acid, which decreases the availability of divalent cations, such as calcium, zinc, and iron by forming a insoluble protein complex that is not readily broken down and may pass through the digestive track unchanged. The poor availability to animals of zinc present in soybeans is attributed to phytic acid. Phytic acid does not interfere with the bio-availability of minerals added to soyfoods. Therefore, mineral supplementation of soybased infant formulae and baby foods is an effective way of increasing the mineral bio-availability of these products.

(d) Gitrogens

There is an unknown component in soybeans which can cause enlargement of the thyroid gland (goiter) in animals and human beings. It can be partially destroyed by heat. This problem can be eliminated by supplementing the soymilk with iodine. In the United States, it is recommended that soybased infant formula be supplemented with 5-75 micrograms iodine/100 kilocalories of formula.

(e) Urease

This enzyme is found in large amounts in raw soybeans. It will degrade urea to form ammonia, a very toxic compound. Urease is only a problem when raw soybeans are feed along with urea to cattle. Urease requires longer heat treatment than trypsin inhibitor to activate it. The assay for urease, that is, the liberation of urease is measured by change in color and pH is simple and quick.

Methodologies used to accomplish project objectives

1. A baseline survey was conducted using conventional survey and rapid rural appraisal to document the status of soybean in selected project sites (Ikoyi, Igangan and Ijaiye) in Oyo State.

2. Training was frequently conducted in local language at the villages.

3. The composition of Nigerian traditional dishes was investigated.

4. Product development research involved: incorporation of soybean with maize (soyvita) cassava (soy gari); production of soymilk and food utilization of soymilk residue.

5. Development of recipes for household level soy fortified food.

6. Soybean oil was extracted, partially refined and was tested for acceptability as a cooking oil.

7. The extruder was used to produce three flours: corn soy blend, extruded full fat flour and extruded defatted flour.

Results and Discussion

One hundred and seventy-four people from three communities: Ijaiye, Igangan and Ikoyi were interviewed. The communities chosen for this study varied in their prior knowledge of soy foods.

Soybean production was minor at the start of survey. About 17% of the farmers cultivated soybeans in Ijaiye, 40% in Ikoyi, while no farmers cultivated soybeans in Igangan. Ikoyi community was the most conversant with soybean utilization. Consumption of soybeans in Ijaiye was not common while no one in Igangan consumed any soybean products during the time of survey (1987). According to the farmers the motivating factors in the cultivation of soybeans are: ready market (26%), multipurpose use (26%), profitability (24%), nutritive value (20%) and knowledge of cultivation (4%).

About 25,059 people were trained using the local language which made the training more effective. Those trained will disseminate information to others. The training encompassed production and utilization. By now, 72 local soybean foods have been developed and food has been chemically analyzed.

Processing effectively eliminated the trypsin inhibitor activity and reduced the levels of phytic acid and tannin (Table 5). Soy-based products had higher protein and mineral levels than the non soy containing foods (Table 4). There was no significant difference in the level of acceptance of a soybean based product in comparison to conventional products by panel members. There is a nutritional superiority of soy-based foods over the conventional foods and also traditional processing methods effectively eliminated the antinutritional factors (Table 5).

New food products like soybean gari, soy vita were introduced in the villages and were acceptable. Also plain and flavoured soybean milk (chocolate and vanilla) were liked. A waste of soymilk production, okara, is now used in the homes. With the increase of the home-level production of soymilk, a corresponding increase in the level of available soymilk residue is obtained.

The protein content of soymilk residue is very high while the low trypsin inhibitor activity (TIA) suggests that the heat treatment was adequate. Presently milk residue is being used to prepare the very popular vegetable soup where it replaces melon flour which is becoming very expensive and generally unavailable. Local dishes such as moinmon, akara, ogi have also been fortified with soybean, there is concurrent increase of the protein content of these traditional foods, with the addition of milk residue.

The study on soybean oil shows that it is highly acceptable to the middle and low income groups. With further refining the high income class may also buy soybean oil. However in the villages, Ikoyi, Igangan, Iroko, Adana and Oniyo, both crude and partially refined soybean oil are acceptable but partially refined oil is preferred to the crude oil.

The products of the extruder have been shown to be nutritionally superior to other soybean foods because of the high protein content (Table 6), these products which were later formulated to local recipes were tested for acceptability in some project sites. Soybean corn blend, was accepted as a gruel and children food, soy full-fat flour, as akara (a popular cowpea product) and defatted soy flour as egusi for vegetable soup.

ACHIEVEMENTS

1. Soybean has been successfully used to increase the protein content of the low protein traditional foods without increasing the cost, or the cooking time and without changing the appearance or taste of foods.

2. A low-cost soybean milk filter press has been fabricated and tested .

3. A low-cost dehuller, manually operated was fabricated.

4. Soybean has been incorporated into cassava, to process soy gari (19% protein vs. <1% protein in the traditional gari). Soybean was added to maize to process soy vita, and "soy musa", a new extruded inexpensive plantain soy baby food was developed.

5. Screw presses constructed in Africa that can process soybeans are now available and their performance is being evaluated.

6. Over 25,000 people from 27 villages have been trained.

7. Soybean milk has now been introduced as a school lunch program.

8. Soybean is used in clinics, and hospitals to rehabilitate the malnourished children.

9. Recipes have been developed and translated into English, Yoruba, Hausa and Igbo.

IMPACT

1. Thirty-five percent of the farmers are now growing soybean.

2. The average area planted with soybean is 0.24 ha.

3. About 54% of the people cook and eat soybean in their homes.

4. Those who eat soybean consume an average of 1.35kg of soybean per week per family.

5. Several new soybean products are now available on the market (Table 7)

6. There are 258 retailers that are selling soybean. Soybean is sold as grain, or as flour (Table 8).

7. Voluntary organizations now do training on their own.

Conclusion

While emphasis presently is being placed on the preparation and utilization of soybean at home, it is likely that there is going to be a shift to commercial centralized processing of soybean.

We can foresee that several soybean products like soybean oil, dairy products, meat analogs, breakfast, and weaning food and other soy-enriched fast foods could enter the market in the nearest future.

With the reduction in the availability of groundnut coupled with its soaring prices, soybean will play a more vigorous role in the formulation of livestock feed, with the possibility of reduction in feed cost and consequently of livestock produce.

For cheap and wholesome products, a central processing of large quantities is necessary. In this respect, we see ultra high temperature processing of dairy product analogs and extrusion cooking playing a dominant role in soybean processing technology in the nearest future. The soybean processing techniques have been well documented. The emphasis is that these technologies are not new to Nigeria per se, as we already have a couple of them presently being used in Nigeria.

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Table 1. Chemical composition of whole soybean.

	Protein (N x 6.25) (%)	Fat (%)	Carbohydrate (%)	Ash (%)
Whole bean	40	20	34	4.9
Cotyledon	43	23	29	5.0
Hull	8.0	1	86	4.3
Hypocotyl	41	11	43	4.4

Table 2. Comparative cost of commodity and protein in selected food sources in Nigeria 1991.

Sources	Commodity N/kg	% Protein commodity	Protein cost N/k
Pork	12.00	12.00	100.00
Beef	20.00	20.00	100.00
Egg	9.00	13.00	69.23
Cowpea	3.75	20.00	18.75
Maize	2.25	9.00	25.00
Soybean	2.00	40.00	5.00
Poultry	16.00	20.00	80.00
Milk powder	16.00	36.00	44.00

N = Naira

K = Kobo

Table 3. **Amino acid profile of soybean protein gram/of Amino acid/16 g of Nitrogen.**

Isoleucine	4.5
Leucine	7.8
Lysine	6.4
Methionine	1.3
Cyrstine	1.3
Phenylalanine	4.9
Tyrosine	3.1
Threonine	3.9
Tryptophan	1.3
Valine	4.8
Arginine	7.2
Histidine	2.5
Alanine	4.3
Aspartic acid	11.7
Glutamic acid	18.7
Glycine	4.2
Proline	5.5
Serine	5.1

Source: FAO (1985).

Table 4. Proximate and mineral contents of selected homemade soy-based products**

	Ogi*		Milk		Moin Moin		Akara	
	Conv.	Soy	Conv.	Soy	Conv.	Soy	Conv.	Soy
Dry matter (%)	99	99	97	97	92	92	95	95
Protein (%)	7	16	21	14	17	24	16	23
Oil (%)	1	2	1	3	10	22	21	25
Calcium (%)	0.02	0.02	0.32	0.04	0.06	0.08	0.05	0.08
Iron (ppm)	138	136	20	75	255	271	274	282
Zinc (ppm)	16	15	43	7	50	43	43	44

* Ogi is maize porridge, Moin moin is steamed cowpea paste and Akara is fried cowpea paste.

** Mean of 3 readings

*** Conv. = Conventional

Table 5. Phytic acid, tannin and trypsin inhibitor levels of raw and processed soybeans*

	Raw soy beans	Soy ogi	Soy milk	Soy moin moin	Soy akara
Phytic acid (%)	2.1	1.3	1.2	1.2	1.1
Tannin (%)	1.9	0.9	1.3	0.6	0.7
Trypsin inhibitor (mg/g)	16	NAD**	NAD	NAD	NAD
Percent soy	100	50%	100%	50%	50%

* Means of 3 readings
** NAD = No Activity Detected

Table 6. Nutrient composition of selected products from the extruder and the screw press

	Defatted cake	Extruded fullfat	Defatted extruded	Extrude defatted
Dry matter %	90	96	94	93
Protein %	48	38	47	47
Fat %	10	21	8	8
Minerals %	3	3	3	3
Trypsin inhibitor Activity %	0.63	0.23	0.17	0.18
Tannin (mg/g)	0.23	0.18	0.21	0.21
Phytic Acid %	2.40	2.39	2.34	2.37

Table 7. Soybean products that are being processed and marketed by companies in Nigeria

Product	Company	Location	% Soybean used
Soy Custard	Lisabi foods	Lagos	30
Soy Milk	Milkman	Ibadan	100
Soy Oil	Oja Farms	Ilorin	100
Soybean Flour (Toasted)	Betamarks	Lagos	100
Soybean Flour (Untoasted)	Betamarks	Lagos	100
Biscuits	Parrot Food	Lagos	30
Soybread	Odichie Bakery	Lagos	25
Soy Oil	Taraku Mills	Benue	100
Soy Powder	Uncle Segun Food Proc & Preserv. Co.	Ibadan	100
Nutrend	Food Specialties	Lagos	15
Nutrimax	Smallette	Ilorin	30
Soybeverage	Farina	Lagos	100
Sogi	Smallette	Ilorin	30
Soy Oil	DLOB	Ibadan	100
Casasoy	Oja Farms	Ilorin	30
Soy Oil	Kofa Agric. Ventures	Lafia	100
Soydrinks	Imo HealthFood	Owerri	100

Table 8. Number of markets* and retail sale outlets for soybean in Ibadan, Nigeria

Date	Markets selling soybean	Retailers' selling soybean	Price (N)	Form of selling
January 1987	2	4	1.50	Seeds
January 1988	6	10	1.50	Seeds
January 1989	13	236	3.50	Seeds and flour
January 1990	17	258	4.25	Seeds and flour

* Only the 17 markets were included in the survey.

USE OF BIOTECHNOLOGY FOR OIL PALM IMPROVEMENT

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ABSTRACT

Tissue culture methods for clonal propagation of oil palms are now well established, but are slow and require much hand work. Modern developments of embryogenic liquid suspension cultures promise to improve efficiency and reduce costs in the future. There are still problems of abnormal flowering in some clonal material subjected to large-scale production, but these are being resolved by using techniques which avoid the problem. At present there is no understanding of the mechanism of induction of the flowering abnormalities.

Less progress has been made in the use of protoplasts or haploids, and DNA transformations are not yet possible. This should become feasible by combination of embryogenic suspensions and the use of ballistic DNA injection techniques, but as yet there are no clearly identified genes which it would be advantageous to transfer to oil palm.

The most important application of molecular biology will be in the development of genetic maps for use by oil palm breeders, using either RFLP analysis or the more recent PCR reaction. These methods applied to existing oil palm germ-plasm collections, augmented by fresh collections from wild grove palms in Africa must be coupled to active palm breeding programmes, with proper trials, recording and selection methods, followed by field trials of chosen genotypes in the areas where the palms will be grown.

Propagation of selected genotypes would be best contracted out to established tissue culture laboratories, especially where requirements for small numbers of plants would make it uneconomic to set up new tissue culture facilities. This could be done through established commercial labs or within an International Institute, such as NIFOR acting on behalf of co-operating African countries. A first step would be the limited propagation of elite *dura* and *pisifera* parents which could be used to set up seed nurseries for improved *tenera* hybrids for local use by growers.

1. INTRODUCTION

In this background paper I shall review the current position relating to the applications of biotechnology to crop improvement in the Oil Palm.

I shall then discuss the relevance of these modern technologies to practical problems in relation to African needs (as I currently perceive them as a complete outsider), and ask which, if any are appropriate.

We can then consider how the relevant technologies might be acquired, how they should be supported, and how they can be integrated into the current practices of oil palm breeding, seed production and plantation management.

2. CURRENT STATE OF THE ART

Applications of biotechnology to crop improvement are almost exclusively concerned with aspects of plant breeding. They range from clonal propagation, using tissue culture techniques, through various manipulations at the cell

genetic level, such as the use of protoplasts for cell fusions or manipulation of haploid cells from pollen culture, to the use of recombinant DNA technology for the transfer of specific genes. Molecular biology also provides very powerful tools for the plant breeder in mapping the genome and identifying individual genes of relevance to the breeding programme.

Progress has been rapid in recent years in the use of biotechnology for the breeding of annual crops, such as tobacco, (a favourite research tool because of its ease of manipulation in culture) oilseed rape, soya beans, tomatoes, rice and maize. The two latter species are in the family of monocotyledons, which in general are less amenable than dicotyledons to tissue culture and the traditional DNA transformation methods using *Agrobacterium tumefaciens* Ti plasmid as a DNA vector. Oil palm is also a monocot, and as such is likely to be a more difficult subject than the dicotyledonous species. It is also a perennial crop with a long breeding cycle, which results in a long time-scale for any genetic studies.

Clonal propagation

At the present time techniques are available for clonal propagation of oil palm using tissue culture. In contrast to other tropical crops such as rubber, banana, cassava and many others, there is no traditional method of clonal propagation available, and the advent of the tissue culture method holds out the prospect for palm breeders to select and multiply clones from individual elite palms from the best available progenies. It has been estimated that yield improvements of 30% should be attainable over the mean oil yield of the progeny from which the clonal selections were made (Meunier *et al*, 1990) . Yield improvements of 20% have already been realized from the first clones produced (Corley 1991).

The methods have been the subject of several recent reviews (Jones & Hughes 1989, Paranjothy 1986, Jones 1990, Wooi 1990). There is now no difficulty for any competent tissue culture laboratory to set up cultures of oil palm, and to recover regenerant plants by following the established published procedures. Indeed there are many such labs in Malaysia, Indonesia, Ivory Coast etc. A comprehensive account of Unilever's experience over the past 15 years, since the first clonal oil palm plants were planted in Malaysia, was given by Corley (1991).

It must be noted that although the techniques have improved steadily since the first plants were regenerated in 1974, propagation of oil palm is still slow, uncertain, and requires constant vigilance and selection of competent tissues from the heterogeneous tissue masses that are characteristic of the type of growth obtained. It is not amenable to simple mericloning methods, and discrimination is required at each subculture to reject non-embryogenic material. Thus it is essential that transfer operators are well-trained and able to assess the state of the cultures. As with most monocot cultures, it is necessary to start with actively growing meristematic tissues, either from young leaf-base tissue, from lateral root initials, or from young inflorescences. In each case it seems essential to induce callus formation to break the organizational integrity of the source tissue before cells are able to form somatic embryos or adventitious buds. In the presence of an auxin (NAA or 2,4-D) a low percentage of calluses will produce somatic embryos. The frequency is greatly dependent on genotype, and is improved by optimization of medium additives. These improvements have been made over a period of 15 years development, mostly in commercial labs which do not publish their results. The early recognition of the proembryogenic cell clusters, and their transfer to

media with reduced auxin content is essential to prevent them reverting to callus and subsequently losing regeneration potential. The embryogenic cultures are then capable of continued proliferation over a long period, producing shoots that can be removed and rooted, and fresh embryoids for further recycling. During this phase there is always the possibility of cultures losing their regeneration potential or undergoing genetic or epigenetic changes, and it is necessary to re-isolate fresh cultures regularly to replace ageing culture lines.

The problems of translating the laboratory techniques into a large scale factory process are not trivial. They require particular attention to hygiene and quality control, and constant attention to detail. It is essential to have good biological management with people who understand the nature of the tissue cultures, can identify the competent cells and can recognize the early signs of any deterioration in quality. A well staffed quality-control laboratory is needed to monitor any signs of microbial or insect contamination, to control the quality of the media in use, and to identify and quickly eliminate any sources of contamination or deterioration of the quality of cultures being transferred (Leifert *et al*. 1991). A laboratory is also required to initiate cultures of new clones, and to regularly re-isolate competent cultures of established clones.

Commercial development of the propagation of clonal oil palm has been seriously delayed by the appearance of abnormal flowers in some clones that have been subject to large-scale production. (Corley *et al* 1986). Intensive work on this problem over the past few years has so far failed to reveal the cause, although some clues are emerging. At the International Plant Growth Regulator meeting in Amsterdam this year, Besse *et al* (1991) reported depressed cytokinin levels in abnormal flower tissues as compared with normal tissues from the same clone. They also reported that regenerants from nodular calluses were almost invariably normal, while the friable "fast-growing" calluses gave rise to abnormal palms. Unilever experience has not been so clear-cut: there have certainly been some normal clones from fast growing friable cultures, and abnormals from the nodular types. Over a period of time the frequency of abnormal ramets developed from nodular polyembryogenic cultures increased, although there was no evidence of friable callus (Corley 1991). In the latter cases it is always possible that some FGC (even if only a few cells) developed on the nodular tissues and gave rise to the abnormal embryoids. There is some evidence for a genetic basis of the problem; cultures from some progenies are more prone to produce abnormal flowers than others. Genetically mantled palms occur naturally at a low frequency and in these cases the condition appears to be heritable, and was reported by Beinaert and Vanderweyen (1941) to be due to a dominant gene. Seeds from mildly affected bunches of abnormal palms from tissue culture (still capable of setting seed), subject to open pollination, have been planted and have now flowered. Some of the progeny were mantled, but the distribution did not suggest the operation of a simple Mendelian dominant gene. (Rao and Donough, 1990). Controlled crosses have been made, but the plants have not yet flowered. In addition, some of the previously abnormal palms have reverted to normal, or at least the frequency of abnormal flowers on the inflorescences is declining. Use of genetic probes has so far failed to reveal any consistent changes in DNA structure (Cheah *et al* 1991), but there is a growing suspicion that there may be changes in the mitochondrial DNA. This might not affect all the mitochondria in the tissues, and hence the abnormality could vary in its level of expression. Reversion to normal could then be explained by rejection of the abnormal mitochondrial population and its replacement by normal mitochondria, perhaps as a result of differential multiplication rates.

Clearly there has been some semi-heritable change in gene expression relating to flower development, resulting in the production of extra carpels from the stamen primordia. This change has been induced in the culture stages. Unilever has examined a wide range of culture conditions, and the Company is now confident that the problem can be avoided by judicious selection of "resistant" ortets, by use of low-risk media, and by limiting the number of transfer cycles. The IRHO group are also confident that provided friable "fast growing callus" is avoided, and only the nodular embryogenic cultures are used, then there is little risk of abnormality developing. Both groups are now engaged in extensive field trials, and are optimistic that large scale production of clonal oil palms can soon be resumed.

Further progress in the development of tissue culture methods was reported by de Touchet, Duval and Pannetier (1990). They successfully obtained an embryogenic suspension culture which could be continuously multiplied in the presence of 2,4-D, but which could be plated out to give rise to individual plants from single somatic embryos. Söndahl (1991, personal communication) has also reported successful plant regeneration from embryogenic suspension cultures of oil palm. This now offers the prospect of developing oil palm suspension cultures suitable for large scale propagation in a fermenter system, much as carrot and alfalfa cultures have been handled previously. Since their presentation in 1990, the work has progressed to the isolation of suspension cultures of a number of embryogenic lines of different genotypes, and the plating conditions are being optimized to improve the yield of single plants. Work is in progress to develop encapsulation methods to aid automated handling of the individual somatic embryos. Success depended on good careful cytological studies of early stages of embryogenesis and the recognition of the embryogenically competent cell clusters at an early stage. (de Touchet, personal communication). If it is possible to develop these methods to a commercial scale, the production costs will be dramatically reduced, and almost eliminate the need for rows of transfer hoods (and their operators) in the production unit.

There may be dangers in such a process. Clones produced so far have been derived from relatively few embryogenic events and, apart from the flowering abnormality have proven extremely uniform, with little or no evidence of somaclonal variation (Wooi *et al* 1982). Large scale production from single cells, each giving rise to individual somatic embryos, may result in a hitherto unseen number of somaclonal variants. The French group are currently field testing clones derived from the suspension cultures for uniformity.

Cell Genetics

a) *Protoplasts* : The successful formation of calluses from oil palm protoplasts was reported by Bass and Hughes in 1984 using a nurse culture technique. No regenerant plants were obtained, but there is now a prospect of producing protoplasts from the embryogenic suspension cultures of de Touchet *et al* which might regenerate plants freely. Sambanthamurthi, Oo and Ong (1987) obtained metabolically active protoplasts from embryogenic cultures, but did not attempt to subculture them. Protoplasts from such a system could be used for either for protoplast fusion experiments (eg interspecific hybridizations between *E. guineensis and E. oleifera*, or other palms) or for DNA transformation work.

b) *Haploids:* Little progress has been reported on culture of oil palm anthers or immature pollen. Odewale (1983) reported callus formation from anther culture of oil palm, but I have not seen the thesis, and do not know whether the cultures originated from microspores or anther wall, nor whether they were

haploid. Work is in progress at PORIM, Malaysia, but again, I do not know the present state of the work.

Molecular Biology

Transformation of oil palm cells with foreign DNA has not been reported. The essential pre-requisites of an efficient plant regeneration system from tissue culture, and a method of transformation, are still lacking. It is unlikely that oil palm, being a monocot, will be transformed by the *Agrobacterium tumefaciens* plasmids, but there are several other promising approaches including the Biolistic approach using direct DNA injection with DNA-coated particles shot into competent cells. Although now popular, most transformants are transient and there are only limited reports of successful permanent integration of functional DNA using this technique in any species. Other methods include electroporation, and PEG mediated DNA transfer. The advent of the embryogenic suspension cultures from de Touchet *et al.* (*loc.cit*) may make these approaches possible. It would be important to determine whether resulting somatic embryos were derived from single transformed cells. Cells already at the multicellular proembryo stage would give rise to chimaeras, and transformed cells may not enter the germ line. In any event it would be essential to evaluate the progeny for stable integration of the foreign DNA, and with the long breeding cycle this would be a very long term programme.

DNA transformation has been used in several crop species to transfer herbicide resistance , resistance to insect pest damage (e.g. using *Bacillus thuringiensis* toxin genes), and to incorporate antiviral genes, either for virus coat protein or anti-sense viral RNA. These have proven effective in protecting against viral infection. Fruit quality has been successfully modified in tomato breeding by the use of anti-sense RNA (Smith *et al.* 1990). (Incidentally the number of authors on this paper is indicative of the size of research resource required to implement effective molecular biology work). The number of successful transformations with commercial applications is still limited, but increasing rapidly, and transformed plants are now in field trials in several countries. Undoubtedly the techniques will improve rapidly and the number of applications will increase exponentially over the next few years.

Molecular probes

Molecular biology has provided very powerful tools for probing and mapping the genomes of crop plants. Restriction Fragment Polymorphisms (RFLPs) enable different genotypes to be distinguished and genetic maps and linkage groups to be established. The recent introduction of the polymerase chain reaction (PCR) for rapid amplification of minute traces of DNA has provided an alternative method which has reduced the time, cost and sample sizes required for these procedures, which can now be done without the need for radioactive probes.(Arnheim *et al.* 1990). However, the very high sensitivity of this method requires extreme care to avoid contamination with extraneous DNA, and results should always be checked with conventional Southern blot analysis before unusual DNA patterns are accepted as coming unequivocally from the sample. At the recent International Oil Palm Conference at PORIM (1991), progress in the diagnostic use of RFLPs in oil palm was reported by Cheah *et al* (1991), and by Mayes and Jack (1991). The technique is now in routine use for clonal typing and checking the identity of clonal ramets with the original ortet. Evidence obtained so far using the probes available suggests there is very little somaclonal variation in oil palm cultures compared with other species, such as potato. It must be noted however that there may be changes in DNA sequences which are not cut by the

restriction enzymes used and do not result in changes in the visible fragment size. At Plant Breeding International (Cambridge) a start has been made in constructing an RFLP map of the oil palm genome for use in the Unilever oil palm breeding programme.

3. RELEVANCE OF THE TECHNIQUES

Relevant technologies are ones which will confer significant benefits to the grower, consumer and/or processor. In the end these can all be reduced to a cost-benefit equation. It is not essential to replace old techniques with modern ones just because they are new and exciting. They must be shown to be worthwhile in terms of cost and productivity. This type of research is expensive and long-term. Evaluation of the benefits of research must be done to allow for recovery of the research and development costs on a proper accounting basis, for example using discounted cash-flow analysis. What benefits might have been obtained if that money had been spent on other programmes, or even left in the bank? In many cases it will be pointless to introduce improved planting material (at higher cost) when the limitations to productivity lie in problems of soil fertility and agronomic management.

There is no doubt that propagation of clonal palms by tissue culture can result in significant yield improvements over conventional seedling progenies. It is also possible to select clones with resistance to *Fusarium oxysporum*. (Corley 1991).

The application of fermenter techniques and automated handling of the young regenerant plants, when available, will significantly reduce the cost of production of the clonal plants, although with high initial capital costs, and the need for high level technical skills for maintenance and back-up.

In order to be effective clonal propagation must be coupled to an active palm breeding program with a wide selection of germplasm and well recorded individual palms in elite progenies. The selection procedures must be based on sound physiological criteria and selection made on the basis of characters known to be heritable (otherwise the selected palms may have had good performance simply because of their growing in a favoured environment, *e.g* good nutrition, optimal water, low competition etc. Such palms would prove to be no better than average when propagated as a clone). Because the selection criteria are uncertain, and many complex factors interact to determine individual palm performance, it is also essential to test clones extensively in the areas where they will be used (Interactions between genotype and environment have been shown to be important (Corley *et al* 1988)), and thorough testing is also required to ensure that there are no abnormalities of flowering, or any extensive somaclonal variation. This is particularly important when any changes are made to the tissue culture procedures.

The prospect of significant improvements from protoplast fusions or other cell manipulations are more remote. Most of the examples from the more easily manipulated species are biological curiosities rather than useful sources of new variation for the plant breeder. While there are undoubtedly characteristics in other palms, for instance differences in oil quality, that might be transferred by somatic hybridizations, the chances of maintaining fertility and high yield, without the introduction of other adverse characters is very low. Even if the hybridization and plant regeneration steps led to fertile offspring, a long back-cross programme would almost inevitably be required to establish the new character in a useful genetic background.

Similarly I am doubtful of the value of haploid breeding in oil palm. With a highly heterozygous outbreeding species it would be expected that the vast majority of haploid cells would be carry many deleterious recessive genes. Thus the regeneration rate may well be very low, and the vast majority of regenerants would be weak. All would be either dura or pisifera, and their value as parents could only be tested by making large numbers of *dura* X *pisifera* crosses and testing the progeny. By this means it might be possible to find parents with complementary genes which would give good *tenera* progeny, but the commitment of resources in field trials and individual palm recording over many years would be enormous. The benefit would be to have truly homozygous parents for production of uniform F1 *tenera* progenies. The same result, of phenotypically and genetically uniform planting material, can be achieved by cloning the best individuals from segregating progenies.

In the oil palm, which is a highly heterozygous outbreeding species, there is plenty of variation available within the existing germ-plasm, and this can be most efficiently exploited by recombination using classical breeding techniques combined with clonal propagation of the best individuals within the segregating progenies. Conventionally plant breeding has sought to produce uniform seed populations from a relatively narrow genetic base, and the introgression of wild-type germ plasm has been limited. The ability to produce uniform clones from elite individuals within highly variable segregating progenies allows the breeders to explore a much wider source of germ-plasm than hitherto. (Hardon *et al.*, 1987). A large reservoir of unexploited germ-plasm still exists within the wild palm groves of West Africa, and it is important that the existing germ-plasm collections should be augmented with new collections of the natural gene-pool. This is an important African resource which must be conserved. Such a policy was adopted by AFOPDA(African Oil Palm Development Association) which has set up a regional network of six African countries, with Nigeria as the leader country. ("Burotrop", 1991)

The majority of characteristics of interest to the palm breeder such as yield, drought tolerance, or disease resistance are either controlled by many genes or by genetic systems that are not yet understood. The current state of gene transfer technology , even in the species where it is possible, is still confined to addition of single genes, either constitutively expressed or possibly with developmentally controlled organ-specific regulators. The successful programmes have been based on a thorough understanding of the underlying biochemistry of the enzyme systems in the pathway which is to be modified by the introduced gene. It is difficult to identify any at present which would confer sufficient benefit to the oil palm grower to warrant the cost of a genetic manipulation programme. There are no known virus diseases, the majority of insect pests are easily controlled, and herbicide tolerance is of little interest except perhaps in the early seedling establishment phase, where again conventional management is simple and effective.

In his wide-ranging paper to the recent International Oil Palm Conference, Davidson (1991) outlined several characteristics which would improve the crop by reducing harvesting costs and harvesting losses and making the introduction of mechanical harvesting a practical option. These include better control over sex-ratio, uniform bunch ripening, and control over fruit abscission, longer bunch stalks for ease of harvesting, and better indicators of bunch ripeness. Some of these characters may be amenable to genetic engineering , but first the relevant genes will have to be identified, the biochemical pathways they control understood, and methods found to introduce the appropriate genes under proper control in a stably integrated way. A start has been made by Osborne (1991) in

understanding the enzymic control of the fruit abscission process in the oil palm. This knowledge could quickly lead to identification of the specific genes involved, and hence to their ultimate modification. In this instance, when the objective would be to interfere with a natural process by inhibiting the activity of enzymes causing fruit abscission, the use of anti-sense genes may be effective. The question must still be asked; is the advantage to be gained worth the investment required to bring about the desired improvement?

To summarize this section, in my opinion the relevant technologies are firstly clonal propagation by tissue culture, coupled to an active conventional palm breeding programme and efficient clone selection and evaluation. Secondly the use of DNA diagnostic procedures (RFLPs PCR) again coupled to the conventional breeding programme and used to develop an understanding of oil palm genetics. We have as yet no linkage maps, very few identified genes and even the chromosome cytology is not well documented. The 32 chromosomes are small and difficult to distinguish, and not easily amenable to karyotyping. The diagnostic techniques are also proving valuable in evaluation of cloned ramets and identification with their ortets.

4. ACQUISITION OF THE TECHNOLOGIES

In my view there is little point in attempting to set up independently a large scale tissue culture cloning operation for oil palm.

No commercial company would contemplate developing, in house, from scratch, a copy of an existing technology. It is far quicker and much more efficient (and therefore cheaper) to buy it in from someone who has already developed it. There is no point in struggling to overcome the problems of scale-up and management when these have already been solved. In most cases, companies developing the new processes will either seek to protect them by patents, or by refusing to divulge the technical details. This is understandable when the large investments and long time-scales involved in developing the technologies are considered. Naturally companies will only indulge in such programmes if they know they can both recover their development costs and subsequently make a profit. It must be remembered that the object of patents is to encourage the use of patented processes, not to inhibit it, and once a patent has been issued it should be possible to negotiate a license to use the process. This is universal practice in most branches of manufacturing technology. Indeed it is probably not worth-while to attempt to set up a separate rival operation, and the modern approach is more and more to contract work out wherever possible. In that way the risks are borne by the contractor, not the customer. There is no requirement for large scale capital investment, no long term commitments, and no problems in maintaining quality. As new techniques are introduced you can benefit from reduced unit prices. There are now numerous tissue culture laboratories with the relevant expertise and multiplication capacity anxious to compete for work. It is therefore possible to negotiate financially competitive contracts for propagation of selected germ-plasm, and if the contractor does not deliver, to put your custom elsewhere. Control over the germ-plasm can be secured by the use of the DNA fingerprinting techniques to ensure there is no risk of plants being transferred to competitors. In any case no reputable propagators would risk the loss of custom consequent upon loss of confidence in the security of material contracted to them. The important resource is the germ-plasm, and the real effort should be concentrated on obtaining the best genetic material for multiplication, and on its rigorous field testing and selection. That then becomes a highly marketable resource, but will have to prove its superiority to rival sources in the market place. West Africa has the great advantage of being the natural

centre of origin of the oil palm. There is a comprehensive genetic collection of both wild and highly developed breeding lines, and a long tradition of oil palm research. Much of the data required for selection of parents for production of progenies suitable for cloning is already in the archives.

Growers and processors want to be able to produce palm oil that will compete in price on the world market where there are steadily increasing supplies available from South-East Asia and South America. The African countries are in a strong position to introduce new genetic variation, including disease resistance (e.g. to *Fusarium* wilt) and to compete strongly with SE Asian material derived from the relatively narrow base of the Deli Duras.

The DNA technology on the other hand, for clonal monitoring and for oil palm genetics to aid the breeding programmes, should be available directly to the palm breeders. To this end it is necessary to acquire the latest techniques, and, because in this field progress is so rapid, there must be regular contact with other molecular biology labs. Regular exchange of people, attendance at international meetings and training workshops will help to maintain an awareness of current methods. The methods in molecular biology are relatively simple, but not cheap. It is necessary to acquire a collection of probes and restriction enzymes, and there are continuous improvements in the methodology rendering equipment out of date within a very short time. Although the preparation of DNA gels is easy, the most important requirement is for experienced workers who can interpret the results and safeguard against the ever-present risk of artifacts. It is also important that the palm breeders are aware of the power of the new technologies, are eager to use them in their work, and can integrate their programmes with the molecular biologists. Plant breeding has traditionally been an empirical art, not necessarily requiring much more than basic genetics. The new techniques require a thorough genetic understanding to be linked to the new molecular information. The implementation of an effective dialogue between molecular biologists, with their highly specialized and largely impenetrable jargon of cryptic neologisms, and the plant breeders traditionally reliant on field recording and simple analysis of components of yield requires education on both sides.

5. USE AND MANAGEMENT OF THE TECHNOLOGY

Having acquired the technology it is essential to apply it to economic advantage and to maintain it with continual updating, and this applies to any biotechnological process which might be adopted. The clonal methods will continue to improve, the factors causing the flowering abnormality will be discovered and evidence of the levels of somaclonal variation will continue to accumulate. This knowledge will determine the ultimate usefulness of oil palm clones. It is important for palm breeders to be in a position to assess the value of the techniques available and to apply them where there are advantages in so doing. Similarly recombinant DNA technology will continue to develop rapidly, and again a constant awareness of the current developments is essential. On the other hand there is little point in trying to develop the methodology for oil palm when there is so little knowledge of oil palm genetics, or of the biochemistry involved in determining the characters which it might be useful to modify. These must be the priority areas for work to provide the background which will make it possible to apply the new technologies as they become available.

The use of clonal oil palm is still in its infancy, and there are many ideas and opinions on how clones should be managed. Optimal planting densities differ between clones (Corley and Donough 1990), particularly if palms with high

bunch index are selected with relatively small leaf canopies and short trunks. It is not clear how many clones should be used in a given planting. Is it safe to plant large monoclonal blocks or would it be better to interplant a clonal mixture? Perhaps 2 hectare blocks of maybe 6 clones would provide security against catastrophic failure of any individual clone. It would be easy to clear and replant the affected blocks, where it is not possible to effectively replace individual palms in a mixed planting. Clones have been found to have relatively closely synchronized sexual cycles. If a whole block is in a male phase there is no need for harvesters to visit that block until it re-enters a female cycle. These are only some of the questions to be answered in optimising the use of clonal material, and many years of field trials and recording will be necessary.

An alternative use of clonal material is for the limited propagation of dura mother palms for seed nurseries producing "clonal seed"; in other words to enhance the production of seeds of what are effectively single progenies of proven parents. We know that limited production of clones will have very low risk of any abnormalities, and such seed nurseries will produce high quality conventional seed which can be planted with confidence until such time as the uncertainties concerning large-scale tissue culture propagation are resolved.

Clearly many of these resources are beyond the range of most individual African countries, and the programmes to date have been geared towards large scale plantations rather than the small growers. Coordinated efforts between African countries to develop clones suitable for local conditions, using the germ-plasm available in NIFOR and within the areas of wild oil palm groves could be of great benefit to growers if the plants can be made available at low cost. In many cases relatively small numbers of plants would be required, and there would be no prospect of setting up a successful commercial enterprise to produce them. However, it would be within the scope of an International Institute to produce and distribute clonal germ-plasm, in much the same way that Cassava clones are produced at IITA. A first step might be to produce small numbers of *dura* mother palms plus a few *pisifera* pollen parents, which could be used to set up seed gardens producing "clonal" *tenera* seed. It must be emphasized that such material would only be worth growing if it had been selected from the best recorded germ-plasm and field tested in the areas where it would be grown. F[illegible]rium wilt resistance and drought tolerance would be two of the most important c[illegible]acters to be sought.

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CYTOGENETICS OF CASSAVA AND RELATED SPECIES

by

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ABSTRACT

Cassava, *Manihot esculenta* Crantz, is a major food crop in Africa. The genus comprises about 98 species, confined to the American tropics, and IITA has a collection of about 22 species. Normal pairing of chromosomes with 18 bivalents at M-I is recorded for these species. Interspecific hybrids with cassava as well as between wild species using 12 of these species are reported. The cytogenetics of these hybrids is discussed.

Unreduced or 2n gametes were frequent in the hybrids and their role in the origin of sexual tetraploids and triploids of cassava is discussed. Besides sexual tetraploids, spontaneous asexual tetraploids and induced tetraploids are also produced in cassava. From the triploid progenies, two aneuploids were obtained for the first time in cassava and their cytogenetic behaviour is discussed.

Introduction

Cassava, *Manihot esculenta* Crantz is a major food crop in Africa. The genus comprises of about 98 species. The species, as wild plants, are confined to the American tropics and no native species are found in the Old World. Morphological criteria, geographical distribution and other factors have been used to systematically classify the large number of so-called types, races or varieties of *M. esculenta* (Magoon, 1967). Inspite of its importance as a major food crop, relatively little is known about the cytogenetics of cassava. All *Manihot* species so far studied, including cassava, have 36 somatic chromosomes (2n = 36). Though cassava is generally considered as a diploid, it has also been postulated that it has an allopolyploid origin, with a basic chromosome number of x = 9 (Perry 1943; Jennings 1963, Umanah and Hartman 1973), and a segmental allotetraploid origin (Magoon et al. 1969).

Cytological studies in *Manihot* species

The cytogenetics of *Manihot* species and interspecific crosses has barely been explored, even though such research must accompany any attempt to utilize genes from the wild species in breeding programs. Until recently, cytological studies in *Manihot* species were confined only to the determination of chromosome number, mostly in cultivated types. Hence, information on the synaptic behaviour of chromosomes in cultivated types, related wild species or interracial or interspecific hybrids, is very scanty.

At present, IITA has a collection of about 22 *Manihot* species. Normal bivalent pairing (18 bivalents) occurs for most clones of cassava and only occasionally non-pairing or one or 2 univalents occurs (for a review, see Bai, 1987). Normal bivalent pairing has also been observed in the wild species like *M. glaziovii*, *M. epruinosa*, *M. leptophylla*, *M. brachyandra*, *M. tristis*, *M. anomala*, *M. pohlii*, *M. tripartita*, *M. gracilis and M. catingae*, wherein also 18 bivalents were observed.

Interspecific Hybridisation and Genome Analysis

Data available on interspecific hybridisation in cassava or other *Manihot* species are limited (see Bai, 1987). This may be due to lack of availability of the species at research centers.

IITA now has a collection of about 22 wild species, and 12 of these species have already been used to produce interspecific hybrids with cassava as well as hybrids between wild species. The main objectives of such species crosses were to gather information on genome homologies and cytogenetic architecture of the species as such information is necessary to effectively incorporate the desirable traits of these species into cassava.

The chromosomes paired mainly as 18 bivalents at metaphase-I (M-I) in most of the hybrids andSwccasionally as 17 bivalents + 2 univalents (Table 1). At anaphase-I (A-I), though disjunction and separation of chromosomes was normal in most of the hybrids, a bridge and a fragment, indicating the presence of an inversion, were very frequent in the hybrids between *M. epruinosa* x *M. leptophylla* and less frequent in the hybrids between the other species (Table 1).

The chiasma frequency in these hybrids varied from as low as 20 in the hybrids between *M. epruinosa* x *M. leptophylla* to as high as 31 in hybrids between cassava x *M. leptophylla*, compared to the chiasma frequency of 34 in cassava clones. These species hybrids are partially male and female fertile.

Production of 2n gametes and their significance

Some of the interspecific hybrids, especially those involving *M. glaziovii* and *M. epruinosa*, as well as some clones of cassava, produce 2n or unreduced gametes ("giant" pollen) as a result of first division or second division restitution (Hahn et al. 1990). The 2n pollen ranged from 0.1% to 35.6% of the total stainable pollen per flower bud. These 2n gametes are instrumental in the origin of the spontaneous sexual tetraploids and triploids in cassava as reported recently (Hahn et al. 1990).

Chromosomal Races in Cassava

Induced polyploids have been reported earlier in cassava by a few workers (Bai, 1987). Sexual tetraploids (2n = 4x = 72) and triploids (2n = 3x = 54) have been obtained in cassava only recently. Sexual polyploids generally are advantageous in that they show less inbreeding, more genetic diversity and higher proportion of tri-allelic and tetra-allelic genotypes, which can lead to higher heterozygosity (Watanabe and Peloquin, 1991).

In the sexual tetraploids, the chromosomes paired mainly as bivalents and quadrivalents. At A-I, chromosome disjunction and distribution to the poles were almost normal in majority of PMCs (pollen mother cells), resulting in 40% to 60% stainable pollen. These tetraploids were partially female fertile as well.

In the sexual triploids, chromosomes paired mainly as trivalents with a few bivalents and univalents. The disjunction and distribution of chromosomes to the poles were irregular resulting in low pollen stainability (10-12%). These triploids were partially female fertile as well.

Besides the spontaneous sexual tetraploids and triploids, somatic tetraploids have also been identified among cassava clones and their origin is attributed to the mitotic anomalies in meristematic cells of axillary buds (Hahn et al. 1991).

Aneuploids

As the triploids were partially fertile, a seedling progeny could be raised from the triploids. By cytological screening of this progeny, two

aneuploid plants have been identified. One of these aneuploid plants has 10 extra chromosomes (46 somatic chromosomes, 2n = 2x + 10) and the other has 8 extra chromosomes (44 somatic chromosomes, 2n = 2x + 8). The chromosome pairing, as observed at M-I in these aneuploids, consists of trivalents, bivalents and univalents. The disjunction and distribution of chromosomes to the poles at A-I in these aneuploids are such that at a given pole, the number of chromosomes varies between 18 to 28. This is significant in that it can produce different types of aneuploid gametes and hence promises the possibility of production of a series of aneuploids in cassava in the near future. The aneuploids are being reported for the first time in cassava. Aneuploids, especially monosomics and trisomics, are useful in the identification of gene(s) that control desirable characteristics, such as quality factors and disease resistance, or regulate chromosome pairing.

In this context, it is significant to study the karyomorphology of the chromosomes at pachytene stage in cassava, in order to identify the extra chromosomes in the aneuploids and to relate any characteristics associated with those extra chromosomes to construct chromosome linkage maps.

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Table 1. Chromosome behaviour at M-I and A-I in *Manihot* species hybrids

Serial No.	Species Female	x	Male	Chromosome pairing at M-I	Chromosome separation at A-I
1.	Cassava	x	*M. glaziovii*	18 II	Normal
2.	Cassava	x	*M. epruinosa*	18 II	Normal/1 bridge + 1 fragment
3.	Cassava	x	*M. leptophylla*	18 II	Normal
4.	Cassava	x	*M. brachyandra*	18 II	Normal
5.	Cassava	x	*M. gracilis*	18 II/ 17II+2I	Normal/ 1 bridge + 1 fragment
6.	Cassava	x	*M. anomala*	18 II	Normal
7.	Cassava	x	*M. tristis*	18 II	Normal/1 laggard
8.	Cassava	x	*M. pohlii*	18 II	Normal
9.	Cassava	x	*M. tripartita*	18 II	Normal
10.	*M. epruinosa*	x	*M. leptophylla*	18 II	Normal/ 1 bridge + 1 fragment
11.	*M. tristis*	x	*M. glaziovii*	18 II	Normal/ 1 bridge + 1 fragment
12.	*M. tristis*	x	*M. brachyandra*	18 II	Normal/ 1 bridge + 1 fragment
13.	*M. tristis*	x	*M. leptophylla*	18 II/ 17II+2I	Normal
14.	*M. tristis*	x	*M. tripartita*	18 II	Normal
15.	*M. catingae*	x	*M. glaziovii*	18 II	Normal
16.	*M. catingae*	x	*M. epruinosa*	18 II/ 17II+2I	Normal

BIOTECHNOLOGY'S CONTRIBUTION TO CASSAVA PRODUCTION AND PROCESSING

by
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1. Introduction

Coming from a Third World Country, Zimbabwe, with its displaced refugees and starving communities never far from our borders or our backyard, let me look at the contribution of biotechnology to cassava production and processing firstly with a severely "bottom-line" appraisal: so far, what has actually been achieved? Thereafter we can take a more speculative look at what could be achieved if we got down to it - and perhaps how to get down to it.

Why cassava? Not because it is easy to apply the methodologies of biotechnology to it - it is not. Why cassava? (A) Because it is Africa's major crop in terms of tonnes produced(1); it is Africa's poor man's crop grown often where nothing else will grow. Yet under good circumstances it is a big yielder: I have heard of over 100 tonnes per hectare claimed in special situations like Indonesia (anecdotal, visiting Washington "experts") where there is plenty of water and sun. (B) Because one third of Africa uses it as a staple diet(2). (C) Because as Africa's population rises, AIDS permitting it, we are being forced back to root and tuber crops simply because they can yield more tonnes per hectare than cereals do. This is the peasant's choice(3) - to return to what will at least fill the stomachs of the family. So cassava is a big crop and may get even bigger. However, nowhere above have I been able to mention cash incentive. No-one is yet looking to make money out of the crop. It is a family crop, usually a women's crop, indeed an "orphan crop" in the sense that it has been greatly neglected by research scientists and funding. So, as we shall see, either we need social advocacy(4) for this as a food security crop or persuasive economic arguments that biotechnology can be enlisted to make a cash crop out of it which will thereby provide the base for industrial and chemical activity.

2. How has Biotechnology helped so far?

Definitions do not help too much: when researchers sniff funding, then definitions, especially of biotechnology, become very elastic. However, in our Masters in Biotechnology programme at UZ(5) we describe biotech as falling into four groups of activities in ascending order of complexity and cost.

A. Traditional biotechnologies, in particular fermentation. Cassava has long been subject to variations of fermentation by an array of time-tested means(6).

B. Lowtech Biotech, in particular the various techniques of tissue culture where all you need is an autoclave, a flow cabinet, some chemicals and relatively constant temperatures (7). Then you can practise the arts of virus-elimination, micro-propagation, anther culture, embryo rescue, somaclonal selections and etc.

C. Immunological Biotechnologies arising from hybridoma techniques, in particular diagnostics based on monoclonal antibody production. For this you need rabbits, guinea-pigs or mice and good tissue culture facilities.

D. Hightech manipulation of DNA fragments, in particular the addition of single gene traits to already successful cultivars. These single genes can come from any other species and often confer resistance to a

particular pest or disease, although it could alter the quality of the crop product. In addition there are certain DNA analysis techniques that can assist the plant breeder, notably the use of RFLPs and RAPDs to follow traits through a breeding programme.

Cassava production and processing has so far been aided by only the first three technologies mentioned above.

3.1 Traditional Technologies

The traditional practice, with many variations on fermenting the peeled and pounded cassava tuber until a tasty and desirable carbohydrate staple is produced, is a major achievement starting from the bland and almost tasteless pure starch. Biotech may improve this with new strains of fungi for fermentation but this is unlikely for some time to come. Accumulation of traditional culture, through centuries of oral transmission has perhaps already reached the apogee of perfection here. Clearly for industrial fermentation to produce specific chemicals quicker and purer, a new digesting fungi and perhaps combinations of fungi are a possible solution but nothing has been achieved so far. Little has been attempted. Some useful work has gone into preparing village-level pilot projects using a non-sterile fermentation to generate a cattle food that is 20% protein(8). In Zimbabwe, an MSc student is enforcing this possibility as there is a real need in our semi-arid regions for a protein-enriched stock feed that will help us get through the long dry season. Incidentally, the Andean Development Bank is fostering such a project in Venezuela.

3.2 Lowtech Tissue Culture

(i) Particularly at IITA, tissue culture has been used effectively to clean up and so **virus eliminate** traditional lines of cassava. This both offers a proper exploitation of a line's potential and provides nuclear stock - a mother culture for *in vitro* and *ex vitro* multiplication and dissemination of high quality material. It also provides a standard under research station conditions defining the potential achievable against which others in National Programmes can compare their own results using IITA developed lines.

(ii) These clean cultivars have been preserved in an African **germplasm bank** at IITA, Ibadan, in Nigeria: South American material is held at CIAT, Cali, Columbia. Some modest national collections also exist in tissue culture. Thus in a world where the germplasm pools are drying up, cassava's long-term future is held in trust at these IARCs.

(iii) **Rapid multiplication**, while costly, is possible in the lab in tissue culture and has the merit that the product remains disease free until it leaves the lab. However I am not aware of any African nation actually doing this for cassava despite the several training courses expertly run here at IITA. My own lab at the University of Zimbabwe has recently received a commission to rapidly multiply our elite clones (the product of years of our own selections out of IITA x local material) so that certain drought-prone areas can be supplied with high quality starting material with a view to providing a food security crop and potential cash crop neither of which existed in these semi-arid areas(10).

Here is an area where the economics and the weaknesses of poor infrastructure seem to have nullified a useful possibility provided by biotechnology. It would be sad if it takes something the size and complexity (and therefore the cost) of IITA to maintain the capacity of

an *in vitro* multiplication system. Indeed even at IITA, mass propagation *per se* is not actually done.

(iv) **Cross-Border Transfer.** IITA has supplied many African countries with test-tubes containing *in vitro* starting material. Some have been successfully multiplied on and some have not. Certainly Zimbabwe benefitted as our breeding programme started from this kind of tissue culture material. It is a very useful service and has the benefit that it can be repeated over and over with the latest lines or to make up for losses. It is a test of the National Agricultural Research Programmes whether or not they have the stability and infrastructure to successfully guide these delicate tissues through customs, through phytosanitary control, into the lab, through variety trials, and out into the field beyond the experimental station.

3.3 Immunological Benefits

When CIAT published data that some of their lines had proven to be embryogenic in the style of Stamp and Henshaw(11), it opened the door to the idea that genetic capacity, having been carefully identified could be bred into some of our African germplasm. To do that it was necessary to transfer those lines from Colombia to Nigeria and beyond. The only safe way to do it without risking the concomitant transfer of South American frogskin virus to Africa, was to send "virus-eliminated" clones in tissue culture to Harrison in Dundee so that he could virus-index the clean stock and, if passed as clean, to transfer it on to IITA, again in tissue culture. Harrison's monoclonals can identify ACMV both the African and the Asian strains and also some of the South American viruses.

Incidentally one has to wonder if all this caution is worthwhile when we are all aware that material has been smuggled, particularly by diplomats, from one continent to another. The frailties and arrogance of human nature can ignore and obviate the most rigorous and internationally agreed procedures!

Although we in UZ originally hoped for South American material of this nature and began asking for it in 1984, we still have not received any of those embryonic lines. Thus we are tempted to join the diplomats. Fortunately, we have been able to identify some lines of our own which are also embryogenic(12).

3.4 Current Status Conclusions

That concludes what I could identify that biotechnology has so far done for Africa's cassava production. It is by no means an unqualified success, although a lot of time, resources and funding has been expended. In that light it is perhaps a disappointingly meagre list of accomplishments. What seems clear from many discussions is that it is not the biotechnology that is limiting the progress (that has been well established here at IITA and in a few other African centres), it is the poorly integrated support, the lack of continuity in facilities and trained scientists that disrupt the steady progress in the application of biotech to production. We may need to look at things like standby generators, guaranteed foreign currency supply for a few chemicals and modest equipments, career structure that keeps good workers at the bench, critical mass of technically qualified groups, removal of political interference in job selections, in order to clear the way for genuine progress. To keep good scientists at the bench, one solution is internal promotions for increasing responsibility without the usual musical chairs to other jobs to gain status and salary. Many of these advices need bureaucratic

muscle to implement, so an advocacy programme is essential to sensitise the appropriate civil servants and politicians.

Those of you who really care about the humble hungry people please consider this last paragraph because these are the real causes of lack of progress at least in my experience.

4.1 What are the Future Possible Prospects?

Can biotech, high or low, really make any economically valid contribution to improving cassava production and processing?

4.1.1. Let me first consider the **contribution that hightech molecular biology might make**. For genetic engineering to be effective we need the following to be in place:

(i) It must be possible to **transform** cells of cassava: that means finding a way to introduce foreign genes into the cell's DNA and have it capable of being expressed there. This has been done in callus tissue(13,14) and transient expression has been demonstrated in some epidermal of secondary embryo tissue(15). However,

(ii) Those cells capable of expressing the genes must then be able to be induced to **regenerate** into normal plants. This has so far not proved possible despite several labs trying. This has been the bottleneck for several years at CIAT, in Paris, in St Louis and in Harare, all of whom have tried quite hard.

(iii) Finally there must be **appropriate constructs available**. Some are ready, at least in some labs, e.g.(16) the coat protein gene for African Cassava Mosaic Virus (ACMV) is waiting in the fridge for the above two conditions to be met. Thus the use of gene-transfers awaits an adequate system combining both transformation and regeneration. Should this be solved we could then envisage:

(a) virus resistance based on a coat protein gene (or one of several other styles); this could increase real yield by 20 - 100%;

(b) non-formation of cyanide(17) based on an anti-sense gene in the CN pathway; this could reduce risk of CN-poisoning by a major factor especially in view of increasing reports of skimping in processing due to lack of fuel or morals;

(c) modified protein content both in quality and quantity; this could replace the lost protein in diets that can no longer afford beef or chicken;

(d) bacterial and fungal resistance if these become practical; this would help improve yields and perhaps increase storage times;

(e) production of medicines and vaccines in the tuber: a dream that is coming closer to reality, ie "pharming" drugs we cannot afford to import is the attraction.

Most of these are currently technically possible if the required time and money should be committed. Each is worthy of attempting though opinions differ about which is priority(18).

4.1.2 The use of molecular techniques to assist the plant breeder has recently come to our attention. Initially it was thought that this was a major investment in time and money and therefore of doubtful value, yet techniques have moved forward so rapidly that it now seems valid for every

crop that a molecular marker map be made (ie an RFLP map) so that breeders can correlate it with their traditional maps, and perhaps use it to quickly follow the movement of genes through their crossing programmes(19). Once the map is sophisticated enough it can also be used to seek out the DNA of genes of interest. That technique has been made more economic and worthwhile by the development of RAPDs which allow those who have near isogenic lines for the presence and absence of a particular trait to go fishing for the gene(20). Once a RAPD marker is located "near" the gene, one can try to find the DNA of the gene by either chromosome walking, or transposon tagging(21). These are 4 to 10 year projects, but if the prize is a gene for nematode resistance or, as our lab is interested in Striga resistance, the investment may be worth it.

I am happy to observe that the IITA biotechnology facility, generously funded by the Italian Government, has made a start in this area. I recall a heated debate as part of IITA's strategic planning, about whether it is worthwhile or not to use RFLPs in cassava and cowpea research. The issue was by no means unanimously agreed, yet a couple of years later there are few who consider that the use of RFLPs will be of no help. The addition of molecular techniques therefore to make the life of the breeder less tedious will surely benefit many crops, including cassava.

4.1.3 Finally many African countries suffer from being only primary producers. This renders their economies hostage to fluctuations in world prices. What are the prospects for biotech assisted value-added industrial use of the starch that cassava provides?

In Zimbabwe a Biomass Users Network and Commonwealth Science Council conference was held to discuss this(22). Participants included farmers, parastatal officers, Government researchers, University lecturers, industrialists and so on. We were interested to learn from Thai experiences where a major export industry was built up, exporting at peak about 10m tonnes a year of dried cassava chips for the pig and cattle feed industry of Europe. When the EC chose to erect a major trade barrier to make it prohibitively expensive, thousands of Thai farmers had no market for their cassava. Quickly the scientists and engineers combined to put up a starch factory. This has proved successful, although it is not clear what the overall economics will finally be. The starch can be used for adhesives, for paint, in textiles, and a range of lesser market niches.

This option is being taken seriously by the Zimbabwe Development Company in its planning appraisals(23). A second factory option is to turn starch into ethanol and use it to thin petrol, or indeed to turn it into butanol and use that to thin diesel fuel. For non-oil countries this can be very attractive as an import substitute. Zimbabwe has for the past fifteen years fermented sugar-cane and put 20% ethanol in its petrol. Brazil has made a success of it although we are aware that the fluctuations of world oil prices make it sometimes uneconomic and at other times a saving. For that very reason, to help insulate ourselves from the vagaries of world trade caprices, a continuous supply of tuber-based fuel is an attractive alternative from the aspect of national security. You are all I am sure aware of the havoc the Gulf war caused in countries like Tanzania and indeed Zimbabwe, simply by doubling our oil bills for some months.

What are the economics of fermenting fuel from carbohydrate? According to Greenfield(24) who has done some of the sums, Australia reluctantly dropped the option as they had to include energy (money) for drying the cassava chips prior to processing. If that factor can be taken out of the equation by sun-drying he thinks the proposition would be viable. Again our ZDC are very interested in this, because we are blessed with long months of sunshine where the chips could dry for free and the

fungi would not be a problem. So what is now needed is not applied biotech as such, but venture capital and adventurous farmers who have the vision to see a new cash crop's potential. We estimate we might need something like 100,000 hectares of cassava to support such a factory. Please note that adhesives, ethanol, butanol are all high value products that could be tracked from the factory in the rural area to the big city for processing or use. Cock in 1985 has provided enough figures to whet the appetite of venture capital(24).

Biotech of course currently contributes the bacteria fungi or yeast and the science to optimise the conditions for fermentation - pH, temperatures, oxygen and so on. A further step would be the engineering of over-expressing enzymes into the microorganism concerned to help control and stabilise the production of what you want. About these we are talking to some Swedish friends about, but it is a long way ahead before we could contemplate practical results.

4.2 Where does all this speculation and hopeful talk leave us? Very much still in "darkest Africa"! We would appear to be a long way from a viable and cost effective use of biotech to improve the quality of life in Africa. We are even further from being able to use any form of biotech to ensure a profit while increasing industrial capacity. It is not the useful biotechnology methods that are lacking, it is the infrastructure to use it, and the knowledge of its potential in the right political and industrial circles and therefore the commitment to "go for it". Perhaps we need entrepreneurs with BScs to actualise the potential. You will notice I sign myself both as a University lecturer and company chairman!

5.1 Conclusions

I have in the past promised that, given a busload of scientists and a container-load of equipment, we could generate the critical mass of scientists and skills needed to do great things with applying modern biotechnology to crying needs. I am happy to see that concept taking root in IITA, and am happy to tell you that with our up-and-running MSc at University of Zimbabwe and a clutch of locally funded PhD researchers(25) the bus is half-full in at least two places in Africa and the container is either there or promised. I realise that we cannot live on dreams forever, yet "where there is no vision, the people perish" is a truism experienced every day in Africa. Thus I remain an optimist and look forward to significant biotech-based developments for the production and processing of cassava so that every day rural peasant and urban unemployed can have the dignity of at least food to eat every day.

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CACAO: NEW RESEARCH ADVANCES AND APPLICATIONS OF BIOTECHNOLOGY

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ABSTRACT

Cacao has been cultivated for more than 400 years and this crop still faces numerous problems with diseases, pests and the lack of high yielding clone materials. Cellular genetics and molecular biology could play an important role to complement existing germplasm evaluation and breeding efforts.

Recent progress has been made on the recovery of somatic embryos from non-sexual explants (petals and nucellus tissues). This progress is now opening the door for large scale micropropagation methods for cacao. Superior donor plants could be selected in the field and subjected to a cloning process. In addition, progress has been made to complete germination and plantlet development for cacao somatic embryos. Cacao improvement programs that would rely on transformation methods can now use the new somatic embryogenesis process derived from nucellus or petal tissues and recover intact plants. Reports have also been made on the recovery of shoots derived from axillary buds. This technique can be very useful for multiplying valuable genotypes for clonal orchards or germplasm banks.

Future work on cacao needs to focus on methods for haploid production, embryogenic cell suspensions, protoplast cultures and transformation techniques. At the same time, refinement of the micropropagation methods for non-sexual explants should be completed.

1. INTRODUCTION

A cacao pod has ca. 40 seeds which are utilized to prepared chocolate after defatting, roasting, and grinding. Cacao butter is always in short supply because the chocolate manufacturers use more fat in the product than the original fat composition of the seed (ca. 50%). The ratio of saturated and unsaturated fatty acids is very important for the chocolate industry since it will determine the shelf stability and the consumer acceptance of the final product (more unsaturated fats will melt at lower temperatures). The fatty acid composition is affected by the environment and higher levels of unsaturated fat is produced from cacao plantations growing at lower temperatures. The modern use of the word "cocoa" refers to the drink made from its seeds and the word "cacao" refers to the tree (Ojeke 1982).

Extensive variability exists for cacao trees in the Upper Amazon region of South America. The Maya civilization was growing cacao plants before the 16th Century (arrival of the Conquistadors). With the decline of the Maya and Aztec civilizations, cacao plantations were established throughout South America (Brazil and Ecuador) and Central America to fulfill the demand from European consumers. According with Cuatreases (1964), the genus *Theobroma* has 22 species and *T. cacao* L. is further divided into two sub-species: (a) *T. cacao* subsp. *cacao* (varieties criollo, Amelonado, Trinitario, and Pentagona) and (b) *T. cacao* subsp. *sphaerocarpum* (variety Calabacillo). The variety Criollo is identified by a thin-skinned pod, light-colored seeds and high quality beans. The varieties Trinitario and Amelonado typically have thick-skinned pods with light purple seeds. The variety Calabacillo has small pods, inferior quality purple seeds and it is considered a source of disease resistance (Hunter 1990).

Africa is the leading cacao producing/exporting area (61% of total) followed by South America (28%). This relative Africa position in the cacao market has been fairly similar since 1951. The total cacao production has increased from 0.7 to 1.6 million tons during the period of 1950-80 (Table 1).

Entering the final decade of this century, cacao prices are at another low point owing to current overproduction with relation to demand. Producers in the western hemisphere now face the added problem of increasing cacao production in the Far East. For example, although Malaysia produced only 26,000 metric tons of cocoa beans in 1978-79, production has increased to 255,000 metric tons in 1988 (Hunter 1990). However, Aderman (1989) predicted that cacao prices will rise this year and stabilize above $3500 per metric ton, and Crotty (1986) predicted, based upon a comparison of actual and simulated cocoa prices, that within the next decade, a metric ton of beans could be worth in excess of $4500. If these projections are true, this is the ideal moment to extend or initiate plantings of new trees in anticipation of rising prices.

The tragedy of cacao growing in the western hemisphere today is that, outside of a few varieties, most of which have not been subjected to rigorous testing, little is currently available for farmers in the way of superior planting material. There is data from field trials in such countries as Puerto Rico, Guatemala, and Trinidad of a number of different clones that show promise, but in the overall scheme of things this is presently of minimal value (Hunter 1990).

Several cacao germplasm collections are available in Africa (Ivory Coast), Brazil (CEPEC, Belem), USA (Miami), Puerto Rico, Trinidad (ICGT), and Costa Rica (CATIE, Turrialba), but little effort is being spent on a systematic evaluation/screening of these gene pools for cacao improvement programs. Very limited information is available on field performance and ecological requirements to recommend planting any specific clone at any particular locality (Hunter 1990).

2. CACAO IMPROVEMENT OPPORTUNITIES

Cacao production still has numerous agronomic limitations, and consequently, there is a great deal of attention for improvement programs to focus on agricultural problems. Total butter production, fatty acid composition, flavor, and theobromine content could also be subject to future cacao improvement programs.

Access to high yielding clones (or hybrids) carrying genes for resistance to major diseases and pests are presently the main emphasis for the majority of cacao improvement programs.

Three fungal diseases cause serious limitations for cacao production in the world. The "black pod" is caused by *Phytophtora palmivora* which also can affect stems and leaves. The "Witches Broom", caused by the fungus *Crinipellis perniciosus* stahel (former Marasmius p.), in indigenous of South America (Ecuador, Colombia, Peru, Venezuela, and Brazil). The "monilia disease" has the fungus *Monilia roreri* as the causing agent and it has caused losses of pods in Colombia, Peru, and Venezuela. Other fungus diseases of minor importance include root diseases caused by fungi of the group *Armillaria*, *Rosellenia*, and *Fomes* (Dublin 1984).

The "Swollen Shoot" is the most serious disease for cacao production in Africa and it is caused by a virus transmitted by mealy bugs. This virus disease has been one of the most limiting factors on cacao production. It was first noticed in Nigeria and later in Ghana, Ivory Coast, and Sierra Leone (Opeke 1982). Other viruses of relative importance are "Cacao Mottle Leaf Virus" and "Cacao Necrosis Virus" (Dublin 1984).

The most serious pest for cacao production in West Africa is the "Cacao Mirids" also called "Capsids" or "Jori-jori". Three species of this insect are found in West Africa: (a) the Brown Mirid (*Sahlbergella singularis*), (b) the Black Mirid (*Distantiella theobroma*), and (c) the Cacao Mosquito (*Helopeltis bergrothi*). The mirids attack the pods and young shoots and suck the sap through a feeding puncture (Opeke 1982). Other pests of minor importance include the thrips that attack young leaves and borers that produce holes in the stems (Dublin 1984).

Selection of high yielding clones or parental lines for hybrid seed production are being pursued by many cacao improvement programs. Pod index (no. pods per kg of dry cacao) has been used as a yield selection criterion in Trinidad (Kennedy et al. 1987). TSH clones with yield potential of 2 tons/ha/year and pod index of 8-9 pods/kg dry cacao have been reported in Trinidad (Hunter 1990).

3. GENETICS AND BREEDING

Very little is known about the genetic and heritability of useful characteristics such as yield factors, vigor and disease resistance in cacao. The genetic of sexual incompatibility and some morphological characteristics such as axil spot and bean color are a little better understood.

Axil spot is a red anthocyanin coloration at the junction of the petiole with the main axis. The color intensity varies from petioles that are completely dark red to those with a spot of red at the petiole junction. This axil spot character, used for the detection of haploids (Dublin 1973a), is controlled by two complementary genes (Harland and Frecheville, 1927).

The bean color varies from white to purple and includes various shades of purple. According to Wellensieck (1932), the purple color is due to the action of a dominant allele and white results from the action of a recessive allele. Similarly, both the number and size of seeds have high inheritability (Dublin 1984).

Pound (1932) was the first to discover the existence of incompatibility in cacao. The Trinidad trees tested by Pound were classified based on their setting capacity as self incompatible (SI) and self compatible (SC). Pound found that pollen SC trees is effective on any stigma, whereas pollen from SI trees only caused setting on SC trees. Self incompatible trees have been found in several other countries including Java, Colombia and Ghana. In contradiction with the early findings of Pound, successful crosses between two SI trees have been reported by several authors. Muntzing (1947) successfully crosses two SI trees in Ecuador and Posnette (1945a), working with a small population of Amazon trees introduced from the upper Amazon, found all tested trees were SI but cross compatible to some extent.

Cacao provides an unique example of incompatibility, where the diploid tissue of the style does not prevent fertilization. All incompatibility

reactions take place in the embryo sac. The incompatibility mechanism in cacao is based on the genetic control of the success or failure of syngamy (Knight and Rogers, 1953 1955; Cope 1962).

The emergence of SC types from SI individuals of the original cacao population may have occurred somewhere in the lower Amazon. All material collected in and near the origin of cacao is SI. The vast population of trees in West Africa, which provides the bulk of the world cacao production, is uniformly SC and is reputed to have initiated from a small population of cacao collected in the low Amazon region.

Genetic improvement of cultivated cacao is mainly based upon exploitation of the heterosis that occurs in hybrids between upper Amazon types and the Amelonado or Trinitario genotypes. Hybrid vigor was first reported in cultivated cacao many years ago. In Indonesia, the Djati Roenggo hybrids, which were famous for vigor and production during the early 1900s were actually derived from spontaneous hybrids of a Forastero type introduced from Venezuela and the local Java Criollo.

The cacao trees from the upper Amazon have greater vigor than existing varieties and offer unique sources of disease resistance. The improved early vigor, ease of establishment, and precocity are so great that hybrids with Amazon types are being used increasingly in most cacao-growing countries. Since several authors have demonstrated the existence of hybrid vigor in cacao, the genetic improvement of this plant has been essentially based on the utilization of group heterosis, which occurs when the Amazon parent is combined with an Amelonado or Trinitario parent. These Amazonian hybrids have several advantages over local cultivars (vigor, earliness, disease resistance) but are highly heterogeneous. This heterogeneity of hybrids is a direct consequence of the heterozygosity of the upper Amazon parent obtaining a homozygous, self-fertile Amazon parent should permit elimination of this heterogeneity and lead to homogeneous hybrids of greater vigor. Hence, a great deal of emphasis has recently been placed on the production of haploid plants for cacao improvement.

The first known haploid seedlings of cacao ($n = x = 10$) were obtained by Dublin (1972). These first haploid cacao trees were obtained following the dissection of embryos from polyembryonic seeds. The ploidy levels were verified by counting chromosomes of young leaves. Haploid plantlets have also been obtained by germinating flat beans under controlled environmental conditions. Under ordinary conditions, these beans have a low germination rate so that the recovery rat of haploid embryos has been very low (Dublin, 1973a). Haploids have also been obtained by screening seedlings derived from monoembryogenic seeds. Haploid seedlings derived from monoembryogenic seeds developed more rapidly and tolerated colchicine treatment better than haploids derived from polyembryos or flat beans.

Homozygous diploid cacao trees derived from haploids develop well vegetatively, produce flowers, and set normal fruits. These homozygous trees, derived from diploidized of haploids (dihaploids), have been used for crossing with Amelonado parents in the genetic improvement of cacao trees in the Ivory Coast.

Interspecific and Intergeneric Hybridization

Many of the wild species of *Theobroma* or *Herrania*, another member of Sterculiaceae, have desirable characters that would be worth transferring into

T. cacao. These include thick pods in *T. bicolor*, resistance to black pod and viral diseases in *T. grandiflora* (Martinson 1966), and high butter fat content (50-60%) in *T. grandiflora*.

Posnette (1945b) was the first to suggest transfer of desirable characters from wild species of *Theobroma* into the cultivated varieties and was the first to work on interspecific hybridization between *T. cacao* and related species.

The results of several interspecific crosses between *T. cacao* and related species of the genus *Theobroma* and *Herrania* have produced very small numbers of fruits. The percentage of flower set is low (Williams 1975), and only a small amount of fruit is obtained (Jacob and Opeke 1971). Interspecific crosses in *T. cacao* generally produce only a few hybrid seeds that are capable of germination. The growth of hybrid seedlings from *T. cacao* x *T. grandiflora* can be improved by grafting the hybrid plant on rootstock of either *T. cacao* or *T. grandiflora* (Martinson 1966).

4. TISSUE CULTURE

To date there have been a limited number of studies on tissue culture of *Theobroma cacao*. In general, all attempts to initiate callus of cacao were successful as callus was rapidly obtained from various organs or explants on a wide range of culture media. On the contrary, all attempts to regenerate plantlets from cacao callus have failed.

Archibald (1954) was the first to investigate tissue culture of cacao. He obtained callus from explants of bark or stem on culture media of Gautheret or White without andy growth regulators.

The second attempt to culture cacao in vitro was reported by Ibanez (1964). Ibanez reported on the action of different sugars (sucrose, dextrose, maltose, lactose, and sorbose) on the respiration rate of cotyledon-free mature cacao embryos under sterile conditions. The best callus growth occurred on MS media supplemented with 11.0 µM IAA, 0.47 µM KIN, and twice the Mwrmal concentration of MS vitamins. A large range of media supplemented with extracts derived from leaves, pod walls, and young seeds were tested in attempts to regenerate plantlets from callus. With the exception of periodic root initiation, no organogenesis was obtained.

Orchard et al. (1979) examined in vitro culture of apical buds of cacao for vegetative propagation. Some growth was observed on both agar and liquid medium but the degree of response varied with the stage of bud development and with hormone treatment. Breakage of dormancy as manifested by bud swelling followed by stipule opening was prompted by both KIN and GA. No intact plants were recovered from cultured dormant buds.

Both Esan (1975) and Pence et al. (1979) were able to obtain somatic embryos in vitro from cultured cotyledon and hypocotyl tissues of very young cacao seed embryos. Esan (1975), in attempts to develop a method for production of cacao plantlets in vitro, used numerous explants including ovules from fruit 6-8 weeks old, immature embryos from 90-day-old fruit, and the embryo axis (axes of cacao bean) from mature unripe pods and anthers. Addition of NAA to the basal culture medium prompted direct somatic embryogenesis rather than an increase of root growth. The ensuing adventive embryos that were spherical or bell-shaped developed through a budding process. Most of these somatic embryos were derived from the hypocotyl portion of the seedling embryo, while some were derived from

the adaxial portion of the cotyledon. The initiation of these adventive embryos was not preceded by callus formation.

Pence *et al.* (1979) initiated tissue cultures of cacao in order to establish the necessary conditions for cacao regeneration *in vitro*. Various explants including leaves, pericarp, ovules, immature embryos, cotyledons from mature embryos, and the axis of mature embryos were cultured on different culture media to determine morphogenetic potential. Callus was obtained with all explants used and on practically all media tested. Immature sexual embryos cultivated in dark or in light on basal medium supplemented with NAA and CW produced adventive embryos, which proliferated by budding from the cotyledon of the immature sexual embryo (Pence *et al.* 1981). When transferred from a solid medium to a liquid medium, these adventive embryos developed roots and primary leaves (Pence *et al.* 1981). Further development and complete normal plantlets were not obtained. In some treatments, up to 80% of the cotyledons of the sexual embryos initiated asexual embryos.

Jalal and Collin (1977, 1979) suggested using callus of cacao to investigate the biosynthesis of polyphenols and flavor compounds in cacao. The polyphenols of the cacao bean have long been regarded as important components of flavor in the roasted fermented cotyledons of cacao. Although some polyphenols were found both in cacao callus and in tissue of the intact plant, most of the polyphenols discovered in the callus were not detected in the plant.

Studies were made to evaluate the *in vitro* synthesis of cocoa butter by cultured somatic embryos (Janick *et al.* 1982). Somatic embryos grown in nutrient medium with increased sucrose concentrations (3-9-15-21-27 and 33%) produced lipids with similar composition of those present in seed embryos.

Subsequent studies on somatic embryogenesis from cultured immature zygotic embryos were reported by Abu-Ampomah *et al.* (1988) and Duhem *et al.* (1989). Direct and indirect somatic embryos were identified and histological sections confirmed the single cell origin of these embryos (Abu-Ampomah *et al.* 1988). The recovery of cacao plantlets was achieved by removal of the cotyledons and increasing the gas exchange inside the culture vessels (Duhem *et al.* 1989).

The first attempts to develop micropropagation methods for cacao were reported by Litz (1986). Axillary buds were induced to proliferate but it was not possible to sustain a rapid proliferation and subsequent growth. In addition, attempts were made to develop a somatic embryogenesis method from non-sexual tissues. Leaf callus of Amelenado trees was induced to differentiate somatic embryos from the globular to late heart stage. It was not possible to stimulate development of cacao somatic embryos beyond this stage. Expansion of the cotyledons was accomplished by gradual necrosis (Litz 1986).

Two successful attempts on culture of cacao axillary buds were recently reported. Flynn *et al.* (1990) described the recovery of some cacao plants in the greenhouse after rooting and hardened off the propagules. Axillary buds were obtained from either orthotropic or plagiotropic shoots of UF-667 or EZX-100 genotypes. The beneficial effect of increased CO_2 (20,000 ppm) in the presence of 150-200 $\mu mol/s/m_2$ of light for promoting *in vitro* growth of axillary shoots of cacao was reported by Figueira *et al.* (1991). Cotyledonary nodes and single-node cuttings from mature plants and shoots were used to induce axillary shoot development.

5. NEW ADVANCES

Until today, a process is not available that would permit the scaling up of individual superior plants from germplasm collection to establish commercial plantations. Considering the existing agronomic limitations for cacao production, DNAP has embarked on a long-term program to develop a method(s) for micropropagation of cacao.

Eleven different explant sources that represent the mother plant (not sexual in origin) were studied in diallelic culture medium design for embryo regeneration. Two types of explants provided some encouraging results: young petals and nucellus.

Petal explants were obtained from cacao clones growing under greenhouse conditions. The best flower bud size was 3-5 mm in length. After sterilization, petals were cultured on callus induction medium for 4 weeks and then subcultured to a regeneration medium. Somatic embryos were isolated after 2-4 months on the regeneration medium. Using more than 9,000 petal explants, a regeneration rate of 4.3% was observed for primary embryos. Some of these embryos were allowed to produce secondary embryos which were then transferred to maturation, germination, and plantlet development/hardening phases (Table 2).

Nucellus explants were excised from young cacao fruits with 7-9 cm in length. After the nucellus was isolated from the seed coat, the portion containing the zygotic embryo was cut and discarded. Twenty nucellus explants were cultured on 100 x 10 mm Petri dishes charged with the primary medium. Primary embryos were visible after 4 weeks of inoculation. The cultures were examined on a weekly basis to check for possible escapes of zygotic embryos due to abnormal positioning. If present, the zygotic embryos would develop quickly giving rise to very large embryos after 2-3 weeks of culture. The embryo regeneration process from nucellus tissues can take place through a direct or indirect pathway. The indirect pathway is characterized by the proliferation of an embryogenic tissue which leads to a large number of embryos. A certain number of nucellus embryos were recultured to produce large numbers of secondary embryos. The regeneration frequency of somatic embryos from the culture of more than 29,000 nucellus explants has been ca. 2.0% (Table 2). The somatic embryos recovered were transferred to Maturation, Germination, and Plantlet Development/Hardening phases to complete the culture process.

Considering a normal flow of the somatic embryogenesis process practiced for petal and nucellus tissues, a total time of 40 weeks are required to transfer plantlets to soil. The protocols currently under development offer great hope for a future application of micropropagation for cacao. This is the first time that embryos and plantlets have been recovered from non-zygotic tissues of cacao which is critical to reproduce the phenotype of the donor plants. The process still need refinements since low efficiency rates are being observed for maturation and germination, and plantlet development. Germination and plantlet development has been a problem in previous studies based on somatic embryos derived from seed embryos. There are conditions and methods that will need to be optimized to allow a large scale utilization of this process for establishing cacao plantations.

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TABLE 1. World production of cacao. Data in 1,000 Mt and relative position in % in parenthesis.

Region	1949/51		1959/61		1969/71		1979/81		Average Annual Growth Rate (1950-80)
Africa	489	(66)	786	(70)	1,100	(73)	999	(61)	2.3
Central America	65	(9)	87	(8)	82	(5)	94	(6)	1.2
South America	182	(25)	225	(20)	282	(19)	461	(28)	3.2
Asia	4		7	(1)	11	(1)	48	(3)	8.7
Oceania	4		12	(1)	31	(2)	34	(2)	7.5
Total	744		1,117		1,506		1,636		2.6

TABLE 2. Somatic embryos and plantlet recovery from petals, and nucellus tissues. Data from explants derived from more than 22 different genotypes.

Culture Phase	Petal tissues		Nucellus tissues	
	Number	% Recovery	Number	% Recovery
Primary explants	9,756	-	29,793	-
Primary embryos	424	4.3	633	2.1
Secondary embryos	2,955	697.0	28,907	4,567
Maturation	1,000	33.8	5,143	17.8
Germination	151	15.1	418	8.1
Plantlet development	48	31.8	88	21.1
Plantlet in soil	15	31.2	23	26.1

STATUS OF COCOA RESEARCH, PRODUCTION AND BIOTECHNOLOGICAL ADVANCES IN NIGERIA

by

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N I G E R I A

ABSTRACT

A brief history of the Cocoa Research Institute of Nigeria (CRIN) and a summary of its objectives, achievements, recent advances and immediate future programmes are presented and discussed in relation to some developments that have affected the Nigerian cocoa industry. Proposals are made to alleviate effects of stalemated situations created through intensive research findings by major consumer developed nations, into industrial cocoa utilization, public consumption habits and to international market forces systems.

Finally suggestions are proffered on how to amend some of the major production, processing and utilization problems posed. A modest justification is presented to support the need for the application of modern biotechnological approaches to cocoa research. Relevant major constraints in Nigeria are also highlighted.

Introduction

Early in the 1940s a Central Cocoa Research Station at Tafo in Ghana then Gold Coast existed. Later this body was elevated to an interterritorial research centre for cocoa and renamed the West African Cocoa Research Institute (WACRI).

The Cocoa Research Institute of Nigeria (CRIN) was a substation of the defunct West African Cocoa Research Institute (WACRI) until 1964 which was founded in 1944 with the main purpose of conducting research into the production of cocoa, a very important export crop and foreign exchange earner in most West African countries particularly Nigeria and Ghana. The headquarters of WACRI was at Tafo in Ghana while its substation was established in 1953 at Moor Plantation, Ibadan, in Nigeria. The main focus of research, apart from other aspects of production, was on the control of the cocoa swollen shoot virus (CSSV), a disease that was causing extensive damage to cocoa trees in the subregion. The germplasm collection in Ghana and Nigeria was basically the same until late in the 1960s.

Since the inception of the Institute, the scope of its research activities has widened considerably and these now cover other export revenue generating corps such as Kola (Cola nitida (Vent) Schott and Endl.) and Cola acuminata (Pal. de Beauv) Schott and Endl.), Coffee (Coffea arabica L. and Coffea canephora Pierre ex Froehner), Tea (Camellia sinensis (L) O. Kuntze) all beverage plants and Cashew (Anacardium occidentale L.) an oil plant.

Cocoa and Kola are priority export, beverage and stimulant crops. They are members of the family sterculianceae and they share a common ecological, soil and climatic requirement. Although cocoa is a relatively fragile plant. The use is limited to localities or regions in Nigeria. Although Kola plays little or no role in world trade, it is of considerable economic importance within Nigeria and between West African countries particularly Nigeria and the Arab Nations. In Nigeria wines and chocolates are produced from both crops. A lot of kola plantation establishment and research is very active.

The main achievement of the kola breeding programme is the improvement of the current low yield from about 250 nuts per tree per annum to over 3,000

nuts per tree per annum and an average potential yield of 40,000 nuts per ha per year. Production problems of Kola include low and erratic yield, protracted juvenile period, rapid deterioration in storage, poor slow and irregular germination, high genetic variability and low caffeine content (3.5%).

Improved technologies have been devised for raising the seedlings and cuttings. The annual world production of Kolanuts (West Africa, mainly Nigeria, and West Indian Islands) stands at 300,000 tonnes. TheSw are exported to Germany, the USA and several Islamic nations particularly Saudi Arabia.

Objectives and functions of the Institute

The objectives and main functions of the Institute include:

(i) the improvement of the genetic potential, agronomic and husbandry practices, including methods of cultivation processing and storage of Cocoa, Kola, Cashew, Coffee and Tea.

(ii) identification of the ecology and methods of control of pests and diseases affecting the crops.

(iii) investigating methods of effective utilization of the products and by-products of the crops.

(iv) the integration of the cultivation of the crops into a farming system

(v) the translation of research results and improved technology into practice in order to improve production and the socio-economic life of the people, and

(vi) development and application of more biotechnological methods to all CRIN schedule crops.

In order to facilitate the attainment of the objectives above research is conducted on an inter-disciplinary crop-based system designed to encourage close cooperation between researchers in all the different scientific disciplines.

For the same purpose, and in order to make our research findings more relevant to the diverse ecological areas in the country and to deal with production problems specific to these areas, six substations of the Institute are located in different parts of the country in addition to the Headquarters at Idi-Ayunre, Ibadan.

These substations are located at Owena in Ondo State, Ochaja in Kogi State, Uhonmora in Edo State, Ikom in Cross River State, Ibeku in Abia State and Kusuku on the Mambilla in Taraba State.

Apart from Mambilla Plateau Substation which is entirely devoted to all the Institute's research work on Coffee (Arabica) and Tea, all the other substations grow the other four crops namely, Cocoa, Cashew, Kola, in addition to Coffee (Robusta).

COCOA PRODUCTION AND QUALITY

Nigeria's cocoa production which between 1940 and 1970 was the leading foreign exchange earner has declined and fluctuated from about 308,000 tonnes in 1970 through a lowest unencouraging level of 80,000 tonnes in 1986/87

season and presently to only about 170,000 tonnes in 1990. Currently Nigeria is the fifth largest cocoa producing country in the world (Table 1). Some of the reasons for this downward trend in production include, among others, the calamity that accompanied the oil boom or "doom"? early in the '70's, when the agricultural sector was relegated to the background. Most government policies and priority programmes have failed to appreciate the fact that the basis of transition from the developing status of our country to an industrialized one depends primarily on the performance of our agriculture most especially the agronomic sector towards food and export crop production.

Accordingly the artificially high exchange rate that made returns from cocoa unattractive and in fact a hopeless venture to young men who would normally have been expected to go into the cocoa production business found salvation in white collar, faster paying jobs and high wages in the oil and oil related industries. Where these failed, some potential cocoa growers resorted to smuggling and more serious nefarious activities.

Table 1: NIGERIA COCOA MARKET REPORT 1991

Production	- 170,000 tons
Hecterage	- 476,000 (2986) (638,000 in 1976)
Export	- 72,000 tons
Grindings	- 20,000
Av. yield (Kg/ha)	- 290
Butter	- 2,100 tons
Powder	- 6,600 tons
Paste	- 10,300 tons
Local Chocolates	- 13,000 tons
% share (Net) of world Export	- 9.02
Hybrid %	- 35 (eq. 160,000 ha)
Cocoa Derived Earning	- 2.33
Av. Farm holding (ha)	- 2.1

Sources:
- Gill & Duffus CMR No. 339, 1991
- Cocoa Growers' Bull., No. 43, 1990
- The Economist Intelligence Unit (EIU) Special Report NO.1185, 1989
- FAO, ICCO, national statistics, 1986

The scrapping of the Nigerian Cocoa Board in 1986 and the opening of the control to open private interest involvement dealt a serious blow on the quality reputation of the country and export again declined (source E.I.U Special Rep. No. 1185, 1989). Other contributory and resultant factors include ageing of existing trees of which more than 80% are over 35 years old, difficult renewal or rehabilitation of the trees and plantation acute shortage of farm labour, old age (average of 60 years) of the farmers, poor and unstable commodity price, expensive and scarce essential imputs.

By 1985 the Cocoa derived earnings of Nigeria as percent of total export earnings was 2.33 compared to Ghana's 58.17, Côte d'Ivoire's 39.02, Cameroon's 24.21 and Malaysia's 1.86 (Source ICCO, 1986).

The most unfortunate policy was the one that failed to link up petroleum revenue with agriculture and in particular cacao.

Considerable shift in the global centres of cocoa production took place between 1970 and 1980. Between 1921 and 1976, Ghana was the main producer but now the Côte d'Ivoire is in the lead (849,000) tons in 1988/89 and 740,000 tons in 1990/91) followed by Brazil (412,000 tons in 1984/85 and 383,000 tons in 1990/91) whereas Ghana now stands a considerable distance behind in only third place (300,000 tons in 1988/89 but 250,000 tons in 1990/91). The production in South east Asia has recorded an impressive upsurge due to increasing and modernized high-tech cultivation in Malaysia which in 1978/79 produced only 26,000 tons but today produces 255,000 tons which represents 29.6 per cent of global production (Gill and Duffus, CMR, No. 339, 1991). By the year 2000 Malaysia would probably be the leading producer nation. This assertion is valid because the pattern of world production is one which is constantly changing; different nations rising to prominence only to be dethroned in no distant date.

The ICCO estimates that in 1987/88 the 50 cocoa producing countries of the world produce a total of 2,167,800 tons of cocoa. Six countries, Côte d'Ivoire, Brazil, Ghana, Malaysia, Nigeria and Cameroon, between them accounted for more than 80 per cent of this total and 94 per cent was produced by the twelve largest producing nations.

Understandably Ghana's dominance throughout its period (65 years) of supremacy together with its early development of and strict adherence to fairly strict quality controls, helped to establish Ghana cocoa as the standard against which all other origins were judged. In 1977/78, however, partially as a result of declining production, Ghana forfeited its position to Côte d'Ivoire, the leading producer today.

Ghana's cocoa is more variable today than it was in the past. The quality of cocoa from Côte d'Ivoire also tends to be rather variable and chocolate manufacturers consider it to be poorer than that from Ghana, Nigeria and Cameroon, mainly due to its high mould and bacterial counts and pesticide residue content.

Nigeria's cocoa quality is accepted to be similar to Ghana's while Cameroon's cocoa is generally used for processing, since it is considered to have a poor flavour but a high butter fat content and a good colour. Brazil's cocoa tends to be more popular in the USA than in Europe but this is due more to historical and geographical linkages between the two countries than to its quality. Malaysia, the only other large producer, sells its cocoa at a considerable discount relative to most other producers because its produce tends to have fairly high acidity levels and the beans are generally smaller than those of other origins, consequently the ratio of nibs to bean weight is lower (E.I.U Special Report, No. 1185, 1989).

COCOA DISEASES

Cocoa is particularly susceptible to a number of diseases and pest attacks. Many of them have been controlled by the timely application of modern fertilizers, fungicides and insecticides, the preparations of which are very expensive and are equally most difficult to obtain. Consequently many plantations are left untreated resulting in collosal production losses. Among the most economically important plant diseases of cocoa in the world and in deed Nigeria today are the pod rot (black pod) caused by Phytophthora palmivora (Butl) and other phytophthora species. These are fungi which attack cocoa pods at all stages of development and can also affect roots, stems and

leaves. The disease occurs most severely in overshaded plantings and is associated with wet weather and humid conditions. It could cause losses and affect up to 70 per cent of the pods. The disease can be controlled by good hygienic management practices, including regular harvesting, removal of infected pods, correct shade management and use of copper based sprays, (Adegbola, 1989).

The other is the swollen shoot virus disease which is spread by mealybugs. It has been extremely destructive in West Africa especially in Ghana, Nigeria and Togo. The Ghana strain of the virus is most virulent. The one in Nigeria is mild. It is an extremely difficult disease to control and the only interim adopted measure is to eradicate all infected trees, this however does not always solve the problem. Work is currently under way to develop trees which demonstrate resistance to this virus (Adegbola, 1989).

COCOA PESTS

Apart from spreading diseases, insect pests can cause considerable damage to cocoa trees and the fruit (pod) in particular. Some 1,500 species of insects are known to feed on cocoa although only a very small number are considered to be serious pests and then usually only in a local area (Idowu, 1989). Probably, however, the pests which cause the most serious crop losses are two species of capside Sahlbergella singularis (brown) and Distantiella theobromae (black) and to a lesser extent termites (Eguagie, 1971 and Idowu, 1989) rodents and birds, while frogs, millipedes and grasshoppers have been established for the nursery (Adenikinju et al., 1989).

COCOA PLANTATION ESTABLISHMENT

Broadly speaking, the costs of producing cocoa can be split into two categories: establishment costs and sustenance or management and post harvest handling costs. Establishment costs tend to be high especially as cocoa takes two to three years to come into production and, depending on the variety grown, a further two to three years to reach productive maturity. The costs of establishment however, depend upon the original condition of the land being planted; it is more convenient to interplant younger cocoa under existing plantation as done during rehabilitation of moribund and uneconomical trees than to have to start by clearing virgin bush (Soyele and Bolaji, 1971). The cash costs involved and the eco-system required vary enormously from locality to locality and indeed from farm to farm, but the labour requirement, which in most cases accounts for at least 60% of the total costs involved in terms of many hours remains virtually the same. During the next three years or so while the cocoa trees are growing the annual input is about 103 man days per ha and at full production approximately 133 man days are required (Soyele and Bolaji, 1971).

Despite all of these production problems, cocoa is an ideal crop for Nigerian small holder farmers, for it can be grown equally well on a small as well as on a large scale. It is relatively easy to establish, maintain, harvest, and process for export. It does not require heavy capital expenditure for establishment through processing on equipment. It has therefore been readily integrated into traditional agricultural systems and although, with the development of the new higher yielding hybrid varieties, more and more plantations are growing cocoa, it is still commonly grown nationwide on small holdings (from units of less than 3 ha). In many areas cocoa competes for land with other crops, especially food crops, although it is common practice on small holdings to intercrop cocoa with food crops during the early stages of development of a cocoa stand under oil palm or plantains in order to provide shade as well as an important second source of income. Robusta coffee, kola sp and some root crops, such as cocoyam (Taro) or cassava

(manihot), have ecological requirements similar to cocoa's and, in areas where pests or diseases have proved to be a serious hinderance to the successful cultivation of cocoa, it is frequently replaced by one of these other cash or food crops.

Status of the world cocoa trade

Cocoa beans now account for about two thirds of world cocoa trade, (Gill and Duffus Market Report No. 340, 1991). This tends towards greater processing of cocoa into semi-finished products for export and has had a marked effect on the structure of the national trade in recent times. In 1946 cocoa beans accounted for over 95 per cent of the volume of the world trade in cocoa, whereas in 1986/87 cocoa beans accounted for only 65 per cent. Consequently, the international cocoa market now has at least two distinct segments, the cocoa bean market and the semi-finished cocoa products market.

Both segments are obviously complementary although to some extent they are, from certain viewpoints, competing markets. It is obvious that depending upon a user's requirements the product market may prove a better source of supply than buying the beans and grinding them. Towards the realignment of the Nigerian cocoa industry in this right direction, the Federal Government of Nigeria, banned the export of raw cocoa and that as of January 1991 only semi processed or processed cocoa will be permitted for exportation. The implication soon became counter- productive for the simple reason that the main existing factories are all operating well below capacity due to a mixture or high finance cost and technical constraints. Consequently, the ban on export of raw cocoa was lifted at the end of November 1991. Meanwhile the Central Bank of Nigeria has provided loans totalling 24 million dollars (USA) for construction of processing plants. The target for the long run is the export of more processed cocoa than the raw cocoa (Gill and Duffus Market Report, No. 335, 1989).

RESEARCH ACHIEVEMENTS

(i) Extension and Liaison Services

In order to arrest the declining trend in our cocoa production, the Institute has carried out a considerable amount of work and has produced cocoa varieties that combine many desirable economic attributes. The Institute's cocoa breeding programme is now in its third phase. Previous breeding programmes have resulted in the release of (a) 15 establishment genotypes referred to as CRIN ELITES. These are used for commercial plantings particularly, in areas considered marginal for cocoa production, (b) the selection of 3 clones that showed tolerance to cocoa swollen shoot virus disease and (c) the selection of 6 clones that showed apparent tolerance to the phytophthora pot rot. Some of these materials are now available to the farmers through the State's Cocoa Development Units (CDU) and Tree Crop Units (TCU) which were established by State Governments for the development of the Cocoa Industry in the cocoa growing states of the country. The Institute has established seed gardens of improved cultivars in various parts of the cocoa belt, these seed gardens are used to increase pod production for distribution to farmers. Also the Institute has articulated methods of assessing good cocoa soils and the most economic fertilizer regimes. For example, nitrogen and phosphorus based fertilizers have been shown to increase yields from 25 to about 50 per cent while the new Amazon cultivars have been shown to respond favourably to Boron fertilizer.

(ii) Protection Programmes

Control of diseases, pests and weeds have been effectively tackled. The Phytophthora pod rot and the cocoa swollen shoot virus (CSSV) diseases, mirids and other insect pests and Chromoleana odorata weed have been identified as the most important of these. At present the most reliable method of control is by pesticide application except for virus diseases. So far seven fungicides, five insecticides and one herbicide have been recommended by the Institute for use in Nigeria. Meanwhile more contact and systemic pesticides are being screened. The best methods and the most effective regimes of application have also been worked out (Are et al., 1971).

(iii) Utilization of products and by-products

The Institute has actively investigated the utilization of by-products obtained from the crops under its mandate. The inclusion of cocoa pod husk up to 17% level in animal feeds has resulted in improved weight gain in pigs and poultry. Also, methods of improving and upgrading soap production from pod husks are being actively investigated. The production of chocolate which is more relatively high temperature tolerant under tropical condition using cocoa beans, kola nuts and cashew is in the process of being perfected. Various types of wines, liquor and grades of alcohols have been produced from juice collected from cocoa fermentation, cashew apple juice and from kola nuts.

(iv) Major cause for concern: Era of Cocoa substitutes

As a direct result of the high prices of cocoa during the late 1970s, manufacturers of chocolate began to incorporate substitutes for cocoa butter and cocoa powder in their products. However, while the use of substitutes has grown it is speculated that their increased future use may be limited, especially as a number of countries now have legislation preventing their use in products labelled chocolate whiles others have strict limits on the extent to which substitutes can be used. Consequently, while it has been estimated that as much as 200,000 tons of cocoa beans per annum are being replaced by substitutes, it is however not expected that this volume will grow substantially. Strictly speaking, three different types of cocoa butter substitutes have be recognized. These are:

(a) Cocoa butter replacers (CBRs). These are fats which are not compatible with cocoa butter.

(b) Cocoa butter equivalents (CBEs). These are fats which have physical and chemical characteristics similar to cocoa butter and are used to replace cocoa butter.

(c) Cocoa butter improvers (CBIs). These are fats which improve the end product in some desired way, such as prolonging the shelf life and raising the melting temperature slightly of chocolates.

Cocoa butter substitutes are being produced by the process of hydrogenation or by fractionation/hydrogenation of the different natural oils being used and generally most are manufactured from a mixture of these natural oils. One of the first oils or fats to be used extensively as a cocoa butter substitute was illipe butter.

It is extracted from the seeds of the shorea tree, which grows wild in Borneo. Another recent one is shea nut butter which is extracted from the

seed of the shorea tree. which is extracted from the fruit of shea tree (Butyrospermum parkii) a savana tree which grows wild throughout West Africa subregion. Several other butter beans, large broad bean often sold dried and used as food, are also being explored. Shorea tree Roxb. ex Gaertn. (Shorea stenoptera Burck.) is a tropical plant and a member of the Dipterocarpaceae.

It has common names like: Borneo tallow, pontianak kernels, illipe and engabang. It occurs in the wild mainly in Indonesia and Malaysia. Its seeds produce the edible butter of high economic value. Over 40,000 tons of the seed is exported annually from Indonesia, Sarawak. Its fat is used like cocoa butter locally for cooking and commercially as a substitute for cocoa butter in chocolate. The butter is 62% saturated, 38% unsaturated fatty acids, 43% stearic acid, 37% oleic acid (Rehm and Espig, 1991).

There are said to be problems with the supply of these seeds, but illipe butter is still widely used today. Coconut oil, palm kernel oil, palm oil and soya bean oil are the most important oils used today in the manufacture of cocoa butter substitutes, although shea nut butter, sal fat and mango seed oil are also used. Coconut oil and palm oil and soya bean oil are generally used in the manufacture of cocoa butter equivalents. Interestingly, a lot of these substitutes are produced in the same countries which also produce cocoa.

Most cocoa powder substitutes available today are based upon carob, which is, in general, being promoted as a healthier alternative to cocoa powder. The promotional literature makes much of the differences between the two products pointing out that car[illegible] unlike cocoa, is free from caffeine and theobromine, is also lower in calories, crude fat and sodium and is richer in natural sugar, fibre and iron. Carob (Leguminosae, Caesalpinioideae) Ceratonia siliqua L., otherwise commonly referred to as St John's bread, locust bean, algarrobo and caroubier, is a subtropical plant with world wide distribution. Its origin is in the Middle East where it is used as an ornamental tree. Main production of the pods is in the Mediterranean area where it is especially important as animal fodder. The juice pressed out ("Kaftan") is used as a syrup. Its kernels provide gum which is traded internationally and utilized in food industry and in pharmaceutical and cosmetic preparations. The seed contains 35 - 45% endosperm (Rehm and Espig, 1991). Carob powder is also mixed with cocoa butter substitutes to make a "chocolate like" confectionery. Again, while their popularity is growing it is believed in some circles that these products account for only a negligible proportion of total confectionery sales. According the popular slogan now is "Eat the real not the likes of chocolate".

PROPOSED SOLUTION TO CACAO "CRISIS" STALEMATE

At present Nigeria and Ghana produce the best cured cocoa into the world market even though they occupy the 5th and 3rd positions respectively on the world production rating. Consequently the question is not how much, rather it is now good is the final product put into the cocoa market.

From the foregoing views expressed above therefore, there is the need for producing countries to either increase their local consumption, produce more of the substitutes at equally comparable cost, preferably through a properly evolved farming systems with cacao so as to reduce and supplement costs of production or to increase cocoa production so as to lower its current price below or to the same level as those of the substitutes. Other alternatives are to evolve and develop technologies needed for the diversification, utilization, incorporation and processing of cacao into more local foods because of its balance nutritional composition. Such cosmetic and fortification processes would thus require a change in the eating habit and tastes of Nigerian especially the younger ones.

At present per caput consumption of chocolate confectionery in Africa tends to be rather low for the obvious reason that special treatment and provisions during manufacture are required in order to preserve its shelf life and stability under very high temperatures of the region. In terms of total volume produced and consumed, Nigeria is one of the very few most important markets in Africa. This is estimated at 13,000 tons per annum. Which ever of these proposed options are given considerations or accepted there would be the need for more and better technological developments which would reduce high costs of labour, chemicals and fertilizers as well as remove or reduce the glaring political dichotomy that exist between producer (production costs) and consumer (free market prices) nations of the world. Furthermore, when cocoa was introduced into Nigeria, the recommendation was that farms should be established on virgin rain forest lands usually without burning.

In other words, the cultivation of cacao provided deforestation or natural forest ecosystem destruction and a replacement by an artificial secondary forest ecosystem.

Indeed, only within recent decades have the tropical rain forests come to be looked upon as a type of crop, to be grown and managed, and where certain inputs are required to guarantee outputs for the future. So abundant were the forests that they have been long regarded as a capital resource freely available for the taking, and disposed in favour of agriculture. Today, with availability of virgin forests running out, cacao establishment is relying more on the rehabilitation of existing old moribund farms.

Knowledge about forest trees and their influence is still meager, compared to that concerning many intensively investigated farm crops. The distribution of forest and its composition indeed within the tropical belt of the world are governed by many interacting ecological factors (lithospheric, biospheric and atmospheric). In a general way temperature and rainfall and latitude altitude are especially important. Today, rehabilitation conditions required for establishing new farms and plantations are very different and they require more inputs. With the slash-and-burn agricultural openings in the tropics the environment is generally less conducive. All the earliest and best cocoa growing areas in Nigeria have become marginal areas for the same crop in less than a century. There is therefore the need for a continuous and concerted global research effort to unravel the over changing interactions between the prevailing conditions and interaction of the climate, the soil and the new hybrid cacaos now being produced by breeders and distributed to farmers for the attainment of excelling yield. This same phenomenon is evidently global. The leading, sensational and celebrated cocoa producing nations of recent past are now being relegated while new ones are proliferating in the South East Asia countries.

THE NEED FOR BIOTECHNOLOGICAL APPLICATION IN THE YIELD AND QUALITY OF COCOA

Cocoa trees reach full productive maturity after roughly six to seven years, having started bearing when the tree is about three years old. Although many of the new hybrid varieties begin bearing earlier and reach maturity sooner than the traditional varieties their production life span are yet to be ascertained. Traditional trees have been known to continue to produce for a number of years, sometimes for as long as 50-60, but in general yields usually start to decline after about 20-25 years, or earlier if the tree has been poorly maintained or has suffered from a combination of severe diseases and pest attacks. Of course yields do depend very much upon the variety grown, the age of the tree and management as well as climatic and environmental factors. Under reasonable conditions traditional varieties grown without fertilizers can yield about 50 kg of cocoa (dry bean) per ha, the amount similarly is dependent very much on the age of the trees, while

some of the new hybrid varieties can yield well in excess of 1,600 kg at full maturity. It has even been reported that some of the new hybrid varieties have produced yields of over 2,000 kg under ideal cultural conditions without the application of fertilizers. Yield variations therefore exist even among hybrid variety populations produced through controlled pollination.

The quality of cocoa is generally judged probably most importantly by its flavour, a complex quality parameter made of more than 200 essences that has defied reproduction, substitutions and biochemical synthesis. Manufacturers and processors have differing preferences as each tries to develop a unique flavour for his products. Flavour is very much dependent on the origin and type, whether bulk cocoa, fine flavour cocoa or the new hybrid varieties, but processing, especially fermenting, and the method of drying and storage can also have a pronounced effect. In particular, manufacturers and processors do not want cocoa which has developed an "off flavour" i.e. mouldy, smoky or bitter. Second, quality is also judged by the number of defects found in a bag of cocoa, such as sticks, stones and other foreign matter and beans that are discoloured, infected by insects (especially weevils), immature and germinated (Soyele, 1971). the third criterion is the physical characteristics of the beans, such as size, butter fat content, shape and the proportion of shell (waste) to nib, etc. All these specifications are so far best attainable through well executed research, standardized technologies and general education but are still suboptimal.

Cocoa Genetic Resources in Nigeria

The main populations of cacao in Nigeria are Amelonado, Trinitario and Upper Amazon types. All introductions of which are from no fewer than sixteen countries. The first eight major countries being: Fernando Po, Ghana, Sri Lanka, Brazil, Ecuador, Wageningen, Trinidad and Surinam at various times between 1874 and 1967. CRIN Breeding Objectives:

> Cacao improvement programmes all over the world are now based mainly on the phenomenon of heterosis inherent in crosses between Upper and Lower Amazon cacaos. In Nigeria, hybrids have been produced through single, three-way, double and Adaptability hybrids from various crosses. The best of these have produced high yielding cultivars and populations from which selections have been made for:
>
> (a) High yield (Pod production and low Pod index)
> (b) Quality
> (c) Establishment abilities (low replanting levels)
> (d) Fast or early maturing (Precocious)
> (e) Disease and pest resistance/tolerance/escapes particularly for phytophthora pod rot
> (f) Rooting abilities
> (g) Adaptability to a broad array of environment
> (h) Agronomically conducive shapes to enhance high density planting and mechanization.

Cacao breeding in Nigeria has had four distinct phases. These are: (i) The First Nigerian Cacao Breeding Programme (ii) WACRI Cacao Breeding Programme (iii) The Second Nigerian Cacao Breeding Programme and (iv) The Third Nigerian Cacao Breeding Programme.

(i) The First Nigerian Breeding Programme spanned the period 1931 - 1956 (Jacob et al., 1971). The main experiments undertaken included selections from local materials in the 1942 and 1945 progeny Trials, Clonal Trial, and Hybrid Vigour trial all at the Institute's headquarters plots, Idi-Ayunre. The main products of these endeavours

were N38, NT (Nigerian Trinidad Hybrid) 39, 114, 164, 215, 216, 310 and 655 as well as the following West African Amelonado selections: HH268, 536 and T38.

(ii) WACRI Breeding programme spanned the period 1938 - 1960 and was executed mainly at Tafo in Ghana. The most significant achievement during this phase was the successful introduction of outstanding Upper Amazon germplasm into West Africa and the development and release of F3 Amazon, a general purpose variety (Ojo et al., 1985). Other important trials under this phase were Series I and Series II which were mainly inter Amazon and local selections respectively. F3 Amazon was found to be superior to Amelonado in establishment, vegetative vigour, yield etc., the Series I were for the most part not superior to F3 Amazon while the Series II Hybrids were even better than F3 Amazon.

(iii) The Second Nigerian Breeding Programme spanned the period 1961 - 1970. The main experiments related to establishment ability, cacao Swollen Shoot Virus (CSSV) tolerance, Phytophthora Pod Rot resistance or escape and dialled crossing programme. The climax of this phase was the development of 15 cultivars which were released as CRIN establishment elites. This phase also witnessed massive introduction of germplasm particularly from Trinidad. About 350 crosses made up of intra-Nanay, intra-Parinari, intra-Iquitos and intra-Pound collections were introduced into Nigeria (Jacob et al., 1971), supplemented by other selections from Ghana, Costa Rica, Fernando Po, Cameroon and Indonesia. Finally, clonal materials were acquired from Kew, Wageningen and Miami.

(iv) The Third Nigerian Breeding Programme originally planned to last from 1971 - 1980 was later deferred (Ojo et al., 1985). Progress on this programme was reported by Ojo and Sanwo (1981). The main achievements are the selection of 10 Nanay and 4 Primary hybrids and the subsequent development and testing of the new CRIN varieties, all from among the Trinidad introduction populations.

Our Cacao breeding programme has to date, relied on exploitation of the variability of earlier cacao selections and introductions. Cacao types used include:

(a) The West African Amelonado derived from introductions made from Islands in the Gulf of Fuinea.

(b) Local 'hybrids' i.e. non-Amelonado cacao collected by Posnetter in Ghana in the late 1930s and early 1940s.

(c) "amazon Cacao". These, together with many other types, were included in the 1944 introduction made by Posnetter from Trinidad: they are designated as "T" clones. Hybridixation within and between these materials gave rise to most of the commercial cacao currently grown by farmers in Nigeria.

(d) 1967 Trinidad introduction made up of hand-pollinated seeds of inter-Nanay, inter-Parimari, inter-Iquitos and inter "P" (Pound's selections).

(e) 'C' clones; these were important breeding materials from the West African Cocoa Research Institute (WACRI) and later included the parents of the series II varieties.

The latter two populations, together with the earlier CRIN selections from Amazon and Trinidad populations formed the core of the breeding

population for the third breeding programme. The characters of interest were yield, establishment ability, quality and resistance to disease. Adaptability Hybrids of Nanay and Parinari crosses:

This trial was an attempt made at simulating WACRI series II hybrid types. The selected Nanay and Parinari single hybrids (Atanda et al., 1975) have been intermated with the West African Amelonado (N38) as to crosses or adaptability hybrids.

Furthermore in an effort to broaden the genetic base of the Nigerian cacao breeding population, extensive screening of available 'C' clones at the Institute's headquarters and Uhonmora substation was done. The resulted in the selection of 21 single crosses (Ojo et al., 1991).

These selections together with their double cross and adaptability hybrid progenies were planted at Ibeku (1983) and at Owena, Uhonmora and Ikom (1984). The materials showed best establishment at Ibeku substation. Both the double and single hybrid progenies showed better precocities compared with the adaptability hybrids (Ojo et al., 1991).

These promising new materials are also being evaluated for other characters including pod value, phytophthora pot rot incidence and Cacao Swollen Shoot Virus (CSSV) tolerance/resistance.

Martinson (1966) reported a successful hybridization between T. cacao and T. grandiflora. High butter-fat contents of some species of Theobroma, especially T grandiflora (60.5%) has been shown by Knight and Rogers (1955). Herrania species contain even higher butter-fat content. Similarly, some of these Theobroma species have very thick and/or hard pods which would be an asset to cacao in resisting infection by the pod rot organisms. The primary goal in making interspecific hybridization between cacao and other wild relatives is the transfer of such desirable characters like thick shell and high better-fat content into various cultivars of Theobroma cacao.

In addition to introducing germplasm from diverse areas for breeding purposes, mutation breeding has been embarked upon as a means of inducing variability in many plant species. Indeed, the use of induced mutation techniques for plant improvement has steadily gained grounds in West Africa in recent years (Opeke and Jacob, 1973).

The determination of correlations between different characteristics of the seedlings and juvinile stages of the crop with the adult forms is meant to facilitate prediction of the future and long term performances of the crops. In cacao, significant correlations have been shown to exist between precocity and potential or future pod production, (Glendinning, (1960), Bartley (1971), and Soria and Esquivel, (168). Similarly, Atanda (1972) proposed the use of cumulative pod production data from 2 - 5 years for determining the potential yield of a cacao cultivar. About 8 - 10 years would therefore be required for evaluation and selection of cultivars. Consequently, selection of cultivars can be made on the basis of their performance in the early years.

IN VITRO STUDIES

The application of tissue culture methods to propagation of cacao was first suggested and explored by Evans, (1951) and later by Archiblad, (1954). since then, there have been reports of various attempts made in more than 30 research laboratories all over the world. Eleven of these laboratories are in producer countries.

These efforts are aimed at supplementing cacao research breeding programmes in all disciplines. These have been reviewed and summarized by Withers, 1984; Pence et al., 1989, Esan, 1992.

During the Third Nigerian Breeding Programme, Ashiru)1969 and 1972) and Jacob (1973) tried to establish some tissue culture work by culturing immature hybrid embryos obtained from normally abortive inter-specific crosses but without success. The pioneering work of Esan (1974 and 1975) reported for the first time, among many others, scientific break-through in cacao somatic embryogenesis, germplasm storage, anthcyanin biosynthesis and culturability of virtually all parts of the cacao plant.

Esan (1975) obtained somatic embryos in vitro from cultured cotyledon and hypocotyl tissues of very young cacao embryos. In his attempt to develop a method for the production of cacao plantlets in vitro, he used numerous explants including ovules from fruit 6-8 weeks old, immature embryos from 90-day-old fruit, and the embryo axis (axes of cacao bean) from mature unripe pods and anthers. The basal medium used for culture of somatic cacao explants was composed of inorganic salts of MS, with WH vitamins and CH. Cacao anthers were cultured on Nitsch medium.

Some growth additives triggered growth responses in specific parts of the embryo. For example, tryptone in a range of 100-1000 mg/l enhanced growth of the cotyledon more than other parts of the embryo. Addition of NAA to the basal culture medium promoted direct somatic embryogenesis rather than an increase of root growth. The ensuring adventive embryos that were spherical or bell-shaped developed through a budding process. Most of these somatic embryos were derived from the hypocotyl portion of the seedling embryo, while some were derived from the adaxial portion of the cotyledon. The initiation of these adventive embryos is not preceded by callus formation. Mutation and germination were not achieved. These impart stimulated greater global enthusiasm and established the much needed trend for a greater tissue culture research through the micropropagation of cacao, the biosynthesis of cacao metabolites and products and in vitro germplasm conservation of cacao (Esan 1975, 1982(a); Withers 1984; Janick 1986).

Micropropagation and in vitro germplasm conservation of cacao has been the main topics of attention at this Institute. Two general techniques have been explored for the micropropagation studies, viz.

(a) the stimulation of apical growth development (terminal and or auxiliary and maintenance of existing developmental integrity including whole embryos and excised embryo axes, and

(b) the initiation of adventitious shoots, roots and somatic embryos (denovo). summaries and highlights of achievements made during the first decade of tissue culture work have been reported by Esan, (1982a).

The utilization of this technique will enhance more collections for longer storage as well as facilitate germplasm conservation and international exchange since virtually all parts of the cacao plant, including somatic embryos produced in vitro, have been cultured and made to grow in vitro (Esan 1975, 1982(b) and 1992 and Pence et al., 1989), except for the roots. With the current rate of progress in plant biotechnology and genetic engineering through the achievement of cacao protoplast isolation, culture, and somatic fusion, tremendous amount of germplasm especially of the wild relatives will be assembled, evaluated and utilized in genetic transformations (Thompson et al., 1987). Such a development will afford new breed of cacao breeders

(conventional and non-conventional) less time to evolve superior genotypes and multiply them rapidly for commercial use.

MAJOR LIMITATIONS

1. Poor information and collaboration network
2. Inadequate manpower, (high and middle level) and infrastructures
3. Poor global collaboration and cooperation among various research groups
4. Poor and irregular funding of travels, training and retraining
5. Non existent or rare forum for interaction and exchange of views and scientific information between colleague scientists and scholars
6. Misplaced priorities and "unfortunate" policies at Government level.
7. Incompatible and divergent priority ratings among scientists working on similar crops.

CONCLUSION

Although cocoa was introduced into Nigeria about a century ago; active research was not embarked upon until about 30 years ago. Much has been achieved during this period in the areas of germplasm introduction, cacao improvement and cocoa production as well as in post harvest primary processing of the produce. However with the advent of biotechnology and its application in cocoa processing, cocoa products development, in vitro propagation and genetic resources conservation, Nigeria has played the role of a pioneer in some aspects and has lagged behind in other areas.

There is the need to develop the plant tissue culture technique and related technologies to perfection such that the plant breeder could evolve superior genotypes, screen and multiply them more easily and rapidly, as well as exploit the genetic attributes inherent in many cocoa wild relatives. Most importantly, there is the need to intensify the commercial production of cacao substitute plants.

Accordingly there is the need to determine and identify microorganisms that could be used to improve the biochemical development of flavour senses of cocoa during fermentation as well as those required for the digestion of pod husk on a large scale so as to make them better consumed by livestock as feeds and also directly by man as food. There is also the need to diversify the products from cacao (the leaves, the fruits and other products of post harvest operations).

Finally cacao is highly susceptible to and heavily colonized by, various populations of microorganisms and epiphytes in succession as it ages, some of these epihytes have valuable economic potentials for use by man and animals. these areas have not been adequately explored.

ACKNOWLEDGEMENT

The author expresses his profound gratitude to the UNIDO for granting the sponsorship to participate and particularly for the recognition accorded. Similar thanks go to the Director, CRIN, Dr S.T. Olatoye for granting the permission to attend and to present this paper, to the Assistant Director, Extension and Planning Dr. G.A. Filani for Library information and finally to Mr E.B. Adeniyi for the typing.

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COFFEE: NEW RESEARCH ADVANCES AND APPLICATIONS OF BIOTECHNOLOGY

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ABSTRACT

Coffee improvement programs can focus on three different areas of application: agronomy, processing industry and consumer benefits. Agronomic characteristics should focus on reducing direct and indirect farming costs. In a modern coffee farm in Brazil, direct costs can reach 63% of the total costs. To reduce coffee farming costs, the new technologies need to address fertilizer efficiency, disease and pest resistance, and crop management aspects that will reduce total labor hours (herbicide resistance, mechanized cultivation and harvesting). The development of coffee varieties with short (3 months), mid (6 months), and long (9 months) maturation cycles would permit a more even distribution of the harvesting and processing activities at the farm level and thus increasing the efficiency of farm labor utilization. The development of a commercial micropropagation process or a true hybrid system in coffee would be highly desirable to counteract frequent disease and pest outbreaks as well as for maximizing the exploitation of agroecological niches and to increase productivity.

Coffee quality depends primarily on the genotype, but is also highly susceptible to environmental conditions. Plantation management, which affects plant microclimate, nutrition level, and seed processing quality has a major impact on the coffee quality. Superior beverage quality is produced from Arabica cultivars grown at higher elevations. Crosses between Arabica and other coffee species confirm that Arabica genes are responsible for superior beverage quality. Storage and postharvest processing also have a major role in determining final coffee quality.

There are several characteristics of coffee that could be altered, resulting in some benefits to the coffee industry and final consumers: increased total soluble solids, larger and more uniform bean size, bean density and texture, uniform maturation, caffeine content, increased levels of compounds responsible for desirable coffee flavor and aroma.

In coffee tissue culture, there have been reports of successful regeneration via somatic embryogenesis of several wild *Coffea* species, five *C. arabica* cultivars and two interspecific hybrids. Recovery of plants via somatic embryogenesis in such a wide range of genotypes demonstrates the potential of using in vitro methods for coffee improvement. Regeneration from embryogenic cell suspensions and isolated protoplasts of Robusta and Arabica genotypes has also been described. Transient transformation utilizing the protoplast uptake method has now been reported. Considering the repeatability of the protoplast regeneration systems available today, the utilization of useful genes for coffee improvement is now a near- to mid-term possibility with a high degree of success. Embryo rescue and anther culture techniques need further developments. Micropropagation on a large scale is now feasible through the use of embryogenic suspensions in Erlenmeyer flasks or bioreactors. Utilization of this technique will make available segregating individual plants to commercial production. Somaclonal variation as a breeding tool for coffee improvement has now been described for Arabica coffee and a few interspecific hybrids.

Stable prices, superior quality and attention to consumer needs will be the most effective long-term strategies for increasing the coffee market.

Biotechnology probably can provide a more efficient way to introduce value-added coffee to the coffee industry. The availability of certain value-added coffee cultivars would open opportunities for market niches for specialty coffee brands. Most agronomic benefits will bring quality and increase net farm profits. Increasing the net return to coffee farmers will contribute to production stability and long-term success of this industry.

1. INTRODUCTION

Coffee is a beverage prepared from seeds (beans) of *Coffea* species after roasting and grinding. The history of coffee can be traced to the 13th Century when it was carried from Ethiopia to Yemen in the Arabian Peninsula. In the 17th Century the beverage became popular in Europe with the opening of the first coffee shops: Venice (1615), France (1644), Vienna (1650), and London (1652).

The genus *Coffea* has ca. 100 species but only two species are of commercial importance. *Coffea arabica* accounts for about 75% of the total green coffee production. This species is an allotetraploid (2n = 44) originated in the Southwest highlands of Ethiopia (Carvalho 1946). Arabica plantations require high altitudes, mild day/night temperatures and defined dry/wet periods. Arabica coffee has preference among consumers due to its superior aroma and flavor. *Coffea canephora* (Robusta coffee) accounts for ca. 25% of total coffee produced. It is a diploid species (2n = 22) with a wide distribution in West and Central Africa. Commercial Robusta plantations began after 1850 in the African Atlantic Coast (Gabon and Angola) and they are typical of lowlands and hot and humid areas. In a few local producing areas, *Coffea liberica*, *C. devewrei*, and *C. racemosa* are also consumed.

Coffee is the most important agricultural commodity generating $10-12 billion dollars per year in the green coffee trade market. It is an important source of hard currency for more than 50 countries located in tropical regions of Latin America, Africa and Asia. In average, approximately 90 million bags (60 kg) are produced per year and the top five producing countries are Brazil (30%), Colombia (14%), Indonesia (6%), Mexico (4.7%), and Ivory Coast (4.3%). Costa Rica is the leading Arabica producing country (1.538 kg/ha/yr) and typical Robusta productivity is Africa and Asia is 600-650 kg/ha/yr (Söndahl 1990).

2. COFFEE IMPROVEMENT OPPORTUNITIES

Coffee research programs can focus on three different areas for improvement: agronomy, processing industry, and final consumers.

(a) Agronomic Characteristics

The net profit of a coffee grower depends on the productivity of the plantation and his direct and indirect costs. In a typical coffee farm in Brazil, direct costs constitute about 63% of the total costs and is equally divided between agrochemicals and labor costs (Medina et al. 1984). To decrease coffee farming costs, coffee improvement programs need to address fertilizer efficiency, disease and pest resistance, and labor utilization. Another potential benefit to coffee farmers is to grow value-added coffee varieties that will address special market needs and permit higher profit margins to the coffee growers. A series of agronomic benefits can be identified for coffee improvement: increase yield, disease resistance, insect resistance, drought tolerance, cold tolerance, resistance to heavy metals, herbicide resistance, early and late maturation cycles, and development of a true hybrid system.

Increased yields can be achieved by new varieties that are more efficient in carbohydrate partitioning between vegetative growth and fruit load or by new cultivars with increase efficiency for nutrient absorption and utilization. Nitrogen and potassium are the two most used elements in a coffee plantation, and thus should be targeted first. Natural resistance to disease and insects would reduce the cost of agrochemical usage. Tolerance to environmental conditions (water deficit, cold, or heavy metals) would improve coffee production in marginal areas and protect farmers against seasonal fluctuations. The use of herbicides would reduce labor costs. Herbicide resistance in coffee should focus on total biodegradable herbicides to minimize soil and underground water residues. Coffee varieties with short (3 months), mid (6 months), and long (9 months) maturation cycles will permit a more even distribution of the harvesting and processing activities at the farm level.

The development of a "true hybrid system" in both Arabica and Robusta coffee would greatly help coffee producing areas. Modern hybrids would have consistent quality and yield. Adoption of diverse hybrids would permit the specificity for different ecological niches. Hybrid system would also permit and efficient method to quickly respond to disease and pest outbreaks whereby a resistant line could be combines with an elite line without great loss in yield.

An alternative to the development of coffee hybrid systems would be the development of efficient and low cost propagation methods for coffee. In this case, single plant selections would be propagated and multiclone plantations could be established. This production strategy can address all the advantages of a hybrid system within a very short period of time and potentially can be a low cost solution. The selection of individual plants well adapted to ecological regions and carrying high levels of tolerance to diseases, pests, and other adverse environmental conditions will maximize yield and stimulate multi-clone use throughout diverse coffee producing areas.

(b) Processing Benefits

There are several characteristics of coffee that could be altered, resulting in some benefits to the coffee industry: increased total soluble solids, larger and more uniform bean size, bean density and texture, uniform maturation, and diverse maturation cycles.

These new traits could be incorporated into existing coffee varieties through breeding or more rapidly by cellular and molecular methods. An increase in soluble solids would provide greater yields for the soluble coffee industry. Alterations of the carbohydrate metabolism in the coffee endosperm leading to the accumulation of a higher fraction of galacto-mannans would accomplish this goal. Uniform bean size is beneficial during roasting. Larger bean size commands a higher price in the whole bean market. Bean density and texture can affect coffee extraction yields. Uniform maturation would minimize the presence of immature beans, which gives an off-flavor to the final product. Diverse maturation cycles would also be beneficial to the coffee processor, since it would decrease the underripe and overripe bean fractions with an overall quality improvement. Another indirect benefit of this approach would be the reduction of the off-flavor "Rio Coffee". Rio Coffee develops due to cherry fermentation mainly after contact after fruit abscission. Different maturation cycles would permit the farmer to harvest (manually or mechanically) at peak maturity with minimal risks of abscission of overripe cherries or the presence of immature beans that negatively affect the beverage quality.

(c) Consumer Benefits

Two aspects that will directly affect coffee consumers are coffee quality and price. Improved aroma and taste are always desirable in the coffee market. It has been proposed that increased levels of sulfur-containing amino acids in the coffee bean will contribute to the enhancement of aroma (Shibamato 1991). Enhancement of reducing sugars and oil content also may improve coffee flavor and aroma. Varying levels of organic acids (high to low) would address different consumer requirements. Coffee flavor is very complex (more than 400 peaks have been identified in gas chromatography) and therefore it will be more difficult to manipulate them. The best approach to ensure premium coffee flavor is to harvest at peak maturity from trees growing in optimum growing conditions (humidity and temperature). Varying caffeine content (high, medium, low and zero) will address some of the consumer needs. Varying caffeine levels would create specialty brands without any extra cost to the processor and without any price or quality penalties to the final consumer.

Coffee quality depend primarily on the genotype, but is also highly susceptible to environmental fluctuations. Plantation management, which determines the microclimate, nutrition level, seed processing methods, and farm storage has a major impact on the final coffee quality. Superior beverage quality is produced from Arabica cultivars grown at higher elevations. Crosses between Arabica and other coffee species confirm that Arabica genes are responsible for superior beverage quality (Carvalho 1988). Postharvest processing and warehouse storage also have a major role in determining final coffee quality.

3. CELLULAR AND MOLECULAR BIOLOGY

(a) Regeneration via Somatic Embryogenesis

Coffee tissue culture was first reported by Staritsky (1970), who described the presence of somatic embryos from orthotopic shoots of *Coffea canephora*. Herman and Hass (1975) reported the development of organoides from *C. arabica* callus cultures. High and low frequency somatic embryogenesis from coffee leaf explants were first reported by Söndahl and Sharp (1977) in *C. arabica* cv. Bourbon. Further studies with mature leaves of *C. canephora*, *C. congensis*, *C. dewevrei* cv. Excelsa, and *C. arabica* cvs. Mundo Novo, Catuai, Laurina and Purpurascens demonstrated the occurrence of the high frequency somatic embryogenesis pathway in a wide range of coffee germplasm (Söndahl and Sharp, 1979).

A histological study of somatic embryogenesis from Arabica coffee leaf explants demonstrated that somatic embryos originated from mesophyll cells (Söndahl et al., 1979b). The development of low-frequency somatic embryos (after 70 days in culture) and high-frequency somatic embryos (after 90-120 days in culture) were further characterized by scanning electron microscopy (Söndahl et al., 1979a). The high frequency embryos developed from distinct friable embryogenic tissue composed of small, rounded, cytoplasmically dense cells measuring about 20 μm in diameter. In contrast, neighboring callus cells appeared as long vacuolated cells about 150 μm in length (Söndahl et al. 1979a).

A novel type of somatic embryogenesis has been proposed by Neuenschwander and Baumann (1991) using a modified version of Söndahl and Sharp (1977) two-step method. Leaf callus is produced on the original "conditioning medium" (MSI) containing 2,4-D (4.5 μM) and kinetin (18.4 μM) and then transferred to a liquid

"induction medium" (MSII) supplemented with half of the original Söndahl and Sharp (1977) plant growth regulator concentrations (0.23 μM NAA and 2.7 μM Kinetin). The somatic embryos produced after 18-24 weeks in this liquid medium displayed a highly synchronized pattern called Self-controlled Somatic Embryogenesis and the resulting embryos germinated at a rate of 94.5% (Neuenschwander and Baumann, 1991). Data from a total population of 185,000 embryos produced by the original protocol (Söndahl and Sharp, 1977) yielded a combined recovery rate of 29% for both maturation and germination phases. The above modified process provides a greater improvement for the conversion rate of coffee somatic embryos and so it could be relevant for the development of bioreactor micropropagation methods.

The histogenesis of callus induction and embryogenesis from *C. canephora* stem explants was described by Nassuth et al. (1980). Parenchymatous cells of the cortex contribute to the formation of callus tissues. Somatic embryo-like structures were present in the callus after 14 days of culture. A histological study of somatic embryo differentiation from leaf discs of Robusta was presented by Pierson et al. (1983). Somatic embryos developed within 90 days from leaf explants of Arabusta, an F_1 hybrid of *C. arabica* x *C. canephora* (4X), cultured on a medium rich in cytokinin and without auxins (Dublin, 1981). Later, Hatanaka et al. (1991) described the effect of cytokinins and auxins on somatic embryogenesis of Robusta leaf cultures. Auxins inhibited embryo formation and 5 μM was the optimum cytokinin (2-ip, 6-benzyladenine, kinetin) concentration. The pattern of embryo development described for Arabusta was similar to the low frequency somatic embryogenetic pathway reported from leaf cultures of *C. arabica* (Söndahl and Sharp, 1977). Somatic embryos have also been induced from egg cells of *C. canephora* (35 days following fertilization) after 90 days of primary culture (Lanaud, 1981).

(b) Cell Suspension Cultures

Coffee suspension cultures were established with the aim of producing aromatic compounds from suspension cells (Townsley, 1974). Cultures were established from friable callus derived from orthotopic shoots of *C. arabica* cv. El Salvador. These suspension cultures were also used for analyzing caffeine and chlorogenic acid content (Buckland and Townsley, 1975) and to compare unsaponifiable lipids in green coffee beans with cell suspensions (Van der Voort and Townsley, 1975). The maximum caffeine content of a 20 to 26 day-old suspension culture was 0.04% on a dry weight basis, which is substantially lower than the 1.2% caffeine content of Arabica beans (Carvalho 1988). The low caffeine content should be interpreted with caution, since caffeine increases as the cell density increases (culture aging). In addition, caffeine is water soluble and diffuses into the liquid medium. In vitro caffeine synthesis from coffee cells was considered relatively high when compared with the synthesis of other secondary plant metabolites (Frischkuecht et al., 1977). Additional studies of caffeine synthesis and biodegradation of purine alkaloids in coffee suspension cultures were made by Frischknecht and Baumann (1980), Baumann and Frischknecht (1982) and Baumann et al. (1983). A recent study on purine alkaloid synthesis from *C. arabica* cell suspension found an increase in the production of caffeine (twofold), theobromine (tenfold), and 7-methylxanthine (twentyfold) when cultures were grown under a light (13 h)/dark (11 h) regime in the presence of adenine (1 mM) and ethylene (5 mM ethephon) (Schulthess et al., 1991). The maximum chlorogenic acid content in *C. arabica* beans ranges from 7.13 to 8.17%. In diploid species, chlorogenic acid ranges is 2 to 70% (*C. salvatrix*) and 10.30% (*C. canephora*) (Carelli et al., 1974). Chlorogenic acid synthesis in *C. arabica* suspension cells is induced by the presence of light (3.25 mM), but it is

completely inhibited by the presence of 1.0 to 10.0 mM ethephon (Schulthess et al., 1991). Cell suspension cultures have been established from embryogenic tissues of *C. arabica* cv. Mundo Novo and plantlets suitable for clonal propagation were recovered (Peña, 1984).

(c) Culture of Embryos, Endosperm, Anthers and Peristem Tissues

Embryos of *C. canephora* x *C. dewevrei* were successfully established by Colonna et al. (1971). Callus from endosperm tissues of *C. arabica* was induced by Keller et al. (1972) with the objective of studying caffeine synthesis. Abundant friable callus from perisperm tissues of *C. arabica* and *C. stenophylla* was induced by Monaco et al. (1977). Perisperm callus proliferated rapidly in the absence of auxin, suggesting that this tissue was not auxin dependent.

Sharp et al. (1973) cultured somatic and haploid tissues of *C. arabica* and obtained callus growth from petioles, leaves and green fruits, proembryo formation from anthers and shoot development (orthotropic shoots). Anthers from several *Coffea* species have been cultured. Friable callus development was observed only with *C. liberica* (Monaco et al., 1977). Anther culture can facilitate the production of homozygous plants (dihaploids) in one single generation, and can provide access to new recombinant forms (gametic array). The availability of dihaploid coffee lines would shorten the time for the development of a "true hybrid seed system" in coffee (4 years vs. 24 years).

(d) Protoplast Culture

Protoplasts have been isolated from leaf-derived friable callus of *C. arabica* cv. Bourbon. Microcolonies were recovered following wall regeneration and cell division in about 30% of the cultures (Söndahl et al., 1980). Small colonies from coffee protoplasts isolated from young leaves have also been reported (Orozco and Schnieder, 1982), but these microcolonies did not survive the first subculture onto semi-solid medium.

Protoplast isolation and embryo development from the Robusta genotype was reported by Schoepke et al. (1987). More recently, protoplast regeneration from coffee embryogenic suspension cultures has been described by several groups (Acuna et al., 1991; Barton et al., 1991; Spiral and Petiard, 1991). Acuna and Peña (1991) described the production of microcolonies from isolated protoplasts after several subcultures on medium containing 0.5 mg/l 2,4-dicholorophenoxy acetic acid (2,4-D), 6-benzladenine (BA) and naphthaleneacetic acid (NAA). Upon transferring protoplast derived calli to hormone-free medium, somatic embryos were recovered which developed normally and germinated. Spiral and Petiard (1991) successfully utilized embryogenic suspensions of Arabica, Robusta, and the interspecific hybrid Arabusta for protoplast isolation and somatic embryo regeneration. Microcolonies were obtained on modified Blaydes medium with 2,4-D, kinetin, and NAA (Blaydes, 1966). Somatic embryos differentiated 8-9 months later on Yasuda medium with BA (Yasuda et al., 1974). It is clear that embryogenic coffee cell suspensions have been essential for coffee protoplast regeneration. The availability of a repeatable protoplast regeneration system for commercial Arabica and Robusta cultivars will be critical for future coffee improvement via somatic hybridization and DNA uptake.

(e) Transformation

Coffee protoplasts were co-cultivated with *Agrobacterium* tumefaciens carrying NPT II and ß-glucuronidase marker genes under control of CaMV 35s promoter. Transient transformation was demonstrated by the GUS histochemical

assay on callus tissues derived from treated protoplasts (Spiral and Petiard, 1991). Protoplasts from Arabica suspension cultures were treated by electroporation in the presence of a plasmid carrying the NPT II marker gene. Callus, embryos, and plantlets were derived from the electroporated protoplasts. Experiments are currently being done to confirm the incorporation of the NPT II marker in the coffee tissues (Barton et al., 1991).

(f) Bioreactor Micropropagation

Initial attempts to develop micropropagation protocols for coffee plants were based on the development of arrested orthotropic buds present at the main axis. Cultures of nodal orthotropic explants of *C. arabica* were reported to regenerate an average of 2.2 plantlets (Custer et al., 1980). In vitro axillary bud development of *C. arabica* cv. Mundo Novo and two interspecific hybrids (*C. canephora* x *C. eugenioides*; *C. congensis* x *C. eugenioides*) produced 4.5 plants per node after 16 weeks in culture (Söndahl, 1982; Söndahl and Nakamura, 1980). Studies on vegetative propagation of Arabusta led to isolation of shoots following the development of arrested buds (Dublin, 1980). A long-term program has been devoted to apply nodal culture method for large scale coffee propagation (Berthouly et al. (1987). It has been reported a yield of 7-9 shoots per cultured node explant of *C. arabica* cv. Catimor every 12-14 weeks. The culture medium consisted of MS salts supplemented with BA (5 μM), indolebutyric acid (0-2.5 μM), citric acid (50-250 mg·litre^{-1}), ascorbic acid (100-300 mg·litre^{-1}) and cysteine (100-300 mg·litre^{-1}). Apical meristems of *C. arabica* have been cultured with the aim of germplasm cryopreservation (Kartha et al., 1981) or as an alternative propagation method (Zok, 1985).

Recent progress has been made for the development of a coffee mass propagation method via somatic embryogenesis in liquid cultures. Embryogenic cell suspensions of Arabica and Robusta coffee were established in Erlenmeyer flasks. Somatic embryogenesis was highly dependent on cell density. High density was inhibitory to growth, but an optimum density of 1.0 g f.w.·litre^{-1} yielded 460,000 somatic embryos per litre after 7 weeks. The projected yield for a 3 litre bioreactor charged with 3 g f.w. embryogenic cells is approximately 600,000 embryos every 2 months of culture (Zamarripa et al. 1991).

Micropropagation of a high-value perennial species like coffee can be an efficient method for propagating individual trees from a segregating population. Micropropagation reduces the time for varietal development and preserves heterozygosity and plasticity in coffee plantations. Micropropagation in coffee must be accomplished via somatic embryogenesis to be competitive in time and cost.

(g) Somaclonal Variation

Somaclonal variation explores the naturally occurring or in vitro-induced variability of somatic cells following plant regeneration (Larkin and Scowcroft, 1981). Most of this variability is due to chromosome alterations, e.g., breakages, translocations, deletions, aneuploidy, polyploidy, gene amplification, transposons, somatic crossing-over and point mutations (Evans and Sharp, 1983). Somaclonal variation is an excellent method for shortening breeding programmes, since it can provide access to genetic variability within existing cultivars (Evans and Sharp, 1986). Somaclones carry few genetic alterations, such that the genetic integrity of the commercial variety is preserved.

Different agronomically important genotypes of coffee are being utilized for a somaclonal variation study including tall stature varieties ('Yellow Bourbon', 'Mundo Novo', and 'Icatu') and short stature varieties ('Red' and 'Yellow Catuai', 'Caturra', 'Catimor', 'Aramosa', and mixed genotypes). Somaclones were derived via high frequency and low frequency somatic embryogenesis pathways (Söndahl et al., 1984; Söndahl and Sharp 1977). Plantlets with 6-8 pairs of leaves were adapted to a field nursery and 6 to 9 months later were transplanted to the field. Control plants of donor genotypes were established at the beginning and end of each somaclone row and as borders. So far, screening for variability is being made at the R_0 generation, during the first harvest. Progenies of different classes of variability are being made for genetic analysis of the most interesting somaclonal mutations.

More than 16,000 somaclones and controls were established in the field. To date, ca. 12,000 (78%) of these coffee somaclones have been screened for variability after 2 to 4 years under field conditions. Any visual deviation from the normal control genotype was tabulated and a total of 1,196 variants (10%) were recorded. Among the several classes of variability observed, the highest frequencies were found for cherry color (red to yellow 42.3%) and tall to compact stature (3.8%). Cherry color is a trait controlled by a semi-dominant gene (Carvalho 1958): deep red (X_cX_c), pale red (X_cx_c), and yellow (x_cx_c). This example illustrates the tendency to detect changes from recessive to a semi-dominant or dominant state. However, traits described as dominant or semi-dominant also shifted to the recessive phenotypic expression, e.g., compact stature (dominant) shifted to tall stature (1.3%), red (semi-dominant) to yellow cherry color (1.2%) and normal green leaves (dominant) to purpuracens leaves (0.3%). One maragogype mutation (Mg - characterized by large beans and leaves) was observed among somaclones derived from 'Yellow Catuai'. One sectorial chimera was observed in one somaclone from 'Catimor'. Another group of somaclones were observed with variability for traits like maturity, susceptibility to ants, susceptibility to *Cercospora*, resistance/susceptibility to leaf rust and bean size.

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ROOT AND TUBER CROPS TISSUE CULTURE RESEARCH ACTIVITY AT IITA

by

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ABSTRACT

At IITA, tissue culture methodologies such as meristem culture, shoot tip and node cutting cultures are routinely used to eliminate virus infection from improved clones of cassava and yam and micropropagation of cassava, yam, sweet potato and cocoyams respectively. Virus-tested improved clones of cassava, yams and sweet potato have been distributed to national programs for evaluation and testing.

The *in vitro* reduced growth storage method is applied to conserve clonal germplasm of root crops. Plantlets can be kept in the same tube for 1 to 2 years. A total of more than two thousand accessions of root crops germplasm are maintained.

Embryo culture technique for the germination of mature embryos of *Manihot* sp. and yams, and immature embryos of cassava are being developed. Somatic embryos matured to cotyledon stage were obtained from several IITA improved clones using *in vitro* young cassava leaf. Some plantlets were regenerated. Anther and unpollinated ovary culture of cassava aiming at producing haploid plants are under investigation.

In vitro microtuber of yam and minitubers obtained from greenhouse grown virus-free yams are alternatives for international distribution of virus-tested clonal materials. However the dormancy and uniform germination of microtubers will have to be investigated.

INTRODUCTION

Root and tuber crops (cassava, yams, sweet potato and cocoyams) are major food sources in Africa. While the tubers are consumed as staples, the leaves except yams are often used as a vegetable which provides protein, vitamins and minerals. According to TAC priority statement, cassava is the most important commodity crop in sub-Saharan Africa.

Root and tuber crops are vegetatively propagated either through stem cuttings or tubers. The seeds of these crops are usually highly heterozyggocity. Often there are shy flowering problems, problems with synchronization of flowering and even problems with the none flowering.

As these crops are vegetatively propagated, the international movement of germplasm material has been restricted by the danger of introducing diseases and pests to non-infected areas. Through its breeding efforts, IITA has over the past years, developed improved clones of cassava, sweet potato and yams resistant to major diseases and pests. Though improved materials were sent to national programs for evaluation in seed form, it was not possible to send clonal materials to the national programs until meristem culture method and virus indexing procedures were developed.

The objectives of tissue culture of root and tuber crops are: to disseminate improved clonal materials to national programs and to conserve clonal germplasm; to evaluate and develop tissue culture procedures to facilitate the

selection/ development of desirable clones; and to transfer technology to the national programs. Cassava is given the highest priority, followed by yams. Minimum resources are devoted to sweet potato and cocoyams.

APPLICATIONS AND RESEARCH ACTIVITIES

The activities of tissue culture of root and tuber crops can be divided into the following three areas: routine activities, research activities, and training and transfer of technology.

1. APPLICATIONS

Over the past several years methodologies for meristem culture, micropropagation and conservation of these crops were developed (Ng and Hahn, 1985; Ng, 1988b). These are now routinely used in our laboratory. However, there might be room for further improvement.

1.1. Disease Elimination:

The procedures for the elimination of virus infections from cassava, yams and sweet potato were developed. A combination of heat treatment of the mother plants for one month followed by meristem culture is effective in eliminating African cassava mosaic virus from cassava and yam mosaic virus from white yam. Whereas meristem culture alone is effective in eliminating sweet potato virus complex from sweet potato. Culture media for meristem culture of cocoyams were also developed. These procedures are routinely used to clean up improved clonal materials for international distribution. A total of 39 clones of cassava, five clones of white yam and
38 clones of sweet potato are free from virus infection and available for distribution.

1.2. Micropropagation and International Distribution

Procedures and media used for the propagation of cassava, yam and sweet potato using single node cuttings were developed and routinely used to propagate virus tested clones. A two-step propagation method was developed for yam multiplication. The single node cuttings were placed on a liquid culture media for one month to induce multiple shoot formation followed by subculturing the node cuttings in solid media for distribution.

The virus-tested clonal materials are distributed to the requesting national programs in plantlet form grown in sterile solid culture media for cassava and sweet potato and plantlets and microtubers in yam. Virus-tested cassava clones have been distributed to 43 countries in Africa to CIAT, sweet potato to over 50 countries throughout the world and yam to 19 countries in Africa and to India and the Fiji Island.

1.3. Germplasm Conservation

A reduced growth storage method is used to conserve the germplasm collections of cassava and *Manihot* sp., yams, sweet potato and cocoyams. This is accomplished by reducing the incubation temperature to 18-22°C in all these crops except sweet potato where a combination of reduced growth media (addition of 3% mannitol to the media) and reduced incubation temperature is used. Under such conditions, the plantlets can be kept for 1 - 2 years before subculturing

takes place. Using this method over 1,500 accessions of yams, 200 of cassava and *Manihot* sp., over 1,000 of sweet potato and 100 of cocoyams are maintained.

2. RESEARCH ACTIVITY

Research activities include embryo/ovule culture, anther and /or unpollinated ovary culture, somatic embryogenesis and *in vitro* tuberization.

2.1. Embryo/Ovule culture

The genetic improvement of cassava aiming at increasing yield, disease and pest resistance involves the germplasm introduction of other *Manihot* sp. and the hybridization between cassava and its related wild species. The ability to germinate seeds from these wild related species plays a vital role in the genetic improvement of cassava. Though in the past years, IITA was able to germinate seeds of these wild related species nevertheless the germination rate was low in some species.

Embryo culture is an important tool which can be used to germinate isolated embryos from those seeds that are difficult to germinate or those that have low germination rate. The immature embryo culture technique is another technique which can rescue hybrids which could not be obtained by conventional methods.

Procedures for the treatment of the mature seeds due to hard seed coat and for the culture of mature embryo of cassava (Ng, 1989), and immature embryos (Ng, unpublished) were developed. Several culture media formulations were used to evaluate the germination rate of both mature embryo and immature embryo of cassava. Murashige ans Skoog's (MS) medium and Gamborg's B_5 (B_5) medium with modifications were used.

The mature embryos germinated easily on all the media tested however the best result was obtained at 1/2 MS medium with 3% sucrose and agar. This medium was used to germinate embryos of several *Manihot* sp.. The immature embryos isolated from open pollinated fruits (three weeks old) were able to germinate on culture media containing coconut water (CW) and the best result so far obtained was on 1/2 MS medium with 15% CW, 5 mg/l IAA, 4% sucrose and 0.7% agar. This medium was used to germinate isolated embryos of a cross between tetraploid and diploid cassava.

Due to disease problem and physiological disorder in some water yam clones that have been used for hybridization, the hybrid seeds could not grow to maturity. Studies on the effects of six different culture media on the growth of mature embryos of two yam species were carried out. Results showed that Nitsch and Nitsch medium gave the highest germination rate (Ngu, Ladeinde and Ng, Unpublished).

2.2. Anther/unpollinated ovary culture

Anther/unpollinated ovary culture for haploid plant production can be used to produce homozygous diploids which could be useful for genetic studies in cassava. Anther culture from a spontaneous tetraploid cassava was used. Protocorm structure and roots were obtained from anthers that were initially cultured on B_5 medium with sucrose, coconut water and gelrite. Callus obtained from this culture medium were transferred to MS medium with sucrose, kinetin, indole acetic acid (IAA) and gerlite or MS medium with sucrose, kinetin, IAA and adenine sulfate and gerlite. Upon transfer to MS medium without growth hormone,

protocorm structure and roots developed (Ng, Unpublished). Haploid plants were obtained from unpollinated ovary culture of onion (Campion and Alloni, 1990). Recently, unpollinated cassava ovaries were also used to explore the possibility to obtain haploid plants from such explant.

2.3. Somatic embryogenesis

Multiplication of cassava using node cuttings has been used by IITA to multiply virus-free materials for distribution. The multiplication rate could be increased if somatic embryogenesis could be induced directly from somatic tissue, followed by the germination of somatic embryos into plantlets. This technique might also provide opportunity for genetic engineering in cassava.

Though somatic embryos and plantlet formation were obtained from Latin American cassava materials (Stamp and Henshaw, 1982 and 1987; Szabados, *et al.*, 1987), work on the African cassava materials was not successful.

Young *in vitro* leaf of eight IITA cassava clones were cultured on MS medium with sucrose and picloram (Ng, 1989). It was found that there were varietal differences in somatic embryo formation in response to the culture media. In general, higher percentage of cultures that produced somatic embryos was obtained in medium containing 10 ppm picloram. Germination of somatic embryos was not obtained. Recently, somatic embryos developed up to green cotyledon stage were obtained from leaf cultures of six IITA cassava clones. Plantlets were obtained in some of these clones.

2.4. In vitro tuberization and minituber production

In vitro microtuber formation can be used as a means for micropropagation in yams. It is an effective way for international distribution of virus tested clonal germplasm. Tuberization in yams was promoted by the increase of sucrose concentration in the culture media and it was found that sucrose at 5% level was optimal in white yam (Ng, 1988a). Microtubers were produced from over 30 accessions of white yam and water yam. Studies aiming to increase the number of microtubers and aerial tuber formation are underway.

Minitubers were successfully produced by transplanting *in vitro* virus- free yam plantlets in sterile soil under screenhouse conditions. The average number of tubers produced per plant ranged from approximately 2 to 3. Mean weight per tuber and tuber weight per plant ranged from 9.51 g to 18.72 g and 17.82 g to 57.09 g respectively.

The tubers were hand carried or mailed to the requesting national programs. They were kept under ambient conditions and after dormancy was broken they were planted directly to the field or seedbed.

Training and Transfer Technology

Training national scientists and technicians is one of our major activities. Over 40 participants coming from Africa, Asia and Latin America were trained in tissue culture of root and tuber crops by IITA.

Technical assistance and information on setting up tissue culture facilities are given to the national programs upon request.

FUTURE PROSPECTS

Routine activities will be continued in all the areas described. Further studies on the culture of immature cassava embryo are in progress. It is aimed at rescuing some interspecific hybrids that may have incompatibility problems. Yam embryo/ovule culture will be continued.

Plant regeneration from callus and anther culture of cassava will be emphasized. *Agrobacterium* mediated transformation in cassava and yams will be initiated. Improvement of somatic embryogenesis and especially on the germination of somatic embryos of cassava is one of the main areas of research.

Further studies on the induction of aerial microtuber formation *in vitro* will lead to a standard procedure for micropropagation and a system for international distribution of virus-tested germplasm. The control of dormancy and uniform germination of microtubers needs to be studied.

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AN OVERVIEW OF IITA'S BIOTECHNOLOGY ACTIVITIES FOR CROP IMPROVEMENT

by

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ABSTRACT

Biotechnology brings new tools, new ideas and new approaches to agricultural research. IITA is exploring the possibilities offered by biotechnology to apply nonconventional technologies for the improvement of its mandated crops such as cassava, yams, plantain, cowpea and maize. One example of an application already in use is tissue culture in disease elimination, micropropagation, international distribution and germplasm conservation.

Areas of research in biotechnology include embryo culture and rescue of hybrids, somatic embryogenesis and plant regeneration, developing biochemical (protein and isozyme) and molecular markers (cDNA and RFLP) as screening tools, phylogenetic studies using RFLP analysis, polyploidization, somaclonal variation, transformation system, haploidization through anther culture, cytogenetic studies and isolation of aneuploids, incorporation/transfer of genes resistant to biotic and abiotic stresses, monoclonal antibodies and cDNA probes for the detection of viruses and their strains, and cryopreservation. Some of these studies are carried out in collaboration with advanced laboratories.

IITA also participates actively in biotechnology networks. Through its collaborative research with advanced laboratories and the training offered to national scientists and technicians, IITA forms a link between the laboratories in the advanced countries and the national agricultural research systems in Africa. Technical assistance in setting up tissue culture facilities is already provided to national agricultural research systems in several African countries.

1. INTRODUCTION

Biotechnology brings new tools, new ideas,and new approaches to agricultural research and it holds promise to help fulfil the increasing need for food production in Africa. A partnership between on-going agricultural research and biotechnology is called for and IITA and other international agricultural research centers are establishing that partnership in certain fields. Several biotechnology research possibilities relate directly to the needs of developing countries, including modifying plants to increase resistance to pests and diseases and to tolerate unfavorable growing conditions, fixing nitrogen more efficiently, providing more protein, preserving germplasm collections, and developing, and multiplying disease-free materials for exchange across national boundaries. Biotechnology can also improve efficiency and facilitate pathogen diagnosis.

Some of the crop improvement objectives for the improvement of IITA commodities cannot be easily realized by means of conventional breeding. Among these are intractable host-plant resistance breeding problems to some diseases and insect pests. New approaches are needed which require the transfer of resistance genes from wild species or even unrelated organisms to improved cultivars. Such intractable problems exist with maize, cowpeas, plantains, cassava and yams. New biotechnological approaches to crop improvement are likely to facilitate more rapid advances in the improvement of plantain/cooking bananas

and yams where seed is difficult to obtain. Scientists at IITA are exploring new possibilities offered by biotechnology in order to apply nonconventional technologies in their breeding work, but they have already been applying biotechnology through IITA's tissue culture laboratory in germplasm conservation and distribution of disease-free *in-vitro* materials.

To consolidate the several on-going but somewhat isolated biotechnological efforts at IITA, a Biotechnology Research Unit has been formed with financial support from the Government of Italy. The new facility focuses on applied biotechnology research to serve all the crop improvement programs at IITA.

2. Production constraints and control strategies

Cassava, yam, and plantain/banana being vegetatively propagated, there are inherent problems with regards to the propagation, conservation and exchange of germplasm. Next to this impediment, other constraints such as the biotic stresses listed below, cannot easily be dealt with by conventional techniques.

2.1. Cassava

The major diseases and pests of cassava are African mosaic virus (ACMV), cassava bacterial blight (CBB), cassava green spider mite (CGM), cassava mealybug (CM) and nematodes (Hahn et al., 1989; Asiedu et al., 1992a). Yield losses due to ACMV infection range from 20 up to 95%. Tuber losses caused by CM and CGM have been estimated at up to 75%. Another problem for the utilization of cassava is that it contains the cyanogenic glucosides linamarin and lotaustralin which upon hydrolysis release hydrocyanic acid (Bokanga, 1992).

Through its breeding efforts, IITA has developed high yielding cassava clones that are resistant to ACMV and CBB, tolerant to CM and CGM, and with low cyanide content (Hahn et al., 1989). However, shy flowering in some cassava clones, particularly those that have desirable characteristics, has limited the choice of parents for genetic recombinations.

2.2. Yam

Disease and pest problems in yams (*D. rotundata* and *D. alata*) are yam mosaic virus, water yam chlorosis, yam storage rot, yam anthracnose, yam tuber beetles and nematodes (Asiedu et al. 1992a).

Shy flowering and the non-synchronization of male and female flowers are bottlenecks in yam improvement (Asiedu et al., 1992b). Thus, the choice of parents for hybridization and recombination of desirable traits is limited. A cytogenetic study on the ploidy levels, tissue culture and genetic engineering will assist in introducing desirable genes into selected plant materials.

2.3. Plantain/banana

African banana and plantain production has recently been affected by the appearance and spread of the virulent black Sigatoka leaf spot disease, which can cause yield losses up to 50%. The black Sigatoka disease is emerging as a Pan-African epidemic and due to the apparent lack of resistance in the African *Musa* genepool, black Sigatoka has become a major threat to food security in the plantain- and banana-growing regions of Africa (Swennen et al., 1992).

The banana bunchy-top virus (BBTV) is confined to relatively small areas in Gabon, Zaire, Rwanda and Burundi(Vuylsteke et al. 1989). Although it causes serious damage in Asia and the Pacific, for reasons unknown, it is not spreading rapidly and has not caused much damage.

The IITA addresses the problem of black Sigatoka through a short-term and a long-term strategy, i.e. distribution of resistant starchy alternatives and plantain/banana breeding respectively. Breeding for resistance to black Sigatoka is fraught with many obstacles specific to the biology of the preferred parthenocarpic *Musa* cultivars. Low clonal multiplication rates, lack of genetic variability, and barriers to sexual hybridization impede genetic improvement. Bananas are indeed one of the few tropical crops that have not yet been bred successfully. Hence the potential of novel, biotechnological approaches to plant improvement may be considerable, although several sophisticated methods still need to be further developed before practical benefits accrue (Vuylsteke and Swennen, 1992).

IITA, in collaboration with INIBAP, has researched and implemented existing *in vitro* culture technologies in order to increase the efficiency of conventional multiplication and breeding procedures, but several bottlenecks remain to be solved before more advanced techniques can be applied to the improvement of *Musa*.

2.4. Cowpea

Cowpea (*Vigna unguiculata* (L.) Walp) is an important food legume crop for the semiarid regions of Africa. It is cultivated as a mixed crop, mostly with sorghum and millet, and forms an important part of the diet of a large part of the African population.

Insect pests, diseases, plant parasitic weeds, drought, and heat are major constraints in cowpea production.

The advanced cowpea lines TVx 3236 and TVx 82D-716, which have high yield potential, also have multiple resistance to anthracnose, bacterial blight, brown blotch, web blight, and scab. It appears that resistance to fungal and bacterial diseases has been well established and that adequate sources of resistance are available within the cowpea germplasm. However, some fungal diseases are known to form new strains. Restriction fragment length polymorphism (RFLP) and/or PCR technologies are potentially useful for determining differences among strains of *Colletotrichum*, for example. Use of RFLPs or PCR technologies on the bacterial DNA could also provide a definitive answer to another unresolved question in phytopathological research on cowpea: whether the *Xanthomonas campestris* pathovars that cause distinct symptoms on cowpea, i.e. bacterial blight, bacterial pustule, and bacterial canker, are distinct species or strains (Singh et al., 1992).

Several improved cowpea lines with resistance to as many as five viruses have been developed.

The control of virus diseases is important for increasing food production in the tropics. In order to devise effective control methods, an understanding of the nature and distribution of virus strains is essential. Unfortunately, resistance is sometimes strain-specific, requiring several gene sources for the development of a suitable cultivar. Awareness of the existence of strains and knowledge of their distribution will facilitate development of breeding and screening strategies.

Most cowpea accessions have been screened for resistance to the major pests. In the case of the legume pod borer (*Maruca testulalis*) and a pod-sucking bug (*Clavigralla tomentosicollis*), it has been difficult to identify useful sources of resistance.

The legume pod borer infests stems, floral buds, flowers, and green pods. The greatest damage is caused to flowers, while the most obvious damage appears on the pods.

Pod-sucking bugs constitute a complex of up to nine different species, with *Clavigralla tomentosicollis* as the most dominant and damaging in most parts of Africa.

Cowpea bruchid is a serious pest of cowpea in storage. Complete infestation often occurs within six months of storage at the farm level. Resistance to cowpea bruchid has been extensively studied at IITA. A moderate level of resistance has been identified in TVu 2027 (Singh 1977). After three months' storage, TVu 2027 showed about 20% infestation while other cowpea varieties showed 66-96% infestation. Similarly, during six months' storage, TVu 2027 showed about 71% infestation while the other varieties were already completely destroyed, indicating the need for better protection for a longer period. This resistance has been incorporated into most advanced breeding lines.

2.5. Parasitic weeds

Striga gesnerioides and *Alectra vogelii* are two major parasitic weeds infesting cowpea. Striga is more common in western Africa and severe losses in yield have often been reported (Aggarwal and Ouedraogo 1989). *Alectra vogelii* has also been reported from western and southern Africa; it causes damage similar to that of striga, with infested cowpea plants suffering serious yield losses.

B301, a cowpea line from Botswana, has shown resistance to a number of striga populations in laboratory tests. The presence of various physiological strains of striga creates an enormous task in breeding for resistance. The use of isozymes and RFLPs is helping with strain identification. RFLPs could also be used to develop an understanding of population genetics in striga and to follow the genetic basis of epidemics.

Drought and heat are major constraints in cowpea production. Most screening methodologies are field based. However, methods for identification of genes for resistance to drought and heat, using RFLPs, have a potential in plant breeding.

2.6. cowpea germplasm and Seed health

There are about 15,000 accessions of cowpea in the germplasm unit at IITA, although some may be duplicates. Because RFLP analysis can characterize the genotype of individuals with high precision, it may be an effective tool for classifying this material.

Another important role for biotechnology is in seed health management. Several fungal and bacterial pathogens are known to be seed-borne. IITA, as well as other institutes in the CGIAR, has as part of its responsibility the movement of both breeders' germplasm and germplasm bank material. There are two ways that biotechnology might help in this respect. Detection of various kinds of

pathogens within a seed lot might be faster and more accurate with biotechnological methods.

An RFLP map of the cowpea genome is expected to help identify specific markers for characters relating to pest resistance and to ultimately aid in the selection of desirable genotypes. Some of the constraints described could be possibly overcome with the help of molecular techniques, including RFLPs and DNA fingerprinting.

3. On-going Projects

3.1. Root and Tuber Crops

3.1.1. Meristem culture and disease elimination

IITA has developed and adopted meristem culture media for cassava, yams, sweet potatoes, and cocoyams. Scientists found the combination of heat treatment of the mother plant followed by meristem culture to be effective in eliminating African cassava mosaic virus (ACMV) from cassava and yam mosaic virus (YMV) from white yam (Ng et al. 1990, 1992). For white yam, where the plants are less tolerant to thermotherapy, chemotherapy in *in vitro* cultures using virazole (a chemical known to reduce viruses) is being explored. Meristem culture followed by virus indexing was effective in eliminating sweet potato virus disease complex. About 100 clones of cassava and its related *Manihot* sp., 500 clones of sweet potatoes, 1,500 clones of yams, and 100 clones of cocoyams have been regenerated from meristem culture.

Plantlets of selected improved clones produced from meristem culture were virus indexed (Thottappilly and Rossel, 1988). Methods for indexing ACMV and YMV are sap inoculation to a sensitive test plant *Nicotiana benthamiana* and Enzyme-linked Immunosorbent Assay (ELISA). Approach graft to *Ipomoea setosa*, complementary grafting to pre-infected clones, ELISA, and Immunosorbent Electron Microscopy (ISEM) were used for sweet potato virus complex detection.

3.1.2. Rapid multiplication and international distribution

Media for rapid multiplication and germplasm preservation of cassava, yams, sweet potatoes, and cocoyams have been developed. A multiplication rate of five-fold can be obtained within four to five weeks using single node cuttings. For yam, *in vitro* microtubers and aerial microtubers were also obtained by increasing the sucrose concentration in the culture media. They can be stored for at least three months, sprouted, and planted directly in soil. Virus-free plantlets in sterile containers with culture media are used for international distribution.

3.1.3. Germplasm conservation

A germplasm conservation method based on reduced growth *in vitro* has been used to maintain germplasm collections of root crops at IITA: 1,000 clones of sweet potatoes, 1,500 clones of yams, 200 clones of cassava and related sp., and 50 clones of cocoyams. Under lower incubation temperature, cassava, yam and cocoyam germplasm can be kept for more than one year when cultured on a normal culture medium. With the addition of 3% mannitol in the culture media and lowering the incubation temperature, sweet potato germplasm can be stored for one to two years.

3.1.4. Biochemical studies on cyanogenesis in cassava

The presence in cassava of cyanogenic glucosides which upon hydrolysis may release hydrocyanic acid (HCN) is a reason for concern for cassava consumers. The biosynthesis of these glucosides, their translocation and accumulation in specific tissues of cassava, particularly in edible root tissues are being studied in collaboration with the Royal Veterinary and Agricultural University of Denmark.

The objective of the study is to provide an understanding of molecular mechanisms for varietal differences in the levels of cyanogenic glucosides in cassava which will lead to the development of new strategies for selecting for low-cyanide clones.

The endogenous enzyme linamarase which hydrolyses cyanogenic glucosides plays an important role in the detoxification of cassava during processing. The wide varietal differences in the levels of linamarase activity observed by IITA scientists in cassava tissues make it possible to improve the crop for this characteristic by conventional breeding methodologies. Molecular biology techniques are needed to establish the biochemical basis of these differences and to develop appropriate screening tools (DNA probes, RFLP markers).

3.2. Plantains and cooking bananas

A plantain/banana tissue culture laboratory was established at the Onne High Rainfall Substation of the IITA in 1983. The tissue culture research and activities performed at that laboratory are fully integrated into the conventional plantain breeding program. A breeding strategy targeting the creation of resistant plantain hybrids was developed at IITA and achieved success within 3 years. The role of tissue culture research and applications in this feat has been considerable. An *in vitro* micropropagation technique for plantains and cooking bananas, developed in the 1980s, is routinely applied to obtain large numbers of plants. Increases in the number of propagules range from 10-30 every two months compared with 6-12 months using conventional field multiplication techniques. Shoot tip culture has been applied successfully in the propagation of over 400 *Musa* germplasm accessions. These materials are also suitable for distribution to national programs through plant quarantine systems because they are considerably lighter and less bulky than conventional propagules, amenable to rapid multiplication if required, and, most importantly, free of non-obscure pathogens.

Embryo culture techniques are applied to enhance seed germination rates. These are further refined by evaluating changes to the culture medium and by the culture of immature embryos. The aim is to improve on the 4-12% germination obtained at present *in vitro* as compared to the 1-2% germination in soil (Vuylsteke and Swennen, 1992).

The occurrence of somaclonal variation (genetic variation among plants regenerated from tissue culture) among *in vitro* propagated plantains has been studied extensively (Vuylsteke et al. 1990). Somaclonal variation is a potential hindrance to the *in vitro* propagation and conservation of germplasm, but has also been highlighted as a potential benefit in terms of creating novel variability for crop improvement such as plant height and disease resistance.

Plant regeneration by somatic embryogenesis in cell suspension cultures has been achieved in *Musa*, but only in wild species (from zygotic embryo explants)

and in a few clones of AA and AAA dessert bananas and ABB cooking bananas (Banerjee *et al.*, 1987; Cronauer-Mitra and Krikorian, 1988; Escalant and Teisson, 1988; Novak *et al.*, 1989). No success has yet been reported in any of the African bananas and plantains.

4. New Initiatives

4.1. Cassava

Wild species of cassava have desirable characteristics for disease and insect resistance and low cyanide levels. Among cultivated species, shy-flowering especially in land-races limits the choice of parents for breeding. Cytogenetic studies of cassava and its wild relatives and embryo/ovule culture will be important new initiatives in cassava breeding (Hahn et al, 1990). Both cultivated cassava and its related wild species all have chromosome number of 2n = 36. IITA scientists have identified six spontaneous sexual tetraploids (2n = 4x = 72) and four spontaneous sexual triploids (2n = 3x = 54) from the diploid interspecific crosses. Some triploids gave over 200 per cent yield increase over normal diploid cassava. Occurrence of natural tetraploids provides new opportunities for polyploidy breeding and genetic studies, making use of different related *Manihot* species. With the recent advances in biotechnology, it would be possible to make use of the tetraploids for anther culture.

By using electrophoretic studies, it is now possible to identify duplicates and differentiate and/or to classify *in vitro* regeneration lines to their characteristics which can be applied for isolation of variants. This may enhance breeding activities (Table 1).

Embryo culture has been used to culture isolated embryos from some *Manihot* species and cassava to enhance the germination rate of the seeds. Media for immature embryo culture of cassava has also been developed.

Somatic embryos matured to cotyledon stage were obtained from young leaves of several IITA cassava clones. Plantlets were obtained in some clones.

4.2. Yams

Many yams are not flowering under natural conditions, and they are polyploids. Therefore, biotechnological and cytogenetic research has a high priority in yam improvement. Future progress will depend in part upon a much better understanding of the nature of polyploidy and flowering in the genus *Dioscorea*. Micropropagation, or the regeneration of plants from callus tissue, is a possible source of variation in the cultivars which are not flowering.

Media and procedures for embryo culture of yams were developed. Microtubers of white yam are produced using inductive media and used for international distribution.

The origin and phylogeny of the Guinea yam as revealed by RFLP analysis of chloroplast and nuclear ribosomal DNA was studied at IITA (Terauchi et al, 1992).

4.3. Plantains/Cooking Bananas

Tissue culture technologies offer solutions to circumvent problems which handicap certain stages in the genetic improvement of plantains and bananas.

The intractable fertilization barriers that hamper the genetic improvement of plantain and banana could be readily surmounted by advanced biotechnological methods, such as recombinant DNA technology and somatic hybridization. However, to benefit from the full potential of such technology, the basic problem of controllable regeneration of plants from single cells or protoplasts must be solved. Several investigators have done research in this direction on *Musa*, mainly aiming at the production of embryogenically competent tissue and the achievement of somatic embryogenesis. In collaboration with the Catholic University of Leuven, Belgium, IITA is conducting research into the regeneration of *Musa* plants through somatic embryogenesis. Embryogenic cell suspension cultures of the widespread cooking banana clone 'Bluggoe' (*Musa* spp., ABB group) were established by culturing meristematic «scalps», taken from proliferating shoot-tip cultures. Plant regeneration proceeded through the developmental pathway of somatic embryogeny, which at all stages showed conspicuous morphological and histological resemblance with zygotic embryogenesis in a wild *Musa* species (Dhed'a et al 1991). Somatic embryos were produced directly from cells in suspension and not via callus. Germinated banana somatic embryos were successfully established in soil.

The relative simplicity of this cell culture protocol may enhance the feasibility of integrating biotechnological approaches in conventional schemes of banana and plantain improvement.

4.3.1. Germplasm exchange and micropropagation

The introduction of valuable genetic resources for the breeding program and the rapid multiplication of selected genotypes is readily achieved using the well established shoot-tip culture technique. Over 300 new *Musa* accessions were introduced, thereby quadrupling the number of accessions held in the IITA collection. These genetic resources were introduced in a joint effort with the Nigerian Plant Quarantine Service and the International Network for the Improvement of Banana and Plantain (INIBAP). At least 33 of these introductions have shown resistance to black Sigatoka. Among these, several AA diploids are useful sources of black Sigatoka resistance in IITA's plantain breeding program. Five black Sigatoka resistant ABB cooking banana cultivars are being rapidly multiplied in vitro and distributed at a rate of 5000 plants annually to Nigerian national programs and farmers as an alternative to the susceptible plantains.

Micropropagation techniques have also been pivotal in the rapid deployment of IITA's plantain breeding program by supplying large numbers of plants of seed-fertile plantain cultivars for use as female parents in breeding schemes. Micropropagation could in the short term play an increasingly important role in the rapid multiplication and distribution of resistant plantain hybrids.

4.3.2. Somaclonal variation

With the increasing use of in vitro culture for plant production, the occurrence of somaclonal variation, that is increased genetic variation among plants regenerated from tissue culture, has been found to be ubiquitous (Scowcroft, 1984). Because in vitro propagation is a frequently used technique for handling *Musa* germplasm in IITA's plantain improvement program, it was necessary to determine the nature and extent of somaclonal variation among a wide range of plantain cultivars.

Factors influencing the incidence of somaclonal variation are investigated in order to identify guidelines for control of in vitro instability.

4.3.3. Embryo culture

In addition to the generally low seed set, hybrid plantain production is further complicated by low seed germination rates. In soil, plantain seeds germinate at a rate of only about 1%.

An improved embryo culture protocol has been developed, whereby germination rates now range from 10-25% (if calculated on the basis of number of embryos cultured). On average, about 700 plantain seeds are handled in vitro each month, resulting in the production of one plantain hybrid per working day.

Techniques of immature embryo rescue are currently under investigation.

4.4. Cowpeas

Among the most destructive insect pests of cowpeas are the *Maruca* pod borer and three pod-sucking bugs. After screening over 8,000 cowpea accessions, no resistance to these pests has been identified in the cultivated *Vigna* but very good sources have been found in wild species, particularly *V. vexillata*. However, it has been impossible to obtain progeny after interspecific crosses. Research is underway by both the scientists of IITA and collaborators in institutes in Italy (The Universita Degli Studi Di Napoli and the Universita Degli Studi Della Tuscia), at Purdue University, and at University of California at Davis to explore the possibility of transferring the resistant gene(s) from the wild species *V. vexillata* to cultivated cowpeas. These studies included wide crosses, cytogenetics, embryo culture, and regeneration system. Other crosses between cowpeas and several wild *Vigna* species (*V. ambacensis*, *V. frutescens*, *V. gracilis*, *V. luteola*, *V. membranace*, *V. nervosa*, *V. oblongifolia*, *V. racemosa*, and *V. reticulata*) have not been successful so far.

In collaboration with the University of Ibadan (Nigeria), it has been possible to rescue hybrid embryos after wide crosses between cowpeas and *V. pubescens*. In another collaborative project with the University of Napoli, Italy, protoplast isolation and multiplication techniques have been developed, somatic fusions have been made, and tolerance of cowpeas to abiotic stresses (e.g. aluminium toxicity and drought) is being studied on protoplasts and free cells.

Initial results on a plant regeneration system have been encouraging. IITA collaborators in Italy and the United States were able to generate roots from calli derived from several explants including leaf discs. They were also able to obtain fast dividing protoplasts which have the capacity to fuse with *Nicotiana* protoplast. Research in this and similar areas is continuing with the prospect of more success in helping to solve the problems caused by insect pests of cowpeas.

Under an agreement with Purdue University, entomological studies are underway to identify bruchid-resistant *Vigna* species and subspecies and make interspecific hybrids, including attempts at embryo rescue of promising crosses. Also, this research involves isolation and characterization of gene products whose genes have potential to confer resistance to insects, focussing mainly on *Maruca*. Candidate genes will include those coding for *Bacillus thuringiensis* protoxins, digestive proteinase inhibitors, and lectins.

4.5. Rice

Some African rice accessions (*Oryza glaberrima*, *O. longistaminata*, and *O. barthii*) have characteristics important for rice improvement in Africa, particularly immunity to the rice yellow mottle virus and resistance to the stem borer *Diopsis* both stressers being specific for Africa. Transferring the desirable traits from African species to the high-yielding *O. sativa* background has not been satisfactory due to insufficient recombination. Approaches through biotechnology will be investigated in collaboration with advanced laboratories.

4.6. Maize

Modern biotechnology is being applied vigorously to maize in both public and private laboratories in the USA. The interest lies especially in mapping of resistance genes to accelerate selection, in transforming maize with the BT gene for resistance to *Lepidopterous* borers, and in transformation with herbicide resistance genes. All of this research is potentially useful to IITA's maize research goals and linkage projects are being developed. An especially African target will be to utilize biotechnology to solve the *Striga* problem on maize in Africa and several approaches are being examined. Linkage projects are being developed with advanced laboratories in the USA and Europe already working with molecular aspects of maize streak virus. The viral coat protein gene transfer approach can be explored for all important African viruses in maize. Research on genetically engineered endophytes (inocuous bacteria that live in maize xylem) is a major activity in the private sector and this approach to delivery of resistance compounds to maize should be considered through joint project development.

Soil-borne fungal pathogens which cause stalk rots, ear rots, and produce aflatoxins have received scant research in the tropics. Their study is difficult because detection methods are inadequate. The development of modern diagnostic/detection methods through biotechnology should accelerate research in pathogen ecology and disease epidemiology. The potential for diagnostics for study of *Striga* biology is to be developed.

In collaboration with the University of California, Davis, USA, IITA has initiated a new project to study "variability in tropical maize downy mildew fungi using molecular markers".

As soon as transformation of maize is possible, there will be much to explore for maize improvement in the tropics of Africa. IITA plans to keep abreast of the rapidly developing aspects of maize biotechnology in the advanced private and public sector laboratories and it will vigorously pursue potential options.

4.7. Diagnostic Research

IITA's Virology Unit will be closely linked with the Biotechnology Unit and will benefit from recombinant DNA techniques. An example is the control of virus diseases by introduction of the viral coat protein gene to plants where resistance is not available. Other developments in biotechnology permit the production of monoclonal antibodies and cDNA probes for the detection of viruses and their strains(Thottappilly and Rossel, 1988). Proper virus diagnosis using monoclonal antibodies can greatly help in the reduction or control of outbreaks of virus diseases. This technology provides a means of meeting the goals of plant disease prevention and control through effective diagnosis.

A laboratory of the U.S. Department of Agriculture (USDA) at Beltsville, Maryland is collaborating with IITA in the production of monoclonal antibodies and cDNA probes for the detection of viruses affecting root and tuber crops. Recently, various strains of viruses affecting IITA's mandated crops were identified. However, their distribution in other countries is not known. This information is crucial for plant breeders.

To help the national programs with the identification of the viruses and strains in their countries, a project proposal was submitted to IDRC (Canada) and approved. The main objective of this project is to produce monoclonal antibodies to detect viruses and their strains in food crops in various African countries. In collaboration with Canada Agriculture, Vancouver, a special workshop was held at IITA in April 1991 and 12 African scientists attended. Because proper identification of viruses is more difficult than for other plant pathogens, many national programs do not have equipment and other facilities to carry out proper virus identification work. Quarantine regulations prohibit them from sending infected plants or plant parts across their borders to IITA, but once monoclonal antibodies are available, the viruses and their strains can be identified within each country. Then, IITA and national breeders can adopt strategies to incorporate virus resistance in plants.

4.8. Germplasm

Germplasm resources are vital to any crop improvement effort and many opportunities exist for identifying novel genetic traits in wild species and landraces. Tissue culture techniques offer solutions to the problem of root crops germplasm conservation and germplasm exchange. Tissue culture allows the transfer of plant materials in a disease-free condition, especially in the case of vegetatively propagated root and tuber crops and plantains/bananas for use in improvement programs worldwide. Cryopreservation will allow the long-term conservation of germplasm particularly for asexual species difficult to maintain by seed. Cryopreservation of virus-indexed genotypes will also help preserve rare genotypes, control genetic erosion, and increase the genetic variability available to breeders through international exchange of healthy materials. Recently, embryogenic cell suspensions of a cooking banana were cryopreserved and plants successfully regenerated therefrom (Panis et al. 1990) at the Catholic University of Leuven in Belgium. Regenerated plants are screened for clonal uniformity at IITA. It is envisaged to transfer this technology to IITA in the near future.

5. Linkage with National Programs

IITA's collaborative research with advanced laboratories gives the Institute the benefit of direct access to the latest biotechnology developments. In turn, it will be able to be more effective in training national scientists and MSc. and PhD. students. The new Biotechnology Unit forms a link between laboratories in the advanced countries and the national agricultural institutes in Africa. This unit will develop strategies to increase the efficiency of breeding programs by finding solutions to problems which cannot be solved by conventional methods.

To strengthen and to broaden collaboration with the national programs and to identify biotechnologically researchable areas, a meeting of African scientists working on cassava, yams and plantain/cooking bananas was held at IITA from August 8-9, 1988; 25 participants represented 14 institutes in 7 African countries. An agreement was reached on a set of recommendations which would guide

IITA in developing research applications of biotechnology. A similar biotechnology meeting was held in February 9-10, 1989 at IITA to establish priorities in cowpea biotechnology research. A conference entitled "Biotechnology: enhancing research on tropical crops" was held at IITA from 26-30 November, 1990 and over 130 participants attended the conference. An African Plant Biotechnology Network (APBNET) was initiated with IITA as its secretariat. Also, this conference identified the constraints in various crops which could be handled by biotechnology.

6. Further needs for the development of biotechnology in the improvement of IITA's mandated crops

An appraisal of the current status of biotechnology in tropical crop improvement makes it clear that only the "lower" technology of plant tissue culture has made some impact yet. The application of the more advanced techniques, listed below, could have great potential in solving specific intractabilities that hamper optimal use of the existing *Musa*, cowpea, maize, yam and cassava germplasm. The further development of these methods and their adaptation to IITA's crops of concern, which often are recalcitrant with regards to both conventional and non-conventional improvement, will require a serious collaborative research effort. For certain research items a *de facto* collaboration has already been established between IITA and other institutes. It is also not necessary for IITA to be engaged in each subject since other institutes involved with research in these crops may have a greater comparative advantage than IITA to carry out particular research subjects. It is envisaged that relevant developments in other parts of the world will also benefit IITA through participation in networking activities.

6.1. Recombinant DNA technology

Transformation of cassava, yam, maize, cowpea and plantain by genetic engineering techniques is worth considering as a potential route for the production of pest and disease-resistant materials.

In the case of cassava, the transfer of a storage protein to fortify the tubers with more and better protein seems to be a researchable issue. A transformation system for the concerned crops still needs to be established. In view of recent progress with *Agrobacterium*-mediated gene transfer to monocots, this is a course meriting due attention. Yam, a monocot, has recently been shown to be amenable to infection by *Agrobacterium* (Schafer *et al.*, 1987).

For *Musa*, no cloned genes are presently available which may directly confer host resistance to fungal disease for any host-pathogen system (particularly for black Sigatoka and *Fusarium* wilt). There are however a number of genes available associated with expression of resistance which could be used experimentally in attempts to confer fungal resistance (Murfett and Clarke, 1987).

INIBAP and IBPGR have recently stressed that an elegant test case would be to control BBTV by transformation of *Musa* with the viral coat protein. Expertise for this approach is available in Australia (Queensland University of Technology). Furthermore, plant regeneration *in vitro* from transgenic *Musa* cells seems a genuine possibility in the near future. Much research has already been performed in Europe on somatic embryogenesis in cell suspensions and callus (Catholic University of Leuven, Belgium; University of Paris-Sud and IRFA/CIRAD, France; FAO/IAEA laboratories, Austria), although more research is warranted in

order to achieve controllable plant regeneration in a wide range of important cultivars.

6.2. Haploidy

In view of the high degree of heterozygosity in cassava and *Musa*, the production of homozygous breeding lines through haploidy would be of use in genetic improvement programmes and would be a tool in the study of the genetics of disease resistance.

6.3. *In vitro* selection for useful mutants

Screening in the field for useful somaclonal variants (e.g., with disease resistance) is not feasible. However, screening at the cellular level and with a selection pressure applied *in vitro* is a more practical approach. In the case of *Musa*, research into cell culture has progressed significantly in the past two years and pathologists are well on their way to purify extracts of the fungus *Mycosphaerella* that could be tested as a screening agent.

6.4. Cryopreservation

Cryopreservation for the long term storage of genetic resources of tropical crops is considered priority research by IBPGR. Such research has been/is supported by the latter at K.U. Leuven (Belgium) and CATIE (Costa Rica) for *Musa* and is progressing at CIAT, Colombia for cassava.

A feasible cryopreservation methodology for the crops of concern to IITA would benefit IITA and national research institutes involved in germplasm conservation.

6.5. Molecular methods to detect genetic diversity

The use of molecular techniques for detecting genetic diversity has raised considerable interest because of the sensitivity of these techniques.

Restriction fragment length polymorphisms (RFLPs), Random Amplified Polymorphic DNA (RAPDs) and, to a lesser extent, isozymes have shown their appropriateness as genetic markers for plant systematic studies and for clonal identification.

IITA has contacted the USDA/ARS laboratory at the Regional Plant Introduction Station, Griffin, Georgia to initiate research on these advanced techniques aimed at the enhancement of the efficiency of plantain breeding schemes. For cowpea, IITA is collaborating with University of Minnesota, USA. For yam, IITA is collaborating with Prof. G. Kahl, University of Frankfurt, Germany to study amplified fragment length polymorphisms.

6.6. Cytogenetics

Only limited cytogenetic studies have so far been carried out on the crops treated in this paper. Because of their heterozygosity and the multigenic nature of many important traits (disease resistance), more profound studies would help in the formulation of efficient breeding strategies.

6.7. Virus diagnostic techniques

Recent developments in biotechnology permit detection of viruses by means of highly specific assays such as serological techniques using monoclonal antibodies. Another highly promising technique is based on nucleic acid hybridization. These modern techniques provide highly efficient tools for the diagnosis of viruses and viroids. The ultimate benefit to be drawn from these activities is a faster, safer and more efficient transfer of improved germplasm in vegetative form. Monoclonal antibodies used in ELISA or dot-blot immunoassays, and the so-called Western blot methods are practical biotechnology innovations for improved diagnosis of virus diseases.

Hybridoma technology provides methods for producing monoclonal antibody preparations of molecular homogeneity. Monoclonal antibodies have been useful in differentiation of strains, quantification of viral antigens, and detection and diagnosis of virus infection. The ability to distinguish minor antigenic differences with monoclonal antibodies has made them useful probes to differentiate strains of a virus. Access to them would be very useful to IITA in order to identify quickly and reliably several of the viruses and their variants or strains, that infect mandate crops.

Detection techniques based on the use of cDNA are often more sensitive than serological methods and their use by IITA virologists will materially increase the capacity to detect virus infected plants. The cDNA probes, and especially non-radioactive ones, are bound to become more popular. A "broad-spectrum" probe could be developed for quarantine purposes and "narrow-spectrum" probes for epidemiological studies.

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BIOTECHNOLOGY IN SUB-SAHARAN AFRICA
Creating and utilizing endogenous technological capability

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ABSTRACT

African countries are experiencing ecological and economic problems that are disrupting the socio-economic and political systems. While the region's population grows at about 3%, food production is growing at 1.8%. The level of malnutrition has risen in the region. The productivity of the industrial sector is marginal to sustain the economies. The region's debt is increasing while the ability to service debt is increasingly declining. These economic changes are leading to irreversible socio-economic, political and ecological problems; the loss of biological diversity, the loss of soil fertility, and deforestation cause economic problems, at least in the long-run.

While African countries are seeing these unfortunate changes, most other regions of the world are enjoying relatively positive economic change. The economic progress of the industrialized world and newly industrializing countries (NICs) is to a large measure a result of the introduction of new technological knowledge and, new forms of institutions to manage the evolution of technologies. The emergence of biotechnology will, at least in the long-run, enhance the ability of these countries to achieve higher economic growth and sustain their economic systems. For Africa, the biotechnology revolution may not positively contribute to economic renewal unless countries in the region build a critical minimum level of technological capability in those biotechnological techniques that may be applied to increase food production, conserve the ecological base, improve health status of the population and increase the region's industrial productivity. The basic premise of the paper, therefore, is that the ability of African countries to renew their economies largely depends on the level of technological capability they build in the biotechnology regime.

1. INTRODUCTION

In the last ten years biotechnology has been the subject of intense debate among those involved in development issues in the Third World. Two positions seem to have emerged in the debate: optimists and pessimists. The optimists view biotechnology as the panacea for Third World problems. They stress that the technology will deal with health, food and ecological problems. To them the potential benefits from biotechnology outweigh its risks. On the other end, pessimists stress more on the risks associated with the technology: the release of genetically engineered microorganisms, the loss of Third World germplasm to the North, increasing use of unenvironmentally-sound herbicides, and the dislocation of Third World traditional exports from international trade. They would like to see the biotechnology revolution stopped.

The two positions express legitimate concerns, however, it is not addressed how Third World countries will either maximize the benefits and reduce the risks or even do away with technology, if that were possible. What is important, at least to us, is how the Third World will draw maximum benefits and reduce the risks from biotechnology. Biotechnology, though still at its infancy, is a two-edge sword. It is therefore important that Third World countries put in

place measures that will enable them to survive the revolution.

Our basic premise is that extent to which African countries will be able to successfully apply modern biotechnological techniques in various sectors of their economies largely depends on the level of technological capability built within their socio-economic systems. This assertion is built on the view, though neoclassical but agreeable, that the range of biotechnological techniques suitable for dealing with the ecological, health and food problems in the regMwn is wide enough. The ability of African countries to apply these techniques, particularly those that are non-proprietary, largely depends on their capability to search, select, acquire and assimilate them.

In the next section of the paper we present an overview of the African economic situation and argue that the economic crisis is a result of the failure of African countries to create institutional space and formulate long-term policies to enhance endogenous technical change. In the third section we examine the role of biotechnology in sustainable development in Africa. We particularly focus on the application of the technology to food production and processing. The technology, we argue, offers new opportunities for producing and processing cassava which is widely acknowledged as one of the main famine crops in most African countries. The fourth section defines the concept of technological capability and suggests particular areas where African countries need to build biotechnological capability. In the last section we examine some other policy issues vital for the development of biotechnology in Africa.

2. TECHNOLOGY IN AFRICA'S ECONOMIC LIFE: A RESIDUAL?

When most African countries attained political independence in the early to mid 1960s they had quite high socio-economic expectations. On their development agenda were issues such as eradicating diseases, increasing per capita food availability, increasing incomes of the majority of the population, increasing volumes of their exports so as to increase their economic competitiveness internationally and providing education for their populations. To achieve these goals, African development planners and political leaders viewed industrialization as the panacea. They invested considerable resources in building infrastructure in urban areas. Industrial development activities were then concentrated in the urban areas. The rural areas where more than 75% of the population reside and provide most of the raw materials for industrial activities and food for the urban populations were neglected in terms of industrialization.

The newly independent African governments invested considerable energy and financial resources in building political structures. However, this was not well planned. It was, and it is still, thought that nuge political structures would enable them to socially and economically progress. While the political structures have increasingly become huge and delicate, the socio-economic structures are falling apart in most African countries. The political structures are now too huge for the economies. African economies are now in a crisis. The region is as poor today as it was 30 years ago.

In the late 1960s and early 1970s most African countries experienced relatively high Gross Domestic Product (GDP) growth rates. On the average, the GDP of most African countries was about 4.69% per year between 1965 and 1970. The per capita incomes grew by an estimated rate of 2.9% per year 1965-1970. On the whole, Africa experienced a relatively stable economic life.

However, in the early and mid 1970s African countries started experiencing frequent and long recessions in their economies. The GDP grew at an average rate of 3.45% between 1973 and 1980, and 1.96% between 1980 and 1987. Since then, the region has continued to experience deep economic recessions. While food production growth rate has declined to less than 2.5% per year in the last twenty years, the human population has grown at a rate of 3% and over. Industrial development has only marginally contributed to the socio-economic lives of the majority of the rural population.

On the whole, most African economies are characterized by a falling agricultural production, declining industrial growth, increasing population, falling per capita incomes and per capita food availability, rising unemployment levels, increasing ecological degradation, declining exports, rising debt and declining capacity to service debt. The consequences of these problems are the increasing social and political unrest seen in most African countries. The following is a World Bank's appraisal of Africa's socio-economic situation:

> The crisis is taking a heavy toll in human terms. In several countries expenditure on social services is sharply down, school enrollments are falling, nutrition is worsening, and infant mortality continues to be high. Open unemployment in the towns, especially of educated youth, is also on the rise. And, threatening Africa's long-term productive capacity, population pressure on the land is accelerating decertification and deforestation; fuelwood is increasingly scarce, and soil fertility is being leached away, although none of these trends has been accurately measured. Last, institutional decay is symbolized by the poor conditions of the once world-class universities, the disintegration of paved highways, and the collapse of the judicial and banking systems. Over-staffed and poorly managed public bureaucracies are deadweights on the productive sectors. Many governments are wrecked by corruption and are increasingly unable to command the confidence of the population at large. In many places political instability, coup d'etats, and ethnic strife are exacting a terrible toll on the helpless people the costs of destabilization in Southern Africa have been enormous.

The last decade has seen significant growth in the corpus of literature explaining the African situation. Two positions have emerged in explaining the causes of Africa's deteriorating socio- economic life. They oscillate between internalists and externalists. The internalists view the crisis as a result of inappropriate domestic macroeconomic policies and unfavorable political systems. On the other hand, the externalists blame the region's problems on the past colonial legacy, recent geopolitical changes in Europe, intellectual property protection regimes in the North as a bottleneck to technology transfer to Africa, and unfavorable international market conditions.

Most African countries are now locked in intense debate on political and economic reforms. There are those who advocate for multiparty political systems as the most suitable for long-term socio-economic evolution. Multiparty systems, it is argued, will restore democracy and lead to economic renewal. On the other end, there are those who argue that African socio-economic systems are still delicate and naive for multiparty political systems.

We do not intend to engage in the debate. What is important to us is that the debate has misplaced one of the most crucial issues for sustainable development: technical change. We can not divorce science and technology issues from the political agenda:

If a developing country is to achieve economic development at a rapid pace it must give economic development top priority in its policy formulation. To realize this goal the government of a developing country must equip itself with a capacity for modern administration and have a willingness to pursue positive policy measures. Therefore, while there can be no doubt about their importance for economic growth, science and technology in themselves will contribute little to economic development where the will of a government or a people to pursue the economic development through the utilization of new science and technology is absent. This is why the problems of science and technology in developing countries must be considered in light of the economic and political structures of these countries.

We are not technology determinists. There is considerable empirical evidence to show that those political and economic systems that are, arguably, stable have achieved that stability through technical change.

The urgent problem in Africa is how to get the economic systems re-established. What is therefore crucial at this moment in Africa is how to get most of the local population engage in suitable technological and entrepreneurial innovations. This is, however, only possible if there is suitable institutional space for technological and socio-economic systems to co-evolve.

It is surprising that African countries have not learnt from the newly industrializing countries (NICs), South Korea for example. It is technical change through suitable institutional and policy innovations that has led to the increasingly high levels of industrial growth in these countries. African countries, at the periphery, continue to treat technical change as an exogenous activity of their socio-economic development. Though the need to engage in different technological innovations is acknowledged in development plans of several African countries, their economic policies and institutions offer little space for technical change. Most of these policies are neoclassical in approach and treat technology as an exogenous variable of political and economic development:

> Traditional theories have led African countries to formulate policies which do not take into consideration the main sources of economic change: genetic resources, technological innovation and institutional reform. The policies in practice all over the continent are too static to accommodate the imperatives of rapid economic change. It is important for these countries to initiate programmes on how to internalize science and technology into the planning process. So far, science and technology are considered as sectors of the academic or educational establishment and exogenous to the process of economic change Conventional approaches to institutional organization, have left Africa with institutional structures that are incapable of responding to scientific and technological imperatives.

The common practice in Africa is to appeal, through international fora, for technology transfer from the North. This is done without much understanding of the nature of technologies being sort. While we do not deny the fact that the transfer of technology is essential for economic renewal in Africa we argue that mere transfer of technology without endogenous technological capability within Africa's socio-economic systems will not save the situation: renew African economies. Other developing regions (India, Korea among others in Asia) of the world are increasingly becoming, to some extent, economically independent, while

Africa continues to depend on the North for economic aid and technology transfer:a politically vulnerable situation. This is happening at a time when there has been significant changes in the academic and political conceptualization of the process of development.

The late 1970s saw the demise of neoclassical economics. The decade also saw the fall of the dependency school, the rise of the newly industrializing countries (NICs) and the emergence of a new paradigm (neo-Schumpterian) that treats technical change as an endogenous activity of the processes of socio-economic evolution. Lall who has written on technological capability in the developing countries observed:

> A number of dependent economies have demonstrated an ability to break out of the constricting circle predicted by dependency theorists. The interesting problems to investigate are those concerning why some LDCs are able to integrate themselves into a dynamic capitalist trade system and others are not. A blanket concept of dependence applied to all LDCs is quite misleading.

Africa, with the largest number of LDCs, has failed to integrate itself into the dynamic, interdependent, and quite complex world economic system. The dynamics of the real world call for interdependence not dependence. It is survival for the fitting: those that can adapt and contribute to the system survive, while those that are not able to take responsibility and integrate themselves into the system find their own niche at the periphery.

Our point of departure in this section is that African countries should build, instead of just adding to, science and technology in their development planning. We advocate for science and technology policies that:

A articulate the urgent or short-term needs of the small-scale agricultural producers in the rural areas, and the poor in the urban slums;

B take into account the roles and capability of women in national development;

C ensure that the natural resource base on which economic growth depends is carefully managed;

D are flexible enough to give space and time for long-term planning so that the needs of future generations are not jeopardized;

E lead to some level of technological and economic independence; and perhaps more important,

F that can be implemented by the particular country depending on its human and natural resources.

The formulation and implementation of such science and technology policies require some level of endogenous technological capability, economic resources and political will.

3. BIOTECHNOLOGY AND SUSTAINABLE DEVELOPMENT IN AFRICA

3.1 Biotechnology and the sustainable development agenda

The World Commission on Environment and Development (WCED) headed by the Prime Minister for Norway Ms. Gro Harlem Brundtland defined sustainable development as "development that meets the needs of the present without compromising the ability of future generations to meet their own needs". The concept of sustainable development, which has become popular in both political and academic circles, recognizes the importance of technical change and environmental management in long-term socio-economic progress. Sustainable development is essentially an evolutionary learning process that involves different policy and institutional innovations different with changes in space (social, political, ecological and economic) and time.

The recommendations of the WCED pose a number of challenges to both developing and industrialized countries. These challenges may be summarized as: how do countries, individually and collectively, 'developed' or developing reform their existing development policies so as to deal with local and global ecological and economic changes; how do countries, particularly the developing ones, select and apply the existing and emerging technologies in meeting the needs of the majority of the poor populations and enhancing natural resource management; and what institutional rearrangements and policy reforms are needed at economic, social and political levels. These are indeed formidable challenges for African countries.

The emergence of biotechnology offers African countries with new opportunities for sustainable development. Biotechnology is herein defined as the application of biological techniques to produce goods and/or services from living organisms or parts of these organisms. The technology, it is recognized by a wide body of literature, offers developing countries with new opportunities for increasing their food production, dealing with the health and ecological problems. The WCED observed:

> Biotechnology will have major implications for the environment. The products of genetic engineering could dramatically improve human and animal health. Researchers are finding new drugs, new therapies, and new ways of controlling disease vectors. Energy derived from plants could increasingly substitute for the non-renewable fossil fuels. New high-yield crop varieties and those resistant to unfavorable weather conditions and pests could revolutionize agriculture Biotechnology could also yield cleaner and more efficient alternatives to many wasteful processes and polluting products. New techniques to treat solid and liquid wastes could solve the pressing problem of hazardous waste disposal.

Recent studies have shown that several African countries are engaged in biotechnology research activities, particularly in agriculture. However, most of these activities are driven by the interests of individual researchers and those of the donor agencies supporting the particular activities. Juma and Makau (1991) note that in Kenya "developments in biotechnology have been research-driven and not much has been done to understand the demand side of biotechnology. In this regard, contributions of biotechnology to meeting the needs of the majority of the population will be limited." The situation is not different from other African countries. Most of the biotechnology R&D activities in Zimbabwe, Zambia and Tanzania are being conducted without adequate extension to the local

populations of these countries. Consequently, the researchers and research institutes are getting more alienated from their local environment. Cooper observed this a similar situation almost twenty years ago: "The scientific communities in the underdeveloped countries are outposts of advanced country science, with very limited links with the economic and social realities which surround them".

Agricultural biotechnology R&D activities in some African countries

Country/Institution	Activity
Zimbabwe	
University of Zimbabwe	Tissue culture to develop disease free coffee, potatoes and tomatoes.
	DNA probe to test for *Salmonella* in foods and water.
Kenya	
University of Nairobi plantlets.	Tissue culture in *Amarathus* to produce disease free and drought resistant
	Tissue culture in citrus and strawberry.
	Microbiological nitrogen fixation for leguminous crops.
Kenya Agricultural Research Institute (KARI)	Tissue culture for rapid multiplication of potatoes and maintaining disease free stocks.
	Specific DNA probe for diagnosis of heartwater disease.
International Centre of Insect Physiology and Ecology (ICIPE)	Application of microbial technologies for biological pest and vector control
Tanzania	
University of Dar-salaam	Identification of rhizobia strains for developing innoculants through fermentation
Burundi	
University of Burundi	In vitro propagation of cassava to conserve germplasm and produce disease-free plantlets.

Source: *African Centre for Technology Studies databank, Nairobi.*

While most of these activities may relate to the needs of the populations of the countries, as we have pointed out above, they have been instituted as research programmes without much understanding of the local socio-ecological framework and extension systems have not being developed to get these activities into economic application by the population.

On the whole, the current biotechnology R&D activities in most Africa countries may not enhance sustainable development. These activities are being conducted without adequate organizational changes in the extension systems and the research framework. Most of them are in fact evolving as part of old research activities of the Green Revolution. They, therefore, lack specified institutional guidance to allow their maturation.

3.2 Applying biotechnology to industrial processing of cassava

3.2.1 Overview on the uses of cassava

Cassava (*Manihot esculenta*), commonly referred to as the "poor man crop" or "famine crop" is perhaps the fourth most important staple crop grown in Africa. It is a shrub normally growing to a height 1-5 meters. It produces starch tubers at the base of the stem. The crop grows in relatively low humid areas:semi-arid. It requires altitude up to 2000m and temperature ranging from 15-40 degrees celsius. Cassava can grow on relatively poor soils compared to maize and beans (pulses).

The crop accounts for one third or more of the total staple food produced in Africa. It is grown mainly for subsistence purposes although in some countries such as Zimbabwe there is some commercial production.

Nutritional composition of cassava roots

Content	% of total
Water	65-70
Starch	20-30
Sugars/fats	5
Protein	1-2
Fibre	1-2
Ash	0.5-1

Source:Bruinsma, D. *et. al.* (1983)

Cassava tubers are a source of starch. Fresh tubers are eaten when cooked or are dried and made into flour that is used to prepare porridge, cakes and dough. Its leaves are used as vegetable and as such its a vital source of protein for the poor rural households. Cassava is also a source of animal fodder. Other products of cassava starch include glues, adhesives, pharmaceutical starch, laundry starch and dusting agents.

Cassava is an important export crop in the world market. European countries (The Netherlands, France, Belgium among others) import over 3.0 million tones of cassava chips and pellets for use in the animal industry. Japan, Canada and USA import starch for various industrial uses. The most important exporting countries are Indonesia, Brazil and Thailand. African countries' production is consumed locally.

3.2.2 Constraints in cassava production in Africa

- decreasing soil fertility;
- poor marketing facilities and relatively low and unstable prices;
- limited technological options for cassava processing in the rural areas;
- increasing post harvest deterioration;
- decreasing production due to attack by pests and viral diseases;
- high levels of cyanide leading to health problems;

Because of these problems and the fact that there is little research being conducted in Africa to improve production and processing, cassava is being relegated to a lower status even in some of the major growing regions. In international research activities on crops, cassava ranks among the last in priority.

3.3.3 New technological opportunities for sustainable production and processing of cassava

The potential for enhancing the production and processing of cassava seems vast with the emergence of new biological techniques: tissue culture and genetic engineering among others. Biotechnology offers opportunities for improving the nutritional value, increasing pest and disease resistance, and enhancing stress resistance of cassava. Research being conducted in several institutions in the USA, Europe and Asia indicate that the range of new technological opportunities for dealing with constraints faced in cassava production and processing is wide.

At the Plant Genetic Systems in Belgium research is going on to develop cassava strains resistant to insect pests by inserting Bacillus thuringiensis toxin genes in the crop. Research is underway at the University of Newcastle's Department of Genetics to develop cassava varieties that have relatively low levels of cyanide through genetic engineering. This is done by increasing the levels of cellular linamarase so as to reduce the glucoside in the tubers.

Researchers at the Chiang Mai University in Thailand have developed ways of producing starch from cassava through microbial techniques. Starch is then used through strain selection techniques to improve yeast strains for food yeast and yeast autolysate. At the King's Mongkut Institute of Technology, Thonburi and the Thailand Institute of Scientific and Technological Research *S. Cerevisiae 281* is used in the fermentation of cassava peels to produce animal feed with relatively high digestibility.

Several of the International Agricultural Research Centres (IARCs) are conducting research on the application of plant biotechnology for cassava production. These involve;

- the application of anther/microspore culture to achieve homozygosity to express recessive traits;

- the application of Electrophoresis in germplasm characterization *in situ* and *ex situ* ;

- regeneration through embryogenesis from leaf cell culture;

- elimination of viral and bacterial diseases through thermotherapy;

- insertion of synthetic genes to increase selected amino-acid production;

- insertion of viral coat protein to confer virus resistance.

At the University of Burundi's Department of Crop Science research is being conducted on the multiplication of cassava plantlets through *in vitro* propagation. Similar work is also going at the Department of Crop Science of the University of Zimbabwe.

Other areas where the application of biotechnology could improve cassava processing include: the production of dextrose from starch through microbial techniques (fermentation) involving *Aspergillus* spp. Fermentation using *Aspergillus flavus* offer opportunities for baking bread from cassava. Moreover, biotechnology could be used in the production of enzymes such as Alpha amalyse, Amyloglucose and Bactase that are often used in food processing. Currently some of these enzymes are imported into Africa. These activities illustrate areas where modern biotechnological techniques could be applied in the production and processing cassava in Africa.

3.3 Constraints on the current biotechnology research activities in Africa

(a) Inadequate manpower

Although several African countries are engaged in biotechnology research their capability to develop and get biotechnology products into the economic domain is limited by, among other factors, lack of sufficient manpower in core areas such as microbiology, genetics, engineering and biochemistry. Most African institutions involved in biotechnology R&D are understaffed. According to Juma (1990), less than 8% of African scientists in agricultural institutions in 10 countries have doctoral training. Robertson (1991) estimates that there are only 400 African biotechnologists and technicians scattered in over 60 countries.

(b) Low R&D funding

Most of the current biotechnology R&D activities in Africa are supported by a small number of donor agencies. Though no compiled statistical data on R&D funding in biotechnology was available to us, the level of funding is considerably low compared to the estimated institutional requirements for effective research in biotechnology. The level of government R&D expenditure compared to the Gross Domestic Product (GDP) is relatively low in most African countries. For instance, Kenya's R&D expenditure on agriculture between 1985 and 1986 averaged only 0.57% of the GDP compared to an average of 9.65% of the total government expenditure, a relatively high portion of the GDP, spent on military services during the same period. On the whole, science and technology R&D activities in Africa are underfunded.

(c) Shortage of equipment; poor infrastructure

Research institutions in most African countries are poorly equipped. In Kenya, one of the few African countries that spends, at least, an average 0.5% of its total GDP on agricultural research, a commission from the National Council for Science and Technology (NCST) and the International Service for National Agricultural Research (ISNAR) found about 80% (9 out of 11) of laboratories and administrative offices of the national research stations inadequately equipped.

In Tanzania, statistics from the UNESCO and the Tanzania Agricultural Research Organization (TARO) indicate that more than 85% of the research facilities are underequipped. The level of capability in terms of equipment for

R&D is likely to lower for most African countries particularly with the structural adjustment policies that have led to reduction in government expenditure.

In terms of general infrastructure, research and extension activities in most African countries are limited by the poor infrastructure. Roads in most countries are underdeveloped. For instance, it may take two days for an extension team in Tanzania to travel a distance of 500 kilometers by a relatively strong car. Other infrastructural facilities such as supply of electricity to laboratories are poor in most African countries:sporadic interruptions in electricity supply affect research work.

(d) Paucity of institutional articulation

Most of the current biotechnology R&D activities in Africa are scattered in public institutions with little or virtually no national and regional coordination. In some countries it is likely that there is significant duplication of research efforts. Public institutions undertaking biotechnology work hardly interact with their counterparts either within the same country or in the region. This means that there is no formal or informal sharing of experiences in different research activities.

In most instances, Juma (1991) notes, biotechnology research activities in the public institutions are initiated and developed by "efforts of individual institutions and people involved in running certain programmes an indication that the projects are not part of a well co-ordinated national programme but rest on the determination of a few individuals or institutions. The long-term government support and commitment that usually accompanies the development of new technologies is lacking in the region."

As mentioned above, most research institutions in Africa are divorced from the socio-ecological and economic needs of the majority of the local population. Research is initiated and developed without a clear understanding of the needs of the population: technology-push. This situation is worsened by the fact that the links between research, extension and the market (consumers) is poor in most African countries, even for conventional agricultural technologies. Scientists and technicians conduct research in the laboratories without much interaction with the extension personnel. This leads to a situation whereby the extension officers are required to deliver a technology package that they can not unpack and explain to the consumers

(e) Paucity of scientific information base

As we noted above, biotechnology is science or knowledge intensive. This means that the ability of any country or firm to successfully engage in biotechnology R&D to no small measure depends on its capability to access new scientific information. In most African countries, scientific information is scattered in the institutions. It is difficult for any one particular research institute to access information from its counterpart within the same country. The storage of information is a problem in some institutions due to lack of (well trained) information officers with some science background.

Important sources of information on biotechnology include publications such as the *Biotechnology and Development Monitor* published by the University of Amsterdam, *Discovery and Innovation* published by the African Academy of Sciences, *Genetic Engineering and Biotechnology Monitor* published by UNIDO's International

Centre for Genetic Engineering and Biotechnology (ICGEB) and a number of others. Most of these publications are available at relatively low costs and form the basis for acquiring new information on biotechnology. However, most African research institutes have not subscribed to these or even if they are mailed to them freely from the publishers they not are stored and used as a vital source of technology.

Most African research institutes are not yet linked to important computer networks such as the Microbial Resources Centres' (MICRENs). This is possibly due to lack of computer facilities and poor infrastructure.

(f) Unfavorable policy environment

Most African countries are seeing major political and economic problems that have necessitated the push for reforms. On the economic front, the Bretton Woods' institutions, the World Bank and the International Monetary Fund (IMF) have forced several countries of the region to institute Structural Adjustment Policies (SAPs). The SAPs essentially advocate for reduction in government expenditure. The level of local funding devoted to biotechnology R&D is likely to decline. Though it is early to determine the effects of these policies, research going on at the African Centre for Technology Studies indicates that the SAPs are likely to erode the limited technological capability accumulated in several African countries and result into severe ecological and economic problems.

There are some fiscal and monetary policies that hinder the process of technology acquisition in Africa. For instance, tax regulations that restrict the importing of computers and software, chemicals, scientific textbooks and other facilities that are essential for research affect the evolution of technological capability in biotechnology and other areas. In some instances the rate of tax may be twice the costs of purchasing equipment from abroad.

Apart from the restrictive economic policies, the political environment in some African countries is not suitable for research. In some countries, preconditions for acquiring research permit are many and too restrictive. It may take longer than the period allocated for the research activity just to get a research permit. On the other front, some African countries are experiencing political strife: an environment not conducive for research.

These factors, and others we have not outlined here, affect the capability of African countries to successfully engage in the biotechnology revolution and exploit its benefits. Dealing with most of these factors requires major economic and political changes that we are incapable of discussing here. In the next section we shall, therefore, only deal those areas that we have "space" to address. Most of these are directly related to the process of creating technological capability in biotechnology.

4. ENDOGENOUS TECHNOLOGICAL CAPABILITY IN BIOTECHNOLOGY

4.1 Technological capability defined

In a recent book John Enos defines technological capability as "something that enables a developing country to exploit, fully, existing techniques, as well as, ideally, to improve upon those that are not perfectly suited to the country involved". He identifies the individual, the institution (organization) and purpose as the main components of technological capability. Technological

capability is embodied in individuals (with skills or experience in particular industrial arts) working collaboratively in institutions to fulfill specific purposes.

In this paper we adopt Enos's definition of technological capability to address the capabilities needed by individuals and institutions to accomplish the purpose of applying biotechnology in African industries to produce and/or process food. We shall be concerned, below, with the "something", be it the individual, equipment or institutional infrastructure that will enable African countries apply biotechnology to meet specific needs (purposes).

4.2 Creating technological capabilities for applying biotechnology in industrial food processing

For African countries to successfully apply biotechnological techniques in food processing and other activities, they will need to build capabilities in different areas either directly related to the biotechnology regime or in general managerial activities. The kinds of capabilities required are:

(a) Scientific capabilities in core biological and engineering disciplines

Biotechnology, as we have pointed out before, is a science or knowledge intensive technology drawing from a wide range of scientific disciplines: microbiology, biochemistry, plant and animal pathology, genetics, and engineering among others. The capacity of any country or firm to economically engage in biotechnology largely depends on its ability to create, harness and efficiently utilize capabilities in these disciplines. Some these capabilities are dispersed in universities and other institutions of learning as well as public and private R&D institutions.

African countries may draw from industrialized countries' universities to build up these capabilities. These may be through collaborative short-term and long-term post-graduate and post-doctoral training in the scientific disciplines essential in industrial biotechnology. Such disciplines include: food engineering technology, industrial microbiology and genetic engineering. The International Centre for Genetic Engineering and Biotechnology (ICGEB) could facilitate the process of training African scientists in such areas by developing a programme that identifies universities in the industrialized countries where the training would be provided. Such a programme may also include funding or scholarship component: a package of financial support to enable the trainees acquire vital literature and conduct research in their home countries in preparation for their dissertations.

African countries should take responsibility to identify and institute biotechnology training programmes suitable for their socio-economic needs. So far, only the University of Zimbabwe has developed a special post-graduate course on biotechnology. Through well structured training programmes China, for example, has managed to build technological capability in microbial technologies and other biotechnology areas: under the "Torch Program". The "Torch Program" was launched in China in order to provide technical and financial support to local industrial enterprises to promote the application of new technologies. It has a significant component for training scientists in different areas of biotechnology.

(b) Industrial development capabilities

The creation of manpower capability in the core scientific areas will only be effective in the long-run if there are industries in which the manpower will be utilized. In general words, it would be uneconomical for African countries to spend resources on training if the acquired capability from the process is underutilized or malutilized. It will be necessary, therefore, for these countries to create industries where the acquired capability through training will be efficiently utilized. Industries involved in food processing activities, such as East African Industries (EIA), need to enter, if they have not, the biotechnology regime. This will require that they acquire bioreactor and bioprocessing technologies. However, the process of acquiring such technologies may be costly and involve some barriers since most of these technologies are developed and owned by private firms in the industrialized countries. The beginning point would be to build on the already accumulated capabilities in non-proprietary technologies in different aspects of food processing. The capacity of local food processing industries to acquire and use protected technologies to a large extent depends on their ability to use those that are available in the public domain.

Establishing biotechnology-based food processing industries, particularly in the rural areas of Africa, will require substantial financial support from external sources: donor agencies from the industrialized countries. African governments should where possible, through their ministries of industry, ministries of science and technology or even ministries of agriculture in collaboration with the finance ministries, help to finance local enterprises in food processing. This is particularly vital during the early stages of development of such enterprises. International finance and development organizations such as the International Commercial Development Cooperation (ICDC), the International Development Research Centre (IDRC) and UNIDO would be instrumental in enhancing the development of such enterprises.

Another important area of industrial food processing where capability is required is downstream processing. This may involve developing pilot projects on fermentation and purification. Downstream processing in the food industry will require specialized bioengineering manpower that is scanty in Africa. These capabilities may be acquired through a collaborative programme between African based food processing enterprises and food processing biotechnology industries in the industrialized countries. Such a programme would involve having experts, in specific areas of bioprocessing, from the industrialized country firms visiting the African enterprises for a specified period to work and train the local manpower. ICGEB could explore ways of developing such training programmes.

(c) Information search, acquisition and assimilation capabilities

We have argued elsewhere that information is probably the most scarce and expensive resource in Africa. Technological information, particularly in scientific journals, is vital for the process of industrialization. African countries still treat such information as less important resource. Their emphasis, through international negotiations, centres on the "the liberalization of property rights regimes to allow them more access to scientific and technological knowledge." Limited effort has been invested in acquiring that technological information (in scientific journals) in the public domain.

Given the knowledge intensive nature of the biotechnology regime, African countries need to institute strategies for searching, acquiring and assimilating

biotechnological information already in the public domain before some of it is turned into private property by firms in the industrialized countries. The thrust for African research institutions should be to subscribe to international scientific journals and establish computer links with their counterparts in the North. In the some industrialized countries, biotechnology firms have instituted means of sharing technological information through venture agreements. This involves exchange of know-how and know-why on protected technologies through constant interaction of technicians or scientists on inter-firm basis.

However, mere acquisition of biotechnological information is not enough. The most important aspect is how to assimilate that acquired information. We do not intend to offer guidelines as to how such information will be used. How the information is used will depend largely on the nature of particular firms or research institutes. In general words, it is important that African researchers and institutions devote time and other resources to assimilating scientific information already in the public domain: for instance, studying scientific journals as part of their research activities. This will need to be accompanied by translating scientific information to reflect their research needs and activities: a process that involves a large measure of learning-by-doing and/or learning-by-using.

(d) Adapting and operating capabilities

The success of any technology in a particular socio-economic system may be measured by the degree to which its adapted and the socio-economic output derived the adaptation of technology. Adaptation does not simply mean the operation of equipment or applying particular techniques to production. It involves, among other activities, managerial development and adjustment, taking the product to the consumers, improving the production process or the product and changing the technology package to suit the local social, economic and ecological needs. In the case of food processing, it may involve producing products that can be purchased by a wide range of the population at reasonable prices, production methods that do not lead to ecological degradation (increased inorganic waste) and improving on the production to suit the local skills and improve the quality of the product.

The process of adapting biotechnology to local conditions in Africa will also largely depend on the ability of local enterprises to develop new organizational means for extension: taking the new products and processes to the local consumers in the rural areas. This will require tertiary education or awareness rising on the technology (process and product) through media or other means.

5. OTHER POLICY ISSUES

5.1 Institutional development

Most of the current biotechnology R&D activities in Africa, as we have indicated above, are being conducted in public research institutions with limited coordination. There is need to identify and develop an institutional set-up that will coordinate these activities. Institutional set-up for this coordination may be viewed from two perspectives: developing national biotechnology centre in each country and/or having a regional coordinating institution.

Kenya and Zimbabwe have, in the last two years or so, being developing proposals for establishing national biotechnology centres.

In Kenya there is division in the views on the nature of the institution. There is a group of scientists and policy-makers that advocate for the establishment of an autonomous centre on biotechnology while another argue for that because of limited funding it is viable to have support go to the existing public institutions already conducting work on biotechnology instead of setting up a new institute. The latter group does not, however, take into account the existing set-up of public research: limited institutional collaboration. A committee has been established to explore ways of establishing an autonomous institute on biotechnology. The institute's mandate will be to coordinate all the biotechnology activities in the public research institutions in the country.

In Zimbabwe the proposal on the establishment of the National Biotechnology Centre (NBC) is still being debated by the scientific community. There are indications that the ministry in-charge of research, science and technology will approve the proposal. The NBC will be headed by a team of scientists drawn from the University of Zimbabwe and other public research institutions.

It will be important for other African countries to explore ways of establishing national biotechnology institutes to coordinate research in the public and private institutes. On the regional front, efforts could also be invested in establishing a regional biotechnology institute that will focus on training and collaborative research in specific areas that relate to the needs of African countries. The institute would operate under the auspices of the International Centre for Genetic Engineering and Biotechnology (ICGEB) of UNIDO.

5.2 Intellectual Property Rights

The issue of intellectual property rights may be considered in two broad ways. First, as it relates to the transfer of biotechnology to African countries. And second, as it relates to the building of endogenous technological capability in biotechnology.

In most African countries the intellectual property protection regimes are still weak. Kenya just recently established an industrial property institute: Kenya Industrial Property Office (KIPO) to oversee the issues of intellectual property protection. Most other countries in the region are yet to institutionalize intellectual property issues. Kenya, Juma (1990) notes, has the most elaborate intellectual property law on biotechnology.

On more a general ground, intellectual property protection regimes of the North are likely to constraint the acquisition of biotechnology by African countries. There is already a significant increase in the protection of scientific information. For instance, Kornhauser *et al.* (1991) notes that there is global decline in the volume of scientific publications on microbial biotechnology corresponding to increase in the number of patents in the same area. This is related to growing privatization of research results particularly in the industrialized countries as they invest more funds into R&D. The extension of intellectual property protection to the genetically-created microbes and seeds is viewed by most opponents of property rights as a constraint to the process of technology transfer and exchange of germplasm. On the other hand, the proponents argue that the extension of patents to cover genetically-created lifeforms is an incentive for innovation.

While the strengthening of intellectual property regimes may have some positive impacts on innovation in general, it limits the ability of developing economies, particularly Africa, to acquire and use new and improved seeds and

other genetically-created biological organisms. These economies have to pay high costs to acquire these improved inputs since the patenting is intended to increase returns from investments in the development of these products. To the extent that African economies currently lack such economic alternatives, their food processing activities may be constrained by intellectual property rights. However, the point we emphasize is that these countries need to use those technologies in the public domain so to build capability in the regime instead of devoting much resources in debating against the intellectual property rights: a debate we are not certain they will win.

5.3 Regional and International Cooperation

There are areas where African countries will need to cooperate among themselves and with the industrialized countries in order for them to successfully industrialize biotechnology in their socio-economic systems.

Training is one such area that we have discussed above. On the regional basis, there is need for African countries to collectively establish a regional treating institute for biotechnology. Such an institute could be coordinated through the framework of the Organization of the African Unity (OAU) and the African Network of Plant Biotechnology (ANPB). Developing manpower capability in biotechnology will require considerable resources and established expertise. Regional training programme or institute would reduce the amount of resources an individual country would invest in developing an institute.

On the international level, cooperation is required in enforcing biosafety regulations such the UNIDO-UNEP-FAO Code of Conduct on Biotechnology. International cooperation will be instrumental in the exchange of technological information, training of African scientists and financial support for R&D in Africa. The ability of African countries to sustain such cooperation depends on the nature of political and economic institutions they put in place. In some African countries there is likelihood that financial support for R&D given by donor agencies such as the World Bank may find way into the pockets of politicians or those who administer the funds.

CONCLUSION

Our thrust in this paper has been to raise some general issues that are important for the development of industrial biotechnology in Africa. We have argued that African countries need to build some level of technological capability to be able to successfully apply biotechnology in the economic domain. The building of that capability requires considerable responsibility on the part of these countries: suitable institutional space is required to allow biotechnology to evolve in the socio-economic systems. On the whole, African countries need to devote some of their economic and human resources to training of manpower, building of infrastructure and biotechnology R&D activities.

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HOW TO REALIZE THE POTENTIAL OF BIOTECHNOLOGY FOR RURAL SMALL-SCALE PRODUCERS IN DEVELOPING COUNTRIES

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1. Introduction

In the past decade a growing interest in small-scale enterprises (SSEs) as a tool to stimulate the overall developmental process in developing countries arose The major reason underlying this trend is disappointment with the performance of modern large-scale industries in developing countries; during the 70s, it became evident that large-scale enterprises did not stimulate a wide ranging developmental process to the extent expected (Teszler, 1989a). Evidence suggests that small-scale enterprises are often inherently more efficient than large-scale enterprises in adding value to local human and material resources. Generally, per unit of investment, small-scale enterprises proportionately create more employment, produce more products that can be used for local consumption, and result in a greater increase in local economic activity than large enterprises (de Wilde, 1988).

In this paper, we will concentrate on rural SSEs which are based around the production of inputs (backward production linkages) and the processing of outputs (forward production linkages) of the agricultural sector. Such industries furnish employment and income for the small-scale farmers, as well as for the land-less for whom casual agricultural labouring generally is the only money earner (Lewis, 1987). The rural small-scale enterprise sector derives its strength from its complementarity; it increases the development potential of the agricultural sector as well as other sectors of economy, it assists in stemming the migratory flow to already overcrowded urban areas and, thus, it contributes to an integrated development process (Ranis, 1990; RSIE, 1988; Teszler, 1989b).

Technological change can be considered one of the prime carriers of economic development (Freeman, 1989) and could also be a crucial factor in the establishment and improvement of SSEs, but only if the technology is appropriate. A technology is considered appropriate when it both takes into account the prevailing conditions and makes the best use of local available resources (ILO, 1980, Bunders and Broerse, 1991). Biotechnology, being a flexible and adaptable technology, could be an appropriate technology for the establishment and improvement of rural SSEs.

Theoretically recognizing how rural SSEs can contribute meaningfully to the alleviation of rural poverty and how biotechnology can enhance the establishment and improvement of such enterprises, does not automatically imply that as such it will be realized. Its realization very much depends on the context; on national and international policies towards rural development and towards R&D methodologies, and on the systematic participation of the rural poor as users, producers and beneficiaries of these new technologies. In order to organize and structure a process to identify, formulate and prioritize appropriate biotechnological innovations for small-scale producers and also, to create an enabling environment for SSEs and to achieve information exchange between relevant people and groups, a model - the interactive bottom-up approach - will be presented in this paper. We will also discuss the consequences of applying such an approach in international research centres.

2. Small-scale enterprises: their importance and problems

SSEs have played an important role in developing countries for a long time. About 10% of the rural population in developing countries is completely dependent on small-scale industrial activities. Furthermore, it is estimated that 10-20% of the male and 50% of the female rural population derives an additional income from rural SSEs (Veenstra, 1991). However, up till the 1980s, this role of SSEs was not recognized by policy-makers in developing countries and donor agencies. Instead, in their industrial development strategies they preferred to concentrate on establishing large-scale enterprises as the cornerstone for development and growth (RSIE, 1988: Teszler and Molenaar, 1989). In particular this has been the case in a number of countries in Sub-Saharan Africa where a presumed lack of entrepreneurial skills has stimulated the establishment of government controlled urban based parastatals as the spearheads of industrial modernization (Teszler and Molenaar, 1989).

However, a modern large-scale industrial sector of any significance is beyond the reach of many developing countries because it (i) involves high investment and maintenance costs, (ii) depends on specialized (urban-based) services, and imported technologies and inputs, (iii) demands good physical and social infrastructure, and (iv) demands access to international markets (Teszler, 1989a). This sector provides no adequate solution to the problem of unemployment, its derived spread effects on other sectors of the economy are limited, and its products do not reach the population at large. As a consequence of the disappointing results obtained by emphasizing large-scale, modernization strategies for industrialization, small-scale industry increasingly came to be considered as a viable alternative or at least as a vital element of an integrated industrial structure, which in turn would promote economic development (RSIE, 1988; Teszler, 1989a).

In general SSEs produce simple implements and consumer goods (such as processed foods, agricultural inputs, clothing, footwear, household utensils and wooden furniture). Usually the quality of these products cannot measure up to the branded output of large-scale enterprises, but the relatively low price they command brings them within the reach of large segments of the population (Teszler, 1989a). SSEs usually make use of existing artisan traditions and knowledge, make use of locally available materials, and apply labour-intensive production methods. SSEs are less dependent than large-scale enterprises on specialized services which in most developing countries tend to be found almost exclusively in urban areas. SSEs are, thus, more independent (Teszler, 1989a).

Problems in the SSE sector occur at three different levels:

A. *Entrepreneurial level:* Entrepreneurial aspirations of the rural poor in developing countries often go unrealized because these people lack access to financing, management skills, knowledge of appropriate technologies suitable for commercialization and an understanding of the potential market for the product (O'Donnell and Hyman, 1987).

B. *Sectoral level:* The basic problem that still needs to be fully appreciated by many policy-makers is the importance of decentralized industrial and service activities in the rural areas, interacting with agriculture and urban industry. In most of the developing countries a profound lack of support still exists concerning the SSE sector in macro-economic, trade and industrialization policies (Ranis, 1990). Furthermore, the SSE sector is faced with low productivity, competition of larger scale industries,

distribution problems, low technological level, and limited development of trade networks (Veenstra, 1991).

C. *Macro-economic level:* The development of a successful SSE sector is often hampered by developments on the macro-economic level; e.g. limited economic growth, limited economic and industrial development, poor physical and social infrastructure, political instability, large population growth and limited institutional facilities.

3. The appropriateness of biotechnology

Biotechnology can be defined as the integrated use of molecular genetics, biochemistry, microbiology and process technology employing micro-organisms, parts of micro-organisms, or cells and tissues of higher organisms to supply goods and services (DGIS, 1989). Biotechnology is neither a scientific discipline nor an industry but a continuum of technologies ranging from highly sophisticated and complex techniques such as enzyme and cell immobilization and recombinant DNA technology to less sophisticated and simpler techniques such as food processing, plant tissue culture and plant inoculation.

Potential of biotechnology

The more advanced biotechnologies can be commercially characterized as high risk, high gain investments, which promise substantial returns on successful investments. Research and development time is usually long and expensive. Often it is the 'high tech' end of the biotechnology gradient that receives the most attention. Yet, despite their current scientific sophistication, they may not be the best fit for a problem in a given location. Even though 'high-tech' biotechnology may ultimately have pervasive effects, the time-lags in research, development, diffusion, investments, education and training for the innumerable potential applications are such that these can hardly occur before the 21st century. Genetic engineering today is still at the stage of computer technology in the 1950s (Freeman, 1989). This is not to argue that 'high-tech' biotechnology has no relevance to the development and improvement of SSEs, but to underline that it is expensive and that the benefits are far from guaranteed on the short and medium term. The emphasis on 'high tech' biotechnology may divert scarce resources from research on 'traditional' biotechnology that may be more appropriate (Persley, 1990).

More immediate results can be expected from 'traditional' biotechnologies. Several of these technologies are well-known, 'mature' and ready for further development towards commercialization. Traditional biotechnological processes are found in the area of food and nutrition, particularly in the manufacturing of foodstuffs and beverages. These processes can be improved: their efficiency and yield can be increased, through the selection of more productive microbial strains, the control of culture conditions, and through the adaptation of the fermentation products to the evolution of food habits and to the consumers' changing tastes (Doele, *et al.*, 1987; Sasson, 1988). Other interesting areas for commercialization of biotechnologies are agricultural inputs which increase yields and/or reduce requirements of capital-intensive inputs; e.g. virus-free plants (plant tissue culture), biofertilizers, biopesticides, livestock feed from crop by-products and mushroom cultivation. These promising biotechnologies, however, usually still need extensive field demonstration and assimilation into local practices before efforts at commercial small-scale production can be made. Some of the technologies may have already demonstrated local commercial success, such as rhizobium inoculant technology and tissue culture, but such success is

on a limited scale and does not immediately translate into expanded activity and international transferability.

Flexible and adaptable

Traditional production methods and technologies are still of enormous importance within the rural SSE sector in developing countries. These methods and technologies are integrated in local social structures and are in harmony with the local culture. The strengthening of the technological capacity of the SSE sector should be built up from the grass-roots level to meet the producers' needs and skills. The recovery of the traditional technological base involves linking modern science with traditional technologies. This linkage also serves to upgrade the traditional technologies selectively through the systematic application of a scientific method, and through the integration of products of science-related technology with those of traditional activities (ILO, 1980).

Since biotechnology can be 'tuned' to very localized problems, it contains elements of an appropriate technology for the establishment and improvement of rural small-scale enterprises. In contrast to most biotechnological research and development itself, many of its applications are inexpensive, uncomplicated and do not require capital- and energy-intensive inputs. Biotechnology is often flexible as to the level and the type of technology used, facilitating small-scale, decentralized application and adaption to the special circumstances of SSEs (Broerse, 1990). Moreover, biotechnology could be linked to indigenous knowledge, existing practice and local initiatives, given the age-old use of some biotechnologies in developing countries (Bunders and Broerse, 1991).

In sum, many of the traditional biotechnologies carry a potential for appropriate application in the SSE sector; they are amenable to labour-intensive, decentralized, small-scale production, requiring a minimal investment in sophisticated equipment and seem adaptable to specific local needs.

4. Constraints for the development of appropriate biotechnology

Recognizing the potential value of biotechnology for small-scale producers does not imply that it will be used. The majority of biotechnological research and development (R&D) efforts are done in areas where the research can lead to commercially attractive applications for which large and lucrative markets already exist, e.g. diagnostics (immunological tests and DNA probes), human pharmaceuticals and animal vaccines, plant improvement (the addition of single gene traits such as herbicide- or pest-resistance to hybrid seeds) and food processing involving mass production of standardized commodities (Broerse, 1990).

As we have seen in the previous paragraph, efforts in biotechnology need not be confined to generating innovations that are high yielding in high input systems, affordable only by resource-rich producers or that are capital-intensive useful only in large-scale urban industries. There are innumerable ways in which international and national research systems can use biotechnology to make substantial contributions to raising productivity in sustainable, low risk, low-input farming systems and rural small-scale industries in developing countries (Joffe and Greeley, 1990). There are, however, many factors which hamper the development of appropriate biotechnology for small-scale producers.

Focus of research in industrialized countries

The most active groups in biotechnology R&D are government institutions (universities and public research institutes) and private companies (research firms and multinationals in the chemical, agrochemical, pharmaceutical and food sectors) in industrialized countries. Much of the fundamental molecular biological and genetic research underlying modern biotechnology has been developed in governmental institutions. The private sector, too, has become interested in biotechnology. Companies, large corporations in particular, have rapidly built ties with universities and public research institutes. Waning government research funding in many developed countries has put increased pressure on universities and public research institutes to engage in contract research. Research contracts add exclusivity of access to public sector research also they provide additional input for private sector development of products. Through contract research and the acquisition of biotechnological research firms, several multinationals have attained a dominant position in biotechnology, a position protected by intellectual property rights (patents) (Broerse, 1990; Clark and Juma, 1991; Junne, 1987; Kenney, 1986).

Governments are also heavily involved in biotechnology. In most industrialized countries (e.g. USA, Japan, United Kingdom, France, Germany and the Netherlands) the development of biotechnology has high priority. Governments endeavour to stimulate and influence development in biotechnology through grants both to industry and public institutions. The priorities addressed by these programmes usually stem from market analyses which identify the problems and interests of industry.

Thus, current biotechnological R&D programmes in industrialized countries are, by and large, guided by the economic considerations of large corporations (and governments). Hence, the focus of biotechnological R&D in industrialized countries is on resource-rich producers and large-scale urban industries.

Limited scientific manpower and funds for operations in Africa

A large proportion of scientists in Africa do not hold postgraduate qualifications. Moreover, between 1960 and 1980, real expenditure per African researcher has decreased by 25 per cent. In contrast, in developed countries, expenditure per researcher increased by 70 per cent in the same period and from a much higher baseline. Due to this lack of qualified researchers and funds, it is very difficult to establish a critical mass in biotechnological research in Africa.

Focus of research in African countries

In policy and research agendas of African countries the emphasis is almost never placed on the generation of appropriate biotechnological innovations for small-scale producers. Scientific research in most African countries started earlier this century during the colonial eras. Consequently, research in general was (and is) usually oriented towards the 'colonial' and export-oriented production on large farms and industries. These resource-rich and large-scale producers are able to communicate their needs to researchers, either directly or through producers' organizations, and assess and adapt the recommendations which come back to them. These producers often have the economic and political power to help researchers and policy-makers in the advancement of their careers.

In addition, it is often assumed that mainstream research benefits all producers, including small-scale producers. Through a so-called 'trickle-down effect' the demands of small-scale producers will also be satisfied. However, this phenomenon rarely occurs, largely because the small-scale producers cannot afford the costly materials and services associated with these innovations. Even when 'trickle-down' does occur, it can have unfortunate and unforeseen side-effects.

Lack of power of small-scale producers

Small-scale producers have much knowledge of their environment and experience of how to use that environment. They are not, as might be assumed, innately conservative. On the contrary, they are usually active and creative in experimenting with local innovations. However, largely bereft of purchasing power, external innovations of potential benefit do not reach them through the commercial process (Bunders and Broerse, 1991). Dispersed, isolated and poor, their influence on the political agenda, even within their own countries, is minimal (World Bank, 1990). Small-scale, resource-poor producers, particularly those in the rural areas, have had no effective organization through which to get access to relevant information from formal research and development, and to articulate their problems and needs.

Lack of communication

In cases where small-scale producers are a specific target for research and development, the innovations are rarely successfully adopted. The many breaks in the chain between research and small-scale producers, and the chasms of education, class, and objectives that stand between the various individuals and organizations involved make successful technology development an unlikely prospect (Ewell, 1989). In general, researchers and policy-makers are ignorant of the problems and needs of small-scale producers. As a consequence, researchers and policy-makers set priorities and goals through conclusions they draw from their own theoretical models and value systems of what ought to be appropriate and not by involving end users directly in the process of problem formulation. 'Appropriateness' on paper is not the same thing as appropriateness in practice. As a result some of the most impeccably 'practical' pieces of research end up with disappointingly few adopters. Meanwhile the majority of African producers continue to rely on their own systems of knowledge and research -procedures and systems of which scientists in the 'formal' sector are often quite unaware (Richards, 1985).

In sum, biotechnology is oriented to the developed world. Many African countries are hardly capable of establishing a critical mass in biotechnology research due to lack of qualified manpower and funds. From scientific discovery onwards, biotechnology leads towards the profitable markets of high technology industries and intensive agriculture. Biotechnological applications currently in development will bring innovations mainly to the group of capital-rich, often large-scale producers (Bunders and Broerse, 1991). In addition, governmental policies and practices often end up favoring large-scale enterprises over small-scale enterprises. In most developing countries these developments are not accompanied by successful creation of employment. In practice, biotechnology seems destined to increase the gap between the rich and the poor as well as the migration to urban areas.

5. Conditions for development of appropriate biotechnology

Discussions on biotechnology focus more on its technological potential than all the means and ways of how poor beneficiaries are to put to use such potential. However, only by the adoption of biotechnological methods by its future applicators the promising potential of this field for developing countries can be put to use. The institutional bases for organization of new technology adoption, such as end-user participation in setting research priorities, the ability of extension services and the marketing infrastructure (for both inputs and outputs) to reach small-scale producers, the targeting of credit, and the distribution of benefits will, thus, be more important determinants of poverty-related effects of technology diffusion than the characteristics of biotechnology. An effective poverty-focused biotechnology intervention holds enormous potential only if developed in an institutional context which empowers poor people themselves and allows them to control decisions on the technology adaptation (Joffe and Greeley, 1990). It is of great importance that scientists and technologists come to the realization that the social, political, and economic environments in which science and technology is to be embedded in the developing countries are enormously complex and risk-prone, and different from those which pertain in the developed world (Doele, *et al.*, 1987). In order to realize the potential of biotechnology for the establishment and improvement of SSEs, a successful approach to technology development will have to fulfill the following four conditions:

A. *Participatory, bottom-up perspective:* An approach to the generation of new technologies should be based on the participation of the target group and a thorough understanding of the problems of the target group, their interests and their production systems. A participatory, 'bottom-up' perspective should be the starting point for national policy, programme and project formulation with respect to applications of biotechnology to ensure that benefits from the resulting productivity improvements are not captured disproportionately by private interests, urban consumers and resource-rich producers. In practice, this perspective requires that the objective of governments is not to 'build a biotechnology capacity at all costs' but to target poverty alleviation first and then to consider any possible role for biotechnology (Joffe and Greeley, 1990).

B. *Technical feasibility:* Innovations should be feasible - that is, the expected output is achievable both scientifically and technically and in terms of its dissemination to the target group. A new enterprise or technology is risky; it takes time away from activities with known outcomes, even if the efficiency of the known activity is very low (de Wilde, 1967). Most entrepreneurs operating in materially poor conditions can only afford a small margin of risk. A combination of appropriateness and feasibility makes an innovation implementable. Furthermore, the biotechnological innovation should have comparative advantage in implementability, problem-solving capacity and cost effectiveness over other options.

C. *Effective policy measures:* Policy-makers should be convinced of SSEs' potential role in development as well as the potential of biotechnology to contribute to the SSE sector. This will ensure that effective policy measures to create an enhancing environment for the development of SSEs and appropriate biotechnology will at least be considered.

D. *Exchange of information:* The exchange of information between different groups is crucial. Decision-making on biotechnology for SSEs is a very complex issue involving different scientific fields and different organizations and social groups. Without effective exchange of information and materials, it is neither possible to plan nor to coordinate the necessary activities, thus strongly hampering effective decision-making. First of all, the results of the research carried out in the different scientific fields are not systematically brought into relation with each other and made accessible to decision makers. Secondly, the different organizations and social groups involved -scientists, expert consultants, donor organizations, policy-makers, extension workers, entrepreneurs and farmers and the organizations which represent and/or work with them- often have different perceptions of what the problems and the appropriate solutions are. All these groups have specific relevant expertise, but lack other types of useful knowledge. Since information exchange between these groups usually is very limited, decision-making on biotechnology in many countries is reduced to an ad hoc process depending on the incidental suggestions of those closest to the decision makers.

In sum, a successful approach to technology development should simultaneously (i) identify, formulate and prioritize appropriate and feasible biotechnological innovations for small-scale producers which have a comparative advantage, (ii) create an enabling environment for SSEs and biotechnological research and (iii) achieve information exchange between entrepreneurs, farmers, scientists, policy-makers, extensionists, and other relevant groups. In the following paragraph, we will discuss an approach specifically designed to organize and structure such a process: the interactive bottom-up approach.

6. The Interactive Bottom-up Approach

The 'interactive bottom-up approach' is a model developed by the Department of Biology and Society of the Vrije Universiteit Amsterdam to assess the use of biotechnology for small-scale producers in developing countries. The approach avoids technology-push by not only drawing on the knowledge and opinions not only of scientists, policy-makers and expert consultants, but also that of end-users and the organizations which represent and/or work with them. Central in this model is the use of two different but closely cooperating teams: a formal interdisciplinary team to bridge the gap between providers of innovations and the potential users, and an informal team on the spot, consisting of people sharing the same commitment, to specify and broadly justify the ideas.

In this model three phases can be distinguished: the preparatory phase, the interaction phase (public debate) and the phase of institutionalization. Depending on the context of the country, the time for preparation and debate may be 5-10 months.

A. Preparation

In this phase, ideas are elaborated. In our case, the idea is that biotechnology can contribute to sustainable rural development. The output of this phase is the formulation and prioritization of problems and research areas, and guidelines for the construction and assessment of new projects. Activities which take place in the preparation phase are:

a. Establishing a formal interdisciplinary team to catalyze and support decision-making on biotechnology for small-scale producers;

b. Preparing an overview of relevant literature;

c. Generating information through interviews with the view to establish the basis of traditional production systems and to enhance these systems through the application of appropriate biotechnology;

d. Establishing an informal team;

e. Exchanging information within the informal team; and

f. Integrating results by specifying and justifying the ideas.

The formal interdisciplinary team (whose members should at least cover the disciplines biotechnology, technology assessment and development studies) will collect information through literature study and interviews from as many different sources as possible. This should encompass information on the problems and interests of small-scale farmers and entrepreneurs and on the links between their activities and that of other groups. The overview should address the national context and the agricultural and industrial sector in order to get a rough idea of the major problems, of biotechnological solutions that are feasible and of the prevailing conditions that need to be taken into account.

The often incoherent information will need to be processed. This may be quite difficult. There will be much information on why things do not work and why they never will without major structural changes. Usually there is no consensus on what solutions are feasible. For the members of the formal team it is difficult to weigh the different problems and solutions against each other. To deal with these problems, an informal team of people, committed to the improvement of small-scale agriculture and industries should be established. The informal team consists of representatives of relevant institutions for agricultural and industrial development. Potential members will already have been identified during interviews. In this team, the information gathered so far can be discussed on the basis of draft reports written by the formal team. Discussions should focus on opportunities and on how to deal with constraints rather than trying to define those constraints more precisely.

The huge amount of information available must result in an integrated view on the role biotechnology could play in rural development. One extreme will be the conclusion that biotechnological projects for small-scale producers simply cannot be justified. This would imply that virtually none of the conditions necessary to stimulate SSEs and to introduce biotechnological innovations pertain or could be met within the foreseeable future. For the stimulation of SSEs three basic conditions can be mentioned: (i) there must be a market for goods produced by SSEs, (ii) there must be some tradition of small-scale artisan or household production and marketing, and (iii) there should be government recognition of SSEs' potential role in development, so that effective policy measures will at least be considered (Teszler, 1989b). No attempt should be made to grow small-scale industry where nothing else will grow, because it probably won't either. In other cases, however, stimulation of SSEs seems possible and some biotechnological innovations will be found to be 'enabling'. The remaining challenge is to use the wealth of information gathered to establish and justify a prioritization of the problem areas to be dealt with by biotechnological innovations.

B. Public debate

The result of the first phase is a reasonably specified and coherent view on the role of biotechnology in serving the small-scale producers in an appropriate and feasible way. However, the results of these activities need to be reviewed and discussed also by those interviewed. They must have the opportunity to criticize the analysis. After all, only those in the formal and informal teams will have had an overview of the full range of opportunities and constraints. It would be misleading to present the integrated results as a consensus document when those who have contributed do not get the opportunity to react. In any case, iteration of analysis may engender new contributions. The fact that many people have been involved in (parts of) the process does not ensure that all opportunities and problems have been identified. Furthermore, a wider discussion is a way of legitimizing the findings of the preparatory phase.

In a public debate, the output of the preparatory phase is discussed openly in order to gain support and to anticipate negative side-effects, constraints and synergies relevant in further developing the innovation. It may lead to the rejection of (part of) the ideas, or to a change of priorities and adaptation of the proposals. Ways of achieving a receptive environment for the effective implementation of the innovation will be discussed. Given that small-scale producers are usually not capable of attracting attention to their situation, a public debate will contribute to the visibility of their needs and problems and arouse public and political support. This will enhance implementation.

C. Institutionalization

On the basis of the proposals prepared during the two previous phases, decisions can be made in different organizations. Producers' and women's organizations, governments of developing and developed countries, international donor organizations, universities, corporations and others may feel that they would like to follow up on the ideas presented. They can prepare new projects, initiate programme studies, adjust existing institutional frameworks and/or establish new ones. There are, however, two potential dangers during this phase. Firstly, once a momentum has been created, the ideas and plans become vulnerable to forces which pull them away from their original design. The different individuals and organizations involved may have 'hidden agendas' and only use the general support for the ideas to realize their own interests (Bunders and Broerse, 1991; Honadle and Klaus, 1979). A second danger is that the ideas of the report are not picked up. In the same way that 'trickle-down' to small-scale producers does not occur naturally, the process of 'trickle-up' to implementing organizations needs specific stimulation. These dangers necessitate subsequent activities of (a part of) the informal team. Its members should stimulate and initiate follow-up activities to ensure that appropriate projects, programmes and institutions are created, and they should monitor the developments.

The design of projects requires specific attention. The information collected in the previous two phases will not be specific enough to allow for a thorough design of project proposals and does not guarantee the appropriateness of the proposed biotechnological innovation in a specific region. Therefore, criteria for formulation and assessment of project proposals are necessary. Clear and well-thought out criteria will improve the identification and planning of the activities, making it more likely that the project results will be produced on schedule and are of the quality specified. This will, in turn, increase the probability that the project and development objectives will be attained. Having formulation and assessment criteria (a checklist against which proposals can be

measured) is a necessary condition. They will, however, not guarantee a project's success since one can never be sure that the original project design or plan will work over time, particularly in a dynamic context.

We are, therefore, developing a set of criteria (presently only in guideline form) which require that proposals demonstrate that certain aspects of the project have been specifically considered. At the same time, they leave room for flexibility in the way these aspects are dealt with. More than in quantitative statements, we are interested in qualitative statements based on the best available information. The guidelines have been developed on the basis of evaluations of earlier innovations in developing countries and recent developments in biotechnology. The guidelines we propose for formulation and assessment of project proposals on biotechnology for small-scale producers in developing countries are as follows. A proposal should:

a. *Demonstrate how the end-user needs have been identified, how they have been involved in the design of the project and how they will be involved during its execution*. Evidence should be provided that a genuine need of the target group is identified. Proposers need to ensure that the research process maintains close and on-going links with, and is ultimately accountable to, its consumers/clients (market- rather than technology-led development).

b. *Outline the anticipated economic, social, environmental and cultural impacts*. Among the considerations should be:

- The *type and scale of the problem* addressed. A thorough description should be given of the problem addressed by the proposed innovation.

- The *input changes* implied by the innovation. The most appropriate biotechnological innovations will usually be those which neither require significant inputs nor significant changes in inputs.

- The *output characteristics* of the innovation. The output of the innovation should meet the demands of the target group.

- The *income-generating effects*. In order to realize income, the outputs of the innovation have to be profitably marketed. Knowledge of the market-supply and demand and the numerous factors affecting prices needs to be acquired, and the market for a new product must be tested.

- The *effect on social and economic relations*. A proposal should consider the scope of the innovation for influencing the broader social and economic circumstances of the people involved. Among other things, attention should be paid here to the labour-using characteristics of the innovation.

- The *effect on the robustness* of the production system. One needs to ensure that the robustness of the production system (stability of the output and sustainability of the production system) is not negatively affected by the innovation.

c. *Demonstrate how the generation of the proposed biotechnological innovation fits into existing rural development policy* and that it has the necessary formal and informal support. The proposers must be convinced that the

innovation will at least be broadly welcomed by various different groups who will be affected by it.

d. *Outline the institutional mechanisms* envisaged both for the research and development process itself and for the dissemination of the innovation to the target group. There must be effective mechanisms to translate R&D into marketable products and to disseminate the innovation to the target group. It is important to consider not only whether there are mechanisms for reaching the target group, but also what support services are needed (such as credit schemes, training facilities, sources of energy and assistance with quality control), if these services are already available and function properly or need to be established or improved.

e. *Indicate whether synergy (or antagonism) with other technological, political or economic measures exist* and how it can be used (or circumvented). Any project should try to make the best use of the possibility of synergies since it will facilitate its preparation/implementation.

f. *Demonstrate that the proposed biotechnological innovation is both technically feasible and safe.* One needs to ensure that the innovation will perform properly not only in the laboratory or research station, but also under prevailing conditions and management procedures of the target group. Ultimately, the end user is the final arbiter of 'technical success'.

g. *Show that the biotechnological innovation has a comparative advantage over other options.* The proposers involved need to demonstrate an awareness of alternative approaches (competition analyses) and to show that the proposed project is the best way of obtaining the stated objective.

h. *Pay explicit attention to technology transfer and the building of indigenous research capacity* to enhance the self-reliance of developing countries. The mechanisms of aspects such as training and intellectual property issues should be described.

i. *Give details of the organization and management of the project.* The project must be managed in such a way that complex multi-disciplinary data can be processed and the right decisions made. Feedback mechanisms which refer to specified achievements must be instituted to guide the project.

j. *Stipulate realistic time-scales for completion of the project or achievement of its objectives* so that a project does not fail simply through exhaustion of funds. A good way of ensuring that the recurrent costs of a technological development project are met, is to build in a self-financing capability right from the start.

Technological, economic, and social viability are all part of successful biotechnological practices and they must all be taken into account since the failure of one means the failure of the whole. Although this may all seem very logical, technological projects are hardly ever designed in this way.

7. Implications for national and international research institutes

The international research community, such as the IARCs (International Agricultural Research Centres), ICGEB (International Centre for Genetic

Engineering and Biotechnology) and IRSIs (Industrial Research and Services Institutes; see box) as well as national research systems in developing countries have to play a decisive role in ensuring that biotechnological research for small-scale producers takes place. They are already involved with applications of biotechnology in addressing concerns of poor countries and poor people. International development research centres are in both a central strategic position and an intermediary position between the major research centres in the developed world on one hand and the research needs in the different African countries on the other hand. However, the approach we advocate for technology development is not currently practiced by national and international research centres. Many research centres face problems in the following areas (UNIDO, 1979; Veenstra, 1991):

- limited knowledge of local conditions and needs;
- little or no participation of end users in priority setting and R&D activities;
- insufficient attention for traditional knowledge and technologies;
- insufficient dissemination of technological information; and
- insufficient 'follow-up' activities and long-term commitment to the end users.

Industrial Research and Services Institutes

The Industrial Research and Services Institutes (IRSIs) have been established by UNIDO in the mid 1960s. IRSIs' goal is to stimulate and influence industrial development in developing countries. In the beginning, IRSIs focussed at middle and large-scale industries, but later they increasingly stimulated the SSE sector. IRSIs function as mediators between the government on the one hand, and UNIDO and the industrial sector on the other. IRSIs give direct and institutional support with the objectives (i) to improve technological management and marketing skills of entrepreneurs, and (ii) to improve and adapt production processes, techniques and products. To reach these objectives, IRSIs perform the following activities:

- supporting services: testing and analysis of products, pre-investment studies, technological information collection and distribution, standards, quality control, need assessment;
- extension services: trouble shooting, process improvement and rationalization, industrial engineering, quality improvement;
- R&D: product improvement, process development and improvement, materials R&D, application R&D; and
- training: managerial and technological skills.
(UNIDO, 1979)

The development research community will, besides ensuring that it has high-grade scientific manpower and attracts sufficient funds for its operational needs, have to focus its research more on a specific target group and its needs and problems, and above all, have to foster an integrated approach to needs assessment, research, development and dissemination of the results. These are the necessary conditions for using new technologies for the benefit of the rural poor of Africa.

When confronted with a participatory, bottom-up approach which includes a wide-ranging list of considerations which go far beyond the ground with which they are familiar, many scientists may feel dismayed. Some of the conditions they may view as obstructionist because they deflate their good intentions or irrelevant because they are not technologically based. Many proposing organizations will simply not have all the skills and resources necessary to meet or even address the guidelines. It should, however, not be assumed that we are suggesting that would-be proposers need to become development experts overnight. They do need, however, to become flexible and inventive in gathering relevant information. They may need to learn, at least, how to communicate with those involved in development. Proposing organizations could, for example, consider collaborating in a 'joint venture' with one or more groups with complementary skills and resources necessary to achieve specific objectives. Such an arrangement could help in (i) the identification of a genuine need of a target group, (ii) the implementation of an applied/adaptive research stage at local level, and (iii) the widespread extension of the finished 'product'. Jointly, these groups could submit proposals which were far more comprehensive (going beyond mere technology), far more realistic and far more likely to attract development funding.

International research centres (IRCs) should be 'research-for-development' centres in the true sense of the word: that is, the centres should be oriented to contribute to an effective development of client groups such as small-scale producers and their production systems, aspects of developing countries which have all too often been ignored. If IRCs are going to apply the 'interactive bottom-up approach' this simultaneously creates both exciting opportunities and dilemmas. The principal opportunity presented by is that the inputs of different groups and activities involved in technology development can be planned and coordinated effectively. But it is the dilemmas which arise with the integrated process which should be of concern since these could be the rocks on which the adventure might founder. We will review the main dilemmas briefly and make some recommendations on structure and organization.

Focus on academic research or on development

IRCs aim to be nerve-centres for strategic research on topics that are important for the development of Africa. This aim raises the fundamental question of whether frontier science and research for development, application, conversion and adaptation of technologies are compatible. The only criterion for excellence is the relevance of its findings for the target group. Yet scientists will feel pressure to pursue science which is challenging for its own sake. In order to retain good scientists, a balance between the two extremes will have to be struck.

In striking such a balance, should the agricultural scientists who, after all, know their own research area best, be free to choose their own research topics? Is such decentralized research planning just as effective in promoting development? Not according to Hobbs (1990). He analyzed decentralized national

agricultural research systems and found that extensive decentralization makes it virtually impossible to formulate a coherent overall program. Decentralization and scientific freedom must therefore be constrained both to ensure that the work has a development perspective and to facilitate regional and local integration (see later). Within the constraints, the scientists should enjoy as much freedom as possible so that they retain enthusiasm, creativity and contacts with the wider scientific community.

Thus, although the scientists' activities will be expected to correspond to accepted scientific standards, scientific or academic progress will not be the only relevant measures to be considered. Research has to be the handmaiden of development. It will be important to develop a yardstick by which the value of science to development can be measured.

The same philosophy can be used to determine the balance that needs to be struck between the IRCs' activities in assessment, research, development in dissemination. Excellence must be judged holistically: excellence in just one aspect (need assessment and identification of research themes, or research itself, or dissemination) does not ensure excellence overall. Irrespective of the value of separate elements in a specific project, it is their integration that is most important. It is the responsibility of IRCs to bring together all aspects of the research and development process: to collate available information, to bring that information together in joint-programmes, to minimize duplication, and to use and to strengthen existing capabilities. Such attention to local conditions and opportunities and applicability of the results must become second nature in IRCs.

Fundamental research - applied research

In conventional organizations, fundamental research and applied research are separated structurally; different people, different organizations, different buildings. In biotechnological research, however, the two are extremes on a continuum. Again, the IRCs, both in their programme and in their structure, will have to strike a balance between the two, to create synergies and minimize disadvantages.

Application-oriented attitudes should be strong in need assessment and extension. Fundamental approaches, on the other hand, are needed in research and problem solving. All these tasks fall within the remit of many IRCs and, if managed properly, the strengths of both approaches should be complementary. What is needed is an integrated approach in which cooperation between technical and social scientists is based on individual contributions.

Local/regional focus - international focus

Another dilemma arises deciding on to where the focus of the target group should fall. With the broad 'research for development' philosophy, the disadvantage is that Africa is too broad a clientele to address. The target group has to be more precisely defined so that IRCs can understand and be responsive to its needs. Ideally, extensive assessment of the needs of all groups in African countries and subsequent prioritization could enable choices to be made. However, that would be prohibitively expensive and time-consuming. Moreover, it would be extremely unrealistic to hold one institute responsible for reaching all end-users. Therefore, we suggest a somewhat pragmatic approach.

The need assessment and prioritization processes should be conducted within just two 'model countries' which are representative of various agro-ecological zones and socio-economic categories of Africa: e.g. the rain forest, the transitional savannah, or the sahel. Within the two countries, the effort should focus on the rural poor. Production of agricultural inputs, farming, processing and consumption are intimately linked and identifying the constraining influence on development of each of these components would provide a major input in helping to define and structure the R&D programme of IRCs. For instance, it would determine whether it will be more effective for the research programme to concentrate on increasing the production at the farm level, on developing or improving post-harvest and processing techniques, on improving nutritional value, or on other areas. IRCs must make an inventory of what has already been performed, of what is already known and of where this expertise is located. The findings can be widely discussed in seminars or workshops to be organized by IRCs. Only then can IRCs acquire their major focus and define their programme. This approach has already been used in identifying and prioritizing research and development in the International Institute for Tropical Agriculture.

Although IRCs must focus on a specific target group, the rural poor, this does not mean that another extremely important group -governments and policy-makers can be ignored. The commitment and support of governments has to be won. Without it, IRCs in the long run might encounter political problems, regardless of their success judged from the perspective of the rural poor. IRCs will have to interface with existing policies (e.g. food policy, economic planning) while defining their own national niche and stressing to the government the value of its information and expertise.

Having focussed on just two countries, IRCs can then look to verify whether key factors which emerged from the assessment phase in the two countries hold valid in some or all of the other African countries. There might then be a tendency to focus research on those aspects that are the most widely applicable. Implementation of the research programme would, however, still focus on the two model countries.

Linkage with the national research systems

A fundamental factor is the relationship of the IRCs with national research systems. In order for research to be best able to take the necessary steps towards integrated development, applied/adaptive research and technologies will have to be transferred to national research systems. IRCs could render important assistance to African countries by delivering the products (physical products and know-how) of its research and development, by acting as a clearing-house or reference centre or, even more importantly, by providing training. The training provision would not only concern high-level biotechnology but also, among others, the skills required for need assessment, prioritization of research and adjustment of solutions to local conditions.

IRCs should also provide training for policy-makers at the national and regional level. For most of them, the threats and opportunities of both modern and traditional biotechnology for their countries will be unknown. Additionally, regulatory officers will be expected to design and implement legislation specifically geared towards biotechnological research or the application of its results, subjects with which most policy-makers will be unfamiliar. Thus, IRCs can play an important supportive role and, at the same time, build a constituency.

The most important innovation at IRCs, will not be any of their specific findings, but the general philosophy and the nature of the results. The centres are themselves, therefore, a model and should be the subject of training activities. Training researchers and others in spreading and applying the 'research for development' philosophy will, perhaps, be the most effective instrument that the IRCs could provide to alleviate poverty.

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THE POTENTIAL FOR BIOTECHNOLOGY IN AFRICA

by

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ABSTRACT

Africa's current problems, especially food security crisis, will find best solution through deployment of modern biotechnology. Biotechnology will have greatest impact in agriculture, health, and industry. In agriculture, biotechnology through cell and tissue culture will lead to improved plant propagation, fast breeding, better control of pests and diseases, and thus better food security. Besides, improved soil fertility through biofertilizers from biological nitrogen fixation (BNF), improved animal, health and husbandry, appropriate food processing and preservation, will enhance production and availability of food. For human health, modern biotechnology will bring about a healthier population through new and more effective vaccines and drugs against endemic tropical diseases of Africa. Industrial growth and environmental protection also stand to benefit from biotechnology.

Problems which impede the introduction of biotechnology in Africa, include inadequate numbers of trained manpower, low funding of biotechnology R&D, poor infrastructure and information acquisition and exchange.

Strategies for enhancing biotechnology in Africa must therefore include capacity building to provide the facilities and support for work in biotechnology. To this extent, efforts should be exerted to embark on education and training of young Africans to produce a critical mass of highly trained biotechnologists. The programme will be facilitated by provision of adequate information and data base on biotechnology. There must also be appropriate networking, linkages and coordination between centres of activity in biotechnology in Africa and laboratories and scientists in advanced countries. Besides, adequate funding of the programmes by national and international organizations should be provided to support the efforts. Most importantly, in adopting the biotechnology option, African countries need to consider its benefits and risks, order of priorities, start with simpler biotechnologies that address the most pressing problems and ensure immediate and short-term benefits. More sophisticated biotechnologies especially those involving rDNA technology, should be embarked on later, as the infrastructure and other facilities improve, for achieving mid-term and long term benefits. Where certain obvious benefits will accrue in the short-term from genetic engineering of plants and animals e.g. for improved resistance to diseases and pests of crops, deliberate effort should be mounted to establish the facilities to enable the inception of such studies, as a crash programme.

Adequate regulations must be established to govern R&D involving rDNA so as to ensure biosafety in testing and release to the environment of genetically modified organisms.

INTRODUCTION

Africa is faced with a food crisis which is the result of a number of (contributory) factors. According to the FAO, the total demand for food and related agricultural products in sub-Sahara Africa will grow at the rate of 3.5% annually, from now to the year 2000 (FAO, 1987). On the other hand, the sub-region's population is estimated to be growing at the rate of 3.8% per annum

between 1985 and the year 2000 and would reach 675 million by then. It also has been discovered that food production in sub-Sahara African countries has either stagnated or has declined in the recent past, and that food supply problems are worsening (FAO, 1991). These population pressures and poor food security are thus adding fuel to the embers of the food crisis. Another contributory factor to the food crisis is the poor management of Africa's natural resources. All the above problems have resulted in hunger, disease, energy shortages, environmental deterioration and pollution, and can only lead to unsustainable development.

Biotechnology, as a tool in science and technology development, is now recognized worldwide as one of the most important developments of the 20th century. This recognition is based on the recent advances in cellular and molecular biology which have provided novel avenues for genetic manipulation of micro-organisms, plants and animals, which directly or indirectly lead to products of benefit to human kind. Some of these include the production of food and fibre, other goods and services such as improved plants and their products, pharmaceuticals, vaccines, human and animal health, and the environment. Although the advancements in biotechnology and commercialization of their products were started mainly in the industrialized countries of Europe, United States of America, and Japan, their potential importance for solving most of the current chronic problems of developing countries has been realized (Anon, 1984; Anon, 1989).

PROS AND CONS FOR BIOTECHNOLOGY IN AFRICA

Although the prospects of employing biotechnology for solving developmental problems in Africa are bright, several points have been made for and against the proposition. First, practically all the commercial applications of biotechnology have occurred in the developed countries, as stated above. Moreover, because of the long standing and well-established scientific base and excellent infrastructure in these countries, they are able to embark on very advanced biotechnology R&D. Besides, almost all the processes and products of biotechnology are under the control of the private sector in these countries which, in any case, already have strong economies, high living standards, and small populations. Biotechnology will therefore produce more wealth for them. Also, a substantial part of the biotechnology effort in these developed countries is directed towards crops, animals, diseases and other problems that are of particular interest to them. Some of the products include those that substitute for the raw exportable commodities from developing countries, thus making traditional food and cash crops from these countries redundant, and causing misery to farmers. These developments will therefore have a negative influence on many developing countries which, instead of benefitting, will be marginalized.

On the ther hand advanced research in and commercialization of biotechnology are either non-existent or just beginning in developing countries of Africa, Asia, Latin America, and the Arab countries of the Middle East. These countries also suffer from large populations, and rapid population growth rates, low standards of living, poor health, many endemic diseases and weak economies. Thus, biotechnology would potentially bring dramatic benefits to these countries and their people. The most attractive way of realizing this potential is for the developing countries to acquire and develop expertise in biotechnology so as to be self-reliant, and find the solutions to their problems. In essence, the need is paramount for them to establish their own biotechnology research programmes, bearing in mind their own interests and priorities.

CAPACITY BUILDING AND ORDERING OF PRIORITIES

Developing countries of Africa desirous to embark on biotechnology as a means of rapid development, must deliberately engage in capacity building in order to succeed (Okonkwo, 1990). Several areas merit attention as follows:

Awareness and planning

It is important for African countries to be aware of the recent advances in biotechnology R&D in developed countries, the impact that biotechnology is having on their economies, and how the African countries' economies are likely to be affected. For instance, some commercialized products of biotechnology from developed countries may compete with or tend to displace some commercial items produced by developing countries, e.g. high fructose corn syrup (HFCS) displacing cane sugar, or starch-based gums displacing gum arabic, etc. Thus, each developing country in Africa must plan a programme for its biotechnology R&D activities against the background of adequate information. It may find alternative uses for some displaced items of commerce, or it may develop its own new items that are attractive to new markets.

Assessment

Planning must include a critical appraisal of national needs and the importance of ordering priorities. In assessing the position on the ground, it is essential to survey, quantify and categorize the available trained scientific manpower, including scientists and technicians, to inform the kinds and levels of activities to start with. The information obtained would indicate in which direction and at what levels future training should be planned for, in order to provide the critical mass of high-level trained manpower for biotechnology R&D. It is also important in planning and assessment efforts, to provide adequate infrastructure for biotechnology R&D including laboratory space, equipment and supplies in Universities, Research Institutes, Colleges and Polytechnics, and in the private sector of the developing country, as well as to provide and maintain the utilities such as electricity supply, water and communication facilities.

BIOTECHNOLOGY R&D PRIORITIES FOR AFRICA

African governments, aided by their scientists and friends, need to identify priority projects where biotechnology R&D can best provide solutions to problems, and yield socio-economic benefits in tangible time. Three areas seem attractive for such efforts, namely Agriculture, Health and Industry.

AGRICULTURE

Since food insecurity is at the root of Africa's problems, agriculture seems an area of high priority meriting initial vigorous attention. In this regard, efforts should be initiated in aspects which are easier to embark on to yield short-term benefits, and complement ongoing conventional agricultural practices. Other problems whose solutions require the use of more sophisticated facilities should be embarked on in time when the infrastructure improves. Areas of biotechnology that would help improve agricultural production include, plant biotechnology, microbial biotechnology, animal biotechnology and aquatic biotechnology.

1. Plant biotechnology

Plant biotechnology through the technique of plant cell, tissue and organ culture holds great promise for achieving short-term benefits in agriculture without the demands of high sophistication in infrastructure and instrumentation. Examples of such applications are:

(a) Clonal propagation: Clonal propagation by cell and tissue culture, is a sure way of achieving mass and cheap propagation of elite crop cultivars for increased food supply (Vasil, 1986; Levin et al., 1988). The technique is also applicable to the mass propagation of forest tree species for afforestation programmes needed urgently to counter the evil ecological and environmental effects of indiscriminate destruction of forests, and to prevent desert encroachment.

(b) Meristem culture and disease elimination: The growing shoot tips of plants consist of meristematic cells which are free of virus and other microbes. Thus, the culture of isolated meristems is a reliable means of eliminating these organisms and of producing disease-free planting stocks of crop plants which enhance agricultural productivity. Examples of crops which have been "cleaned" by this method are cassava, sweet potato, yam, cocoa, and strawberry (Anon, 1984).

(c) Embryo culture: Embryos from wide crosses involving plants from widely separated species, often abort due to cross-incompatibilities. Such embryos may contain unique and useful combinations of genes that may be expressed as disease or other environmental stress resistance. By "rescuing" the embryos and culturing them in vitro, they can be saved and the beneficial traits realized.

(d) Pollen and anther culture for haploids: In vitro culture of pollen, anthers and ovaries provides a rapid (weeks to a few months) and reliable method for the production of haploid (infertile) plants. The latter can be readily converted, by induced chromosome doubling, to yield diploid, fertile, homozygous plants which are important for developing new breeding lines, new cultivars, and hybrid vigour. Recessive genes which may code for some important characteristics are easily expressed in these lines. Conventional methods of producing homozygous pure breeding lines are extremely labour-intensive and time-consuming, often lasting for many years (Vasil Swd Nitsch, 1975; Heberle-Bors, 1985; Vasil, 1990).

(e) Germplasm conservation: Tissue culture procedures also provide a means for conserving genetic resources in the form of gene banks. In vitro cultures of accessions, especially those of endangered but valuable species, perpetuate these species, and facilitate international exchange of materials. The system also helps to maintain the unique plant diversity of Africa and to back-up other efforts in the application of this methodology for development.

(f) Resistance breeding: Cell and tissue culture methods enable breeding for stress tolerance such as drought, alkalinity, acidity, and salinity. This helps to bring into cultivation many African lands which have been marginalized by such stresses.

All the biotechnological techniques and applications mentioned above should be within the reach of African countries, as they do not require very

sophisticated laboratory appliances. Tnus, they can be applied in the near- and mid-term for immediate or short-term benefits.

The following three areas of plant biotechnology require sophisticated and expensive laboratory facilities and specially trained scientists to engage in them. However, in view of the fact that they hold so much promise for improving agriculture and food security in the short term, they merit inclusion in an African plant biotechnology programme for rapid development. Special training of scientists and capacity building in these areas must be planned and mobilized by African countries desirous to tap these systems. These include genetic engineering for herbicide resistance, insect resistance, and virus tolerance.

(g) Genetic engineering for herbicide resistance: Weeds cause heavy losses in crops. Herbicides (weed killers) which are often used to eliminate the weeds are environmentally unsafe and their residues cause problems for animal life. New classes of herbicides such as glyphosate (Roundup) etc. are non-selective and kill all plants. However, these latter herbicides are rapidly biodegraded and thus do not contaminate underground water. Resistance to such herbicides is controlled by single genes which have been cloned and stably integrated into the genomes of crop plants which (transgenic plants) then show resistance to the herbicide. Thus, when the herbicide is applied in the farm, it kills only the weeds. The transgenic crop plants containing the herbicide resistance gene are not affected (Shah et al., 1986; De Block et al., 1987; Schell et al., 1989).

(h) Genetic engineering for insect resistance: Insect pests constitute a major hazard to crops. It has been found that the bacterium, Bacillus thuringiensis, produces a protein which, when ingested by certain insects (mainly in Lepidoptera and Diptera), generates an active, lethal toxin in their guts, and kills them in a short time. The toxin does not have any effect on human beings, other animals and beneficial insect groups such as bees. The Bt gene coding for the protein has been cloned and successfully integrated into the genome of several plant species (Fischhoff et al., 1987; Vaeck et al., 1987; Schell et al., 1989). Such transgenic plants containing the Bt gene are protected from insect attacks and damage.

(i) Genetic engineering for virus tolerance: Viruses also contribute to heavy yield losses and damage to many crop plants. However, it has been found that plants infected by wild strains of viruses acquire resistance to subsequent infection by a more virulent strain. This phenomenon termed "cross-protection", has been demonstrated for a large number of viruses. It has also been shown that cross-protection is provided by the coat protein (CP) of the mild, inducing virus. These findings have led to the production of transgenic plants in which CP genes of certain viruses have been incorporated (Tumer et al., 1987; Nelson et al., 1988; Fauquet and Beachy, 1990; Beachy, 1991). Such transgenic plants show resistance to infection and damage by a number of viruses.

Another attractive objective or priority area in plant genetic engineering research is the possibility of inserting nitrogen fixing (Nif) genes from bacteria that grow naturally in some crops" roots into cereal crops. Such transgenic cereal crops would thus acquire the capacity to generate their own nitrogen fertilizer through atmospheric nitrogen fixation. This would facilitate rapid food production and minimize costly fertilizer imports. However, no positive results of success in this kind of research has been reported.

2. Microbial Biotechnology:

Microbial biotechnology is an area of much potential and interest to Africa in the application of biotechnology to agriculture and also to industrial development. The areas of interest are biofertilizers, industrial enzymes, single cell proteins, bio-insecticides, and biogas generation.

A. Biofertilizers: Some soil micro-organisms have direct beneficial effect on the plant and thus can be classified as bio-fertilizers. Examples are nitrogen-fixing micro-organisms, mycorrhiza fungi, and plant growth promoting rhizobacteria. Use has been made of micro-organisms that can fix atmospheric nitrogen to produce nitrogen-rich compounds which then become available to crops. Examples are blue-green algae (cyanobacteria), Azotobacter, Klebsiella, Rhizobium, and Bradyrhizobium, and Actinomycetes. While some of these are free-living, rhizobia infect roots of host plants where they form nitrogen-fixing nodules. Another example is the symbiotic association between Anabaena azolla and the water fern, Azolla sp. (see Johnston, 1989).

Effective biological nitrogen fixation (BNF) avoids the use of chemical nitrogen fertilizers which pollute the soil, and provides a cheaper and cleaner means of nitrogen fertilization to enhance the productivity of degraded African soils. Already, Rhizobium inoculant production is being commercialized in Africa (Ssali and Keya, 1985). Further research is required to fully commercialize the production and distribution of Rhizobia inoculants, and the application of Azolla-Anabaena associations to enhance the fertility of African soils.

B. Industrial enzymes: Many enzymes of importance to industrial processes are cheaply produced by employing micro-organisms. Notable examples are industrial carbohydrates such as a-amylase, b-amylase, pullulanase, amyloglucosidase, and enzymes of the cellulase complex. These enzymes find application in brewing industry as aids in saccharification (sugar formation from) of starches. This is an area of interest to many developing countries of Africa in search of local maltable grains for their breweries. Besides, microbial production of pectinases used in wine clarification as well as in jam and marmalade industries, is another example. Pectinases are also important for manufacturing detergents, in baking and in beer brewing. Enzymes of the cellulase complex such as endoglucanases, exoglucanases, and b-glucosidase, hold great promise as tools for the hydrolysis of cellulosic wastes into sugar syrup capable of serving as substrate in a variety of industrial fermentations. Potentially cheap sources of cellulose are available, as it is a major component of municipal wastes and of residues from paper industries. One formidable problem in the utilization of ligno-cellulosic waste products is how to achieve efficient hydrolysis of the recalcitrant cellulose component. The problem could however be surmounted by application of certain cellulase-complex enzymes from procaryotes and fungi.

C. Single cell proteins: Single cell proteins (SCP) production refers to the conversion of a large variety of raw materials e.g. methanol, ethanol, sugars, petroleum hydrocarbons, industrial and agricultural wastes into microbial biomass which can be processed into protein-rich food and animal feed. In the African context, SCP can be an important supplement in local starch foods which need protein enrichment for better nutrition e.g. "garri" and "ogi" in Nigeria. Cheap industrial and agricultural wastes are

abundant for use in formulating the required feed stock. Examples are crude oil wastes, mollasses and waste products from sugar refineries. African local strains of yeasts such as Saccharomyces cerevisiae are available to act on the above substrates, while others like Endomycopsis and Candida utilis could be grown on starchy wastes (Obi, personal communication).

D. Bio-insecticides: As stated earlier, Bacillus thuringiensis produces a protein which, when ingested by an insect, generates an active, potent and lethal toxin in its guts. This knowledge has been used in converting colonies of B. thuringiensis (serotype H14) and B. sphaericus (strain 1593) into powder form and the powder used as an insecticide. Such colonies could be produced using agricultural wastes as feed stocks. The powders have been successfully assessed in field trials for the control of mosquito larvae (vectors of malaria) in Nigeria (Obi and Obeta, personal communication). Further tests are continuing, to evaluate toxin production by other local species and strains of Bacillus. Applications of B. thuringiensis colony powder has been extended to the control of the pesticide-resistant blackfly vectors of river blindness in West Africa (Bunders, 1990). More research along the lines given above are called for in order to scale-up the production of the bacteria, as well as lead to the discovery of other entomo-pathogens which could be used as bio-insecticides.

E. Bioenergy (through biogas) production: Biogas production is based on the anaerobic conversion of hydrocarbons such as sugars, cellulose and organic matter into methane and carbon dioxide by mixed populations of thermophillic micro-organisms (Bunders, 1990). Biogas digesters supplied with manure or wastes from livestock, crop residues, etc., produce biogas which is used as an energy source for domestic purposes, and the remaining organic residues which are used as compost. Thus, in the promotion of the use of alternative sources of energy, to help in the conservation of dwindling supplies of petroleum, biogas generation holds great promise for rural areas of African countries. Numerous biogas generation plants are operational in China, India, and other Asian countries, using cow dung as substrate. Biogas use also relieves pressure on over-dependence on fuelwood, a major course of deforestation.

3. Animal Biotechnology:

Biotechnology could contribute to the improvement of livestock production in Africa in several ways. These include animal reproduction research with particular reference to cryopreservation of sperm cells in semen from elite bulls at super-low temperatures (-196°C) and subsequent use in artificial insemination. Also important are experiments on superovulation, in vitro ova culture and fertilization, culture of the embryos followed by transfer to "surrogate" mothers.

These techniques are useful in rapid improvement of economic traits of animals, including milk production, rate of meat-animal growth, preservation of genetic material (e.g. semen, ova, embryos) of economic importance long after they are made available for breeding, and years after the animals that first produced them are dead.

Also of importance is animal health where intensification of research is called for to promote production of vaccines against various animal diseases. In

the long term, attention should be paid to new vaccines produced through recombinant DNA technology.

4. Aquatic Biotechnology:

The river, lakes and oceans are great reservoirs of food and of biological resources for mankind. They are expected, therefore, to form a major source of food for the future. However, the aquatic environments worldwide are prone to easy pollution, hence damaging the valuable water-based resources. There is, therefore, an urgent need for education and training in aquatic biotechnology, including aquaculture, water clean-up, and pollution control. This need is most urgent in Africa and other developing countries which together produce 45% of the world's 85 million tonne fish annually. In the short-term, improved management of fish farms, fingerling selection and production under modern methods, production of other aquatic food organisms such as prawns, crayfish, and oysters, should be encouraged.

Research and production of transgenic fish through rDNA methods qualifies as a long-term goal. India is using rDNA techniques to improve the productivity of the small-bodied tilapia fish. Their scientists are attempting to clone the bovine growth hormone gene and micro-inject it into the fertilized tilapia egg to induce formation of larger fish.

HEALTH

In the health sector, attention should be focussed on major health problems, especially those that can be solved through biotechnology R&D. Relevant areas include (1) Development of rapid diagnostic techniques, (2) Vaccine production, and (3) Development of therapeutic agents from local plant sources.

1. Development of rapid diagnostic techniques

The following aspects are of importance: (a) Production of culture media, (b) Diagnostic agents, and (c) Monoclonal antibody production.

A. Production of culture media: Several possibilities exist for the production of culture media from local raw materials in African countries. These media can then be tested extensively for their efficiency of plating. They can be easily commercialized as there is a good market for them in developing countries.

B. Diagnostic agents: Production of reagents for diagnosis of viral, rickettsial and chlamydial infections of man and animals are of importance to developing countries. Such reagents as antigens, antisera and complements, can be prepared. However, such preparations require extensive tissue culture and biotransformation studies.

C. Monoclonal antibody production: The basis for monoclonal antibody (MAb) technology is the fusion of an antibody-producing mammalian cell (e.g. spleen) with myeloma (cancer) cell to produce a hybridoma cell. When stimulated by an antigen such as a virus, the hybridoma will grow and produce a specific antibody, named monoclonal antibody, against the antigen (Bunders, 1990). The MAbs are then harvested from the liquid medium in which the hybridoma cells were cultured. The MAb technique may be used for diagnostic purposed to identify pathogenic organisms (e.g. viruses, bacteria, and parasites). One major advantage of MAb tests in

diagnosis is their speed, accuracy and specificity. MAb can also be used in therapeutic treatment since it can serve to deliver cytotoxic drugs to tumor cells alone because of surface differences between normal and malignant (or tumor) cells.

2. Vaccine production

In developing countries, including Africa, potent vaccines can be prepared using local strains of organisms. Producing a vaccine locally has many advantages. For example, it ensures availability at all times; it gives better protection since it would be from a local strain; and deterioration of imported vaccines due to improper storage (e.g. in the hot, humid, tropical environment) would be minimized.

3. Research on local therapeutic agents

Many African countries lie along the tropical and subtropical latitudes. These regions house a vast flora of amazingly diverse plant families and species many of which are yet to be named, described and classified. Some of these plants are rich in useful primary and secondary chemical compounds including therapeutic agents. The flora of these regions needs to be extensively and exhaustively explored in order to discover those that contain substances of medicinal/ therapeutic and industrial importance. Compounds that could be isolated and purified include anti-tumor drugs, sweeteners, dyes, flavour, and aroma/ fragrance compounds. Some of the plants containing these compounds (e.g. Thaumatococcus danielli producing the protein sweetener thaumatin, and Dioscoreophyllum cuminsii also producing the protein sweetener monelin have habitats in the tropical forests which are being decimated by human deforestation activities, accidental or deliberate fires, etc., thus threatening them with extinction. Such endangered species can be rescued by germplasm preservation techniques in tissue culture, and studies could lead to their cells being cultivated and the important components being produced by the cultured cells as has been achieved for shikonin, pyrethrin, etc. (Anon, 1984; Bunders, 1990).

INDUSTRY

Under the section on microbial biotechnology, attention was drawn to the role of microbes in facilitating various processes including biogas generation, production of bioinsecticides, and production of industrial enzymes. These could correctly be described as industrial biotechnology productions. However, the major application of biotechnology to industry is exemplified by fermentations, and these ought to be promoted in Africa. The most important industrial biotechnology in this regard is alcoholic fermentation. The importance of alcohol in industry and other sectors of the economy, including use as organic solvent, in beverages, energy source, and as automobile fuel, etc., is immense. Other microbial biotechnology processes involving fermentation that yield products of industrial importance include those for the production of amino acids, antibiotics, and other primary and secondary metabolites e.g. citric acid.

BIOTECHNOLOGY AND FOOD PROCESSING

Although the food crisis in Africa is caused by declining food production, on the one hand, and by uncontrolled population growth in the region on the other; it is known that a considerable percentage of the food produced is lost due to post-harvest spoilage. If biotechnological methods can be deployed to prevent such losses, most of the food that is produced can be saved and made

available to the people, thus reducing the food crisis. Several avenues are worthy of investigation, such as microbial fermentations of food, detoxification, (Okafor, 1981; Okafor and Ejiofor, 1990) etc.

CONSTRAINTS IMPEDING AFRICAN BIOTECHNOLOGY

Many problems and constraints impede biotechnology R&D in Africa. These include low funding, poor infrastructure such as lack of adequate laboratory space, equipment and their spare parts and maintenance, unavailability of reagents and other chemicals and supplies, unreliable water and electric power suppliers. Manpower with the specialized skills for modern biotechnology R&D, especially for rDNA technology, is grossly inadequate. Another major drawback is the lack of relevant and current literature and data base on biotechnology.

In recent survey, by questionnaire, of biotechnology scientists in sub-Sahara African countries, conducted by the author (Okonkwo, 1991), just over 100 responses were received from 19 countries. Of these, 90% were in plant biotechnology (employing cell and tissue culture procedures), 30% were in microbial biotechnology, 7% in animal biotechnology, and 2% in human health biotechnology. Less than 5% were engaged in recombinant DNA research. There were overlaps; for example, some scientists were engaged in both plant and microbial biotechnology. In view of the above, only mostly non-sophisticated biotechnologies are being and can be undertaken in Africa at the present time, with only minimal attention being given to genetic engineering.

SOLUTIONS TO THE CONSTRAINTS AND MODALITIES FOR ADVANCEMENT

1. Education and Training

Most African countries do not yet have the critical mass of scientific manpower to undertake studies in modern biotechnology, which is a prerequisite for the latter's deployment to solve problems. Thus, it is necessary to first plan for biotechnology R&D by increasing the quantity and quality of scientific personnel in the fields of physiology, biochemistry, genetics, microbiology and molecular biology. This will provide a pool of scientists that can embark on more specialized biotechnology research and training activities. In this regard, it is advisable for developing African countries to revise the curricula of their national colleges, polytechnics and universities so as to include course in modern biotechnology.

Modes of training programmes to be planned for include postgraduate fellowships to support capable students in research leading to M.Sc. degree (2 years), and Ph.D degree (3 years), post-doctoral fellowships (1-2 years) for advanced training in biotechnology in outstanding international research laboratories in developed and developing countries. Besides, it is advisable to organize periodically, on a regional or inter-regional basis, intensive training courses (1-2 weeks) on basic and advanced techniques used in biotechnology research, taught by experts in the fields. The courses should be mounted in laboratories that are well-equipped and staffed for the particular discipline taught, for example, the International Agricultural Research Centres (IARCS), Universities, and/or National Research Institutes, etc.

2. Information Acquisition and Exchange

Scientists in Africa are isolated and frequently ignorant of the latest advances in most areas of science and technology (S&T). This is the result of

unavailability of scientific information such as journal publications, books, symposia proceedings and monographs on topical subjects. Deliberate efforts should be made, as part of capacity building, to establish data bases in African countries, and, with assistance from relevant international organizations, gain access to international data banks. A system should also be established for them to acquire journals, books and other relevant up-to-date literature at reasonable cost. Assistance on the modalities can be obtained from agencies such as UNESCO, ICSU's CODATA, and the Third World Academy of Sciences (TWAS).

3. Networks, Linkages and Coordination

National and regional efforts at capacity building in biotechnology must be supported by adequate networking, linkages and coordination. At the national level, wherever possible, it is desirable to establish a Centre for Genetic Resources, Genetic Engineering and Biotechnology which will serve as a focal point for organization and coordination of biotechnology R&D in the various institutions and private sector in the country. It will also serve as a vehicle for regional and international linkages for acquisition and exchange of information on biotechnology matters. This centre must, however, not operate in isolation, but should be integrated effectively with other national agricultural, industrial, health and S&T programmes for complementarity. Besides, those African countries with critical mass of scientists engaged in biotechnology R&D should each establish a national biotechnology society. Through scientific activities such as annual conferences, seminars and symposia, the society will be able to catalyze and build up research strengths, provide a forum for discussion of research projects, results achieved, and for assessment of possibilities for commercialization of products, and for consideration of future commitment of scientists to R&D, collaboration, in-depth studies and fast achievement of results, without duplication of efforts.

Networking has been widely accepted as a useful method for coordination of scientific activities and for exchange of information regionally, inter-regionally, and inter-nationally. Networks in biotechnology R&D are beginning to develop in Africa. For instance, two such networks which have developed recently are worthy of note.

The African Plant Biotechnology Network (APBNet) was established in Nairobi, Kenya, in January 1989, with a coordinating office at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, and with sub-regional offices for West, Central, East, North and Southern Africa. The Coordinator based at IITA has been collecting and collating data (obtained through questionnaires) on manpower, which have been embodied in a directory of African Plant biotechnologists, first published in 1990 (Ng, 1990).

Similarly, ICSU-UNESCO's African Biosciences Network (ABN) has established a sub-network for biotechnology, following an international symposium on the food crisis in Africa, at Yamoussoukro, Côte d'Ivoire, in July 1989, and appointed a Coordinator (Okonkwo, 1989). Again, a survey (through questionnaires) was undertaken, to assess the statio of manpower, research interests, infrastructure positions and constraints in biotechnology in sub-Sahara African countries. The information has been supplemented through visits, by the Coordinator, of selected African countries. A first edition of the directory has recently been produced (Okonkwo, 1991), and periodic updating is planned.

Development of other networks has also been recorded, such as UNESCO-UNEP's Microbiological Resource Centres (MIRCEN) networks for Applied Microbiology and

Biotechnology starting with activities in Nairobi, Kenya, and spreading to other parts of Africa; also reported is the francophone Animal Biotechnology network.

It is hoped that with the cooperation and collaboration among these networks, a comprehensive knowledge and documentation of the statio of biotechnology R&D in Africa will emerge. This will enhance effective planning, exchange of information, and collaboration in research and training activities.

4. Other International Support Activities

A number of international organizations have been playing significant roles in promoting and supporting S&T capacities in developing countries. Most of them have strong biotechnology components in their programmes. Some of them are inter-governmental organizations (IGOs) and agencies while others are non-governmental organizations (NGOs). Notable organizations playing active roles in supporting biotechnology activities include the IGOs such as UNIDO, UNESCO, FAO; and NGOs like ICSU and the Consultative Group on International Agricultural Research (CGIAR). All these organizations, by communicating and interacting with developing countries, are helping them to define, assess, and fashion programmes in S&T in general and biotechnology in particular.

For example, UNIDO's International Centre for Genetic Engineering and Biotechnology (ICGEB) is already making significant impact in strengthening and supporting biotechnology activities in developing countries. By initiating and catalyzing the establishment of affiliated centres at national, sub-regional and regional levels, ICGEB is bringing biotechnology research to the grass roots. It is hoped that many more affiliate centres will be formed in Africa in the near future. The main areas of emphasis of ICGEB include training and education (by sponsoring training courses and making research fellowship awards), supporting communication and information exchange through sponsoring of seminars, symposia, and conferences/workshops on biotechnology; and establishing computerized informatics in biotechnology. It also awards research grants to support worthy proposals on biotechnology problems, submitted by scientists through affiliated centres.

Among the NGOs, the CGIAR, a conglomerate of 16 IARCs sponsored by the FAO and the World Bank, carries out research through internationally-recruited scientists and technologists to improve agricultural productivity in developing countries. Most of them are located in developing countries worldwide and apply tissue culture procedures in their agricultural biotechnology research thrusts. Some of them are currently upgrading their facilities for full attention to most aspects of modern biotechnology. They also organize various workshops, training courses and symposia in plant biotechnology for the benefit of scientists in developing countries.

The International Council of Scientific Unions (ICSU), an umbrella NGO of some 20 scientific unions, 75 national academies of science and research councils, and 26 associate scientific organizations, has its goal as fostering and encouraging cooperation in international scientific activity for the benefit of mankind, with special concern for the world's less developed countries. ICSU created the International Scientific Committee on Biotechnology (COBIOTECH) in 1986 with the objectives of stimulating activity and cooperation with appropriate organizations including the industrial community as regards advancement of research and education and transfer of information and resources in biotechnology (Gerhardt, 1990). COBIOTECH covers the entire breadth of biotechnology, and its activities are in three dimensions of research, education and information

transfer. It has made a major input in its informational activities by publishing in 1991 a resource book titled "Biotechnology worldwide" edited by Coombs and Campbell (1991). The book contains reports on the state of biotechnology in 50 countries worldwide. COBIOTECH interacts and collaborates with other regional and international organizations such as the Federation of European Biotechnological Societies (FEBS), UNESCO, UNIDO/ICGEB (to which it is now an affiliate member), and UNDP.

5. Funding (National Governments & International Agencies)

A major impediment to development in most African countries is the paltry funding provided by governments for R&D (still at 0.2 - 0.3% of GNP). Developed countries commit 2 - 2.5% of their GNPs. However, recent advancements in S&T in a number of developing countries correlates with their increased funding of S&T (see Table 1).

Table 1: Expenditure of funds on R&D by some developing countries
(After Hassan, 1990)

Country	GNP	
	Previous	Now
Bangladesh	0.2	1.1
Brazil	0.6	2.1
Iran	0.5	2.0
Pakistan	0.17	1.0
Philippines	0.2	1.5
South Korea	0.6	2.0
Venezuela	0.4	2.0

Interestingly, no African country qualifies for inclusion in that group. Unless African governments deliberately upgrade their budget allocations to S&T to at least 1% of their GNPs by the end of this century, progress in agricultural research, including biotechnology, and S&T in general, is bound to be stultified.

Additional support should be sought from the well-known UN organizations which have been giving grants to developing countries, such as UNIDO, UNDP, UNEP, UNESCO, FAO, and WHO; as well as others like the World Bank, ADB, GTZ, IRDC, and TWAS, etc.

6. Policy Issues

Many governments in African countries are either unaware of or insensitive to the contributions made by their indigenous scientists in science and technology, not to talk of worldwide activities in the biotechnology field. They also seem not to appreciate the infrastructural constraints under which their scientists work. It is therefore essential to educate policy makers as well as the public in Africa on the potential benefits of biotechnology and the need for government and policy makers to support S&T efforts in Africa and to adequately fund biotechnology programmes. Attention has been drawn elsewhere to this need (Hassan, 1989; Okonkwo, 1990). Bodies such as the Organization of African Unity (OAU), the Economic Commission for Africa (ECA), and the African Development Bank (ADB) should join in the campaign to get African leaders to emulate other developing countries such as Iran, South Korea, Brazil, Venezuela, etc. that have upgraded their allocation of funds to S&T to at least 2% of their GNPs. The ADB should emulate the World Bank which now has its own biotechnology R&D programme.

In the specific area of biotechnology, in view of its immense potential for contributing to rapid development in Africa, a deliberate effort should be mounted to achieve rapid results. For example, there is need to popularize the role of biotechnology in solving socio-economic problems in Africa. Electronic and print media should be used to emphasize the benefits of biotechnology R&D. The production of new vaccines, new high-yielding, disease-resistant crop varieties, and biofertilizer development, for example, should be highlighted.

7. Risk Assessment and Biosafety Regulations

It is essential that the public and policy makers in African countries should be aware of, not only the benefits of biotechnology but also the risks, especially with respect to genetic engineering research. It is possible for genes to "escape" from genetically engineered varieties, if care is not taken, and enter wild plants, e.g. in herbicide resistance. Therefore, all experiments involving recombinant DNA technology must have proper containment or quarantine conditions for the research and trials preceding the release of genetically modified organisms (GMOs). To this extent, individual African countries should establish functional and well-informed national review bodies and institutional biosafety committees to assist government in formulating regulations to aid monitoring and regulating the release of GMOs into the environment. Other important regulatory matters which the countries should attend to are: the establishment and observation of code of conduct; guidelines governing intellectual property rights and patents; including limits to patentable materials; and ethical issues, in biotechnology R&D.

The IARCSs, where they exist, also need to establish international biosafety committees and ensure that they function in accordance with the host country's regulations.

Finally, international development agencies need to ensure that biosafety reviews are conducted prior to the release of GMOs in any projects they sponsor.

All the above regulations will ensure the conduct of biotechnology experiments and sage application of results without risk to environment. Besides, advice on risk assessment and on formulating the regulations and guidelines should be sought from international agencies and from developed countries with experience in such matters.

CONCLUSIONS

From the above presentation, it seems clear that each country in Africa stands to benefit from biotechnology R&D by training her manpower for effective application of the "new" technology to agriculture, health care delivery, industrial production, and the environment. In doing these she must consider the benefits and risks, order her priorities, start with simpler biotechnologies that address the most pressing problems, for short-term benefits, and embark on more sophisticated ones later when their infrastructure improves, for long-term goals.

In agriculture, biotechnology will assist in improved control of pests and diseases, and thus better food security. For short-term benefits, improved plant propagation and fast breeding for many useful traits, through plant cell and tissue culture, improved soil fertility through biofertilizers from BNF, improved animal health and husbandry, and increase in food supply through appropriate food processing, seem most attractive.

In the human sector, biotechnology will bring about a healthier population through the introduction and use of a new generation of biotechnology-produced vaccines and drugs against most tropical, endemic diseases of Africa.

Besides, mid- and long-term benefits of biotechnology will accrue from genetic engineering of plants and animals for improved resistance to disease and pests. Deliberate efforts should be mounted to embark on these more sophisticated biotechnology R&D by planning for and deploying the conditions that will make them flourish.

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BRIDGING THE GAP BETWEEN RESEARCH AND APPLICATIONS IN THE THIRD WORLD

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Introduction

A previous study concluded that the successful transfer of biotechnology and related know-how from the research laboratory to the applied sectors of industry, agriculture and health does not usually occur in developing countries. [1] The main reason why technology transfer did not take place is that a gap separated the research establishment from the applied sector, precluding the transfer of results from research to applications. Two factors create and sustain the gap; (1) mechanisms that would facilitate such transfer either do not exist or are poorly developed in these countries, and (2) economic, legal, and social barriers prevent university-industry cooperation and serve to impede contacts between the two from being developed in the future. Yet, developing countries will be unable to achieve even a small measure of self-sufficiency in biotechnology unless they are able to utilize results from indigenous research. Bridging the gap between research and applications is therefore of vital importance. Accordingly, the objective of this paper is to consider what governments and international agencies can do to bridge the gap between research and applications and identify specific measures that can be implemented by them relatively quickly and easily. To reach the objective, it is necessary to provide a background. Accordingly, the generic reasons that prevent the transfer of biotechnology within developing countries are identified and briefly discussed; measures that can be taken by governments to overcome these problems are indicated; and the paper concludes by proposing specific assistance that international agencies, especially UNIDO, can provide that would promote the transfer of biotechnology within a developing country.

Before commencing with the substantive part, I note that this paper was prepared for a conference on the utilization of biotechnology for food industry in Africa. But the problems of technology transfer discussed and analyzed here (and in the preceding study) are generic to the Third World; the gap between research and application is present in most countries of Latin America, Asia and Africa. Methods for overcoming or bridging the gap may vary in details from country to country and from industry to industry, but the general prescription for corrective actions that may be taken by governments and international agencies will be similar everywhere.

I. The Problem of Transferring Biotechnology in Developing Countries.

In 1988 I undertook a project on behalf of the United Nations University to assess the effectiveness of technical assistance provided by three major United Nations (UN) agencies to four developing countries, Egypt, Thailand and Venezuela (case countries), in the field of biotechnology. [2] The study's unexpected major finding was that practically all technical assistance being provided by these agencies was aimed at building up the research capabilities of developing countries; very little, if any, aid was dispensed to industry or agriculture. Further, although the international agencies made some attempts to ensure that the research they supported engendered results useful to the applied sector, in fact no technology transfer took place. In other words, results from biotechnology research being performed in the case countries had no impact on the

pressing problems plaguing these countries, nor were they used to advance their economic development.

At first glance, it seemed as if the UN agencies were remiss in how they directed their technical assistance. However, my analysis of the situation existing in the case countries indicated that there are fundamental and complex reasons at the national level that dictate the eventual disposition of technical assistance. Five major reasons may be mentioned and discussed:

A Although support being provided to science by the governments of the case countries is minimal, scientists in each country were well-organized and were able to influence government policy-making. Their collective voice was especially forceful when they can make a strong argument for a particular course of action; in this instance, the need to build a strong scientific base upon which biotechnology may grow and progress. Also, the fact that scientists are government employees in the case countries enabled their spokespersons to have direct access to the decision and policy makers who represent their countries at international agencies. In effect, when assistance is sought from international agencies, scientists, being unified and well-represented, were in an excellent position to voice their preferences.

B Second, conversely, no bioscience-based industry exists as such in the case countries. For example, firms other than food industry that dependent on fermentation production methods, such as pharmaceutical companies, are well placed to adopt biotechnology. This type of firm does not exist in the case countries - so-called pharmaceutical plants were in fact subsidiaries of multi-national companies that have only a packaging function. As a result of the lack of biotechnology-based industry, there is no industry interest group or spokesperson who presents the views or demands of those in the applied sectors to either national governments or international agencies.

C Third, all case countries lacked effective mechanisms for transferring results and findings from the research laboratories to those who might be in a position to use them in industry or agriculture. Referring to Figure 1, universities and other public research institutes did not have units that take on applied research or applied research and development (R&D); nor do they have specialized technology transfer offices. Similarly, industrial firms did not have applied research and development laboratories, nor did firms possess technology acquisition departments whose job it is to ferret out useful applications from research done at public and private universities. The best intentions of international agencies to fund research having specified applied objectives will therefore come to naught, since there is no way for the results to reach those who may apply them.

D Fourth, the possibility of "fixing" the technology transfer process is low because both the academic and business environments in each of the case countries discourages university-industry cooperation and, in general, are not conducive to entrepreneurship. From the academic side, scientists are disinclined to take up applied research or to foster contacts with industry. On the business side, the countries have inadequately designed or outdated tax and intellectual property laws, which fiscally penalize business successes while making no allowance for risk taking, thereby directly or indirectly deterring industry from financing risky

development. In addition, rules specifically governing biosafety were absent in the case countries. This means that while research may proceed relatively unhindered (see below), conditions under which testing and manufacture may take place are unspecified, and no government agency would assume responsibility for overseeing these activities.

E Fifth, powerful social disincentives existed in universities that blocked scientists from taking on applied research or associating with industry. Simultaneously, industrialists faced formidable bureaucratic barriers when they attempted to have directed research done at university laboratories or engage local scientists as consultants to solve problems or improve processes. As a result, they find it easier and quicker to buy required technology or expertise from American, English, Japanese, Taiwanese and other foreign sources rather than try to access indigenous research however funded.

The difference in technology transfer between a industrialized nation (U.S.) and a developing country (Ecuador) may be illustrated by drawing on personal experience. At present I am the principal investigator of a year-long project which aims to assess the status of marine biotechnology in the U.S. In the course of that project, I took time out to evaluated project proposals submitted to UNIDO for funding by Bolivian, Ecuadorian and Peruvian authorities. One of the Ecuadorian projects related to marine biotechnology.

In the U.S., Dr. Miriam Polne-Fuller, working at the University of California at Santa Barbara, has been performing basic research for over six years on the interrelationship between a certain marine amoeba and the giant kelp on which it lives. She observed that the amoeba was able to digest the tough kelp leaves. Intrigued, she wondered if the amoeba also had the ability to digest manmade substances, such as plastic. Laboratory investigation indicated that the amoeba was indeed able to break down several types of manmade polymers, albeit at low efficiency. Polne-Fuller then spent the next year developing the organism, using classical breeding and selection techniques, until she had a fairly efficient strain. At that time, she informed the University's technology transfer office about her findings. Recognizing its possible applied significance, the technology transfer office initiated the process to patent the organism. The technology transfer office also prepared a statement for its monthly newsletter describing the experiment and findings. The newsletter is sent out to companies throughout the U.S. every month.

The technology transfer office at Occidental Petroleum Company read the statement and recognized the implications of the research for the company, which is one of the largest manufacturer of certain plastics in the world. The importance lies with the fact that more and more American states are adopting legislation that promotes or orders recycling of plastics in the community and the manufacture of packaging that is biodegradable. Occidental contacted the university's technology transfer office, and it acted to bring the researcher together with the company. After negotiations, the company agreed to fund applied research at the university to develop an industrially useful amoeba. As the first step, the university and company researchers will work jointly to develop radioactive polymers, so the anabolic process of the amoeba can be clarified. No such probes exist today, so this is a new departure for both sides. In any case, if further progress is made, the company will take over the development process and if commercialization is achieved, the university will receive royalties. According to University policy, about 70% of proceeds are returned to Polne-Fuller's laboratory.

An alternative scenario, and one that is perhaps more common in the U.S., is that the researcher would have formed her own company and sought to raise funds via forming a general or limited partnership with entrepreneurs or making private or public offerings of stock. The small start-up company could also have sought capital from the state government or from one of the federal agencies. On the state level, for example, the State of Maryland has an innovative program called Maryland Industrial Partnerships, which funds cooperative research between universities and companies at a maximum of $ 50,000 per year for three years. In addition, some universities, such as the University of Maryland, have established so-called incubators, where a small science or technology-based company may rent space for offices and laboratories at exceedingly favorable rates and access university resources, such as computer centers, data bases, library and expert assistance. On the federal level, all major agencies, including the National Science Foundation, the Department of Agriculture, the Department of Defense, etc., must set aside 5% of the funds they use to support research and development for so-called small business innovative research, which in effect supports high risk research and development by companies having fewer than 500 employees.

Now to return to the researcher in Ecuador. This scientist, who holds a regular appointment at the Catholic University in Quito, has been researching diseases that afflict shrimp aquaculture in that country. Since Ecuador is the second largest producer of aquacultured shrimp in the world, and since the most serious constraint on intensive aquaculture is disease, [3] the potential of this researcher's work can easily be recognized.

As of September 1991, the Ecuadorian scientist had been researching for several years marine vibrios that cause disease among aquacultured shrimp. About six months ago, he was able to develop a vaccine that elicited strong protective reaction in shrimp against vibrios. To all appearance, he had a product that had promise for aquaculture and that was ready for testing and advanced development. However, work has stopped because he has been unable to raise the funds necessary to continue development. The aquaculture industry in Ecuador is not willing to provide funds, even though it would seem to be in its interest to do so, because the project is perceived to be risky (in terms of successfully producing a marketable product); marketable results under the best of circumstances cannot be achieved for at least three years, so no short-term gain are likely; and companies do not receive credit in the form of tax breaks or other incentives from the government for funding this risky research. Private persons and companies are not interested because they perceive this project as being risky, and they would not receive any tax credit or other financial incentive if the venture failed (on the other hand, if the venture was successful, the profits would be taxed at about 60%). Finally, the government has no program that promotes small industry. In the end, this researcher's only possibility may be to try to raise funds from international sources. However, he has no contacts with international business or international agencies and would, in any case, lack the business expertise to be able to negotiate an equitable contract. The likelihood is high that a business in Taiwan or Korea, where shrimp aquaculture is also important, would contract with him to develop a product, but would in the end deny the researchers equitable compensation.

The experience of the Ecuadorian researcher indicates some of the difficult problems facing the prospective scientific entrepreneur who believes he has invented something useful, but is prevented from capitalizing on his invention. It also provides guidance for corrective actions by governments and international agencies.

II. Overcoming Barriers to Technology Transfer.

A government has primary responsibility for designing and implementing methods whereby barriers to technology transfer may be overcome. In addition, it takes cooperative, complimentary actions from the two parties directly involved in the transfer, the technology producer and the technology user, to ensure that research results are applied. The role and responsibilities of each of these three parties must be clarified.

A. Government and Technology Transfer.

A government may encourage technology transfer by direct and indirect means. These means have been discussed and analyzed elsewhere [4][5], so they will be only briefly reviewed here:

a Strengthening the research base. Universities in developing countries are mostly supported by governments; researchers are mostly government employees. As is well known, public universities in most developing countries are underfunded and researchers are underpaid (when compared to persons in the private sector who have approximately the same education and responsibilities). In a time of severe budgetary constraints it is difficult to correct these shortcomings; nevertheless, it must be done before a country can build the scientific/technical base upon which a science-based industry, such as biotechnology industry, can grow and flourish. Accordingly, a significant strengthening of research can only come about if governments take the hard decisions to raise new funds or divert scarce funds from other programs in order to strengthen scientific institutions and finance necessary research.

b Promoting small business initiatives. After the scientist-inventor has conceived a concept, the concept has been researched, and it has been verified as commercially promising in the laboratory, a point is reached where funds for the advanced development or the concept is required. This point is crucial because development will cost on the order of seven to ten times as much as research. However, developmental funds are exceedingly difficult to raise since investors will perceive a venture at this early stage of development as being a risky investment. A government is probably the only source in most developing countries for this kind of funding; if it is not available, the budding venture dies. Accordingly, governments must make funds available for development and pilot plant operations, either as grants or low-cost, long-term loans.

c Tax laws that favor investment and risk taking. Tax laws may be designed to encourage investments in R&D, which is very important to science-based businesses. Governments may do this through three methods. First, laws may be designed to promote investments in R&D by allowing the company which invests its profits in R&D to credit a proportion of this amount against owed taxes. Second, investors who invest in risky R&D should be able to credit losses occurred in doing so against owed taxes. Third, tax laws should be designed so they do not unduly tax profits from profitable investments, thus appearing to penalize success.

d Intellectual property law. There has been much debate on the propriety of patenting life forms and the fairness of allowing patents on procedures having wide applicability, such as the polymerase chain reaction (PCR).[6] This is not the place to debate these issues, it is however appropriate to

note that investors are not likely to invest capital to develop inventions unless they are assured of exclusive, long-term rights to those inventions. Further, companies, whether multinational or national, are unlikely to develop new or unique products in countries where they cannot protect these products adequately through licensing or patenting. Accordingly, governments cannot ignore the intellectual property issues as they concern biotechnology inventions. If governments wish to set up biotechnology-based industries, they will find it well worth their while to consult with the World Intellectual Property Organization in Geneva on this matter, and to analyze the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure in terms of its own national interests.

e Biosafety regulations. Usually research is not effected by lack of regulations since universities are often autonomous, enabling them to adopt their own rules governing R&D. In addition, they can easily adopt rules promulgated elsewhere such as, for example, the National Institutes of Health guidelines or the generic regulations formulated by the Organization for Economic Cooperation and Development (for a general discussion of the subject of biosafety and developing countries, see Trigo and Jaffé [7]). However, the biosafety issue is problematic when we move past research. Most developing countries have not promulgated regulations that specifically address biotechnology research, development, testing or manufacture. In some cases, laws or regulations that deal with the environment, occupational health or industrial activity may have implications for bioscientific or bioindustrial activities, but national agencies will or do not enforce them because they lack the expertise or resources to do so. In view of this uncertain situation in most developing countries, companies will be unable to adequately plan for the development, testing and manufacture of biotechnology products. If they are unable to perform these vital activities, they will in effect be precluded from entering into biotechnology R&D in most developing countries. Accordingly, governments must adopt equitable and adequate biosafety regulations that govern research, development, testing and manufacture.

B. Technology Producer.

The major indigenous technology producers in the developing countries are universities and public scientific or technical institutes. It is imperative that they establish technology transfer units. Often, this will mean that universities will have to break out of the mold set perhaps hundreds of years ago, that of the academic ivory tower. Universities are changing everywhere, even the ancient universities of Europe that once epitomized the academic ivory tower. One reason for this change is that the scientific research done at these universities can no longer be designated as basic (as contrasted to applied research done usually by industry). In biotechnology especially, findings from so-called basic research can have almost immediate applied implications. For example, when a researcher clarifies the molecular control in a cell that produces a protein, he or she is at the same time mapping out a production process that is of interest to industry. Unless the researcher, and the university employing that researcher, is willing to forego a possibly significant financial reward, the university must track the research being done at its laboratories and assess its applied impacts.

Of course, models already exist for such outreach activities. In particular, most national agricultural research institutes have outreach programs that introduce the fruits of their research to farmers. Agricultural outreach programs have been remarkably successful in most parts of the Third World, witness the incredibly rapid spread of the green revolution. There is no good reason why governments should forego trying to emulate the success of agricultural outreach programs in other applied areas. Accordingly, public research institutions in developing countries must set up technology transfer units. These units would have four objectives; to seek out research being done at the home university that may have commercial promises, to obtain patent protection for inventions from research, to present inventions to potential users in the appropriate industries, and to make contractual arrangements between the university and the technology user wishing to apply an invention. It would not be expensive for universities to set up these units; most universities could do it using existing personnel and facilities. Governments should promote the concept of technology transfer units among its country's universities.

C. Technology User.

In industry, results from research can theoretically benefit two types of technology end users; established, traditional firms and new biotechnology-based industries. The first type of firm exist throughout the world, manufacturing goods in industrial sectors such as food, chemicals, natural resource extraction, manufacturing, etc. However, by far most firms in the Third World have capabilities only to manufacture, package and market goods. They do not possess applied research or advanced development units, which limits their ability to absorb or adopt results from indigenous or foreign scientific research. It should therefore be of high priority to the many companies that could benefit from being able to apply research findings to set up industrial technology transfer units. These counterparts to the university technology transfer units would have four responsibilities: to seek out research whose results could be applied by the parent organization; to negotiate with the university for rights to use these results; to perform the advanced development that would enable the parent company to utilize research results; and to contract with the university for further research required to solve problems or improve processes. Since most firms in developing countries would not have the resources to set up industrial technology transfer units, funds for this purpose would have to be provided by governments through grants and loans, as noted above.

The second type of enterprise, the biotechnology-based company, is common in the industrialized world, but only a few of them have been established in the Third World. For example, the only biotechnology company in Peru, Bioingenieria Aplicada, exploits for profit the indigenous natural resources of that country on a sustainable basis and under environmentally sound conditions. It is also the first company in Peru to set up a direct link between industry and university. It is important to encourage the establishment of biotechnology companies in the Third World because they will probably be the main user of research results from indigenous universities and the most important vehicle for the development and commercialization of biotechnology products in developing countries. However, as is the case of technology transfer units in industry, governments have the major role in creating the economic climate conducive to the entrepreneurship of biotechnology-based industry because only they can adopt measures that encourage people to make the risky investments for establishing that industry.

The Role of International Agencies in Technology Transfer.

While a government has the major responsibility for promoting and supporting capability building in biotechnology in its country, international agencies can provide specialized technical assistance to it which hastens the process and lessens its risks. The technical assistance most likely to have positive effects is that which enhances capability building.

In general, there are five major ingredients to capability-building in biotechnology:[8]

- Training - in the first instance, scientific excellence is reached by well educated and trained scientists. Since most Third World countries lack expertise required for biotechnology, and cannot afford to send their scientists elsewhere for training, the providing of training opportunities by international organizations is a very important function.

- Specialized training - we must recognize that capability-building consists of more than training researchers in biotechnology techniques. Scientists and regulators in developing countries must become skilled in particularly two specialty areas. First, they must gain fundamental skills in assessing and managing risks. The objective would be to help scientists and regulators from developing countries gain sufficient skills in the fundamental methodologies that underlie these activities so they can return to their home countries and adapt them to fit local circumstances and conditions. Second, they must become knowledgeable of the intellectual property law that applies to biotechnology products and processes. There are, however, far fewer possibilities for this kind of training than in biotechnology techniques.

- Information - researchers must have an adequate and timely access to information to work efficiently. They will otherwise remain behind scientific progress achieved elsewhere, duplicate work already done, and take approaches that have been proven wrong or unfruitful. Because of scarce resources, Third World researchers are often unable to subscribe to journals, buy books, or connect to data bases. The assistance that international organizations can provide to scientific institutions in developing countries to act conduits for scientific/technical information would be a very important function.

- Equipment - almost all sophisticated equipment used in biotechnology R&D is manufactured in industrialized countries. Third World governments usually do not provide more than minimal funds to their researchers for equipment purchases. It is therefore important that donor nations and assistance agencies provide Third World laboratories with some funds for the purchase of equipment and for their continuing upkeep.

- Supplies - most chemicals and reagents used in biotechnology R&D are manufactured in industrialized countries. They are often expensive and labile. Little financial support is provided by Third World governments for the purchase of supplies. It is therefore important that donor nations and assistance agencies provide resources to researchers in developing countries so they can buy expendable supplies.

While international organizations play a secondary role to governments when it comes to capability building in biotechnology among developing countries, they

can perform important functions that assist the process. Specifically, the can supply some of the major ingredients listed above:

First, UNIDO, the ICGEB and other international agencies ought to provide additional training opportunities to Third World scientists. Several kinds of training should be made available. Some scientists are already well trained in most aspects of biotechnology; they may need only a refresher course or a short course on a specialized aspect of biotechnology to, for example, use the polymerase chain reaction. An intensive two week course should suffice in these instances. Others may require a detailed introduction and some practice on more complex subjects to, for instance, learn how to construct hybridomas to make monoclonal antibodies. In this case, a two or three month course should prove sufficient. Yet other scientists may have done their initial work in a classical natural science discipline, perhaps botany, and need to complete a thorough and wide-ranging curriculum in order to undertake intricate projects in plant biotechnology. This person would benefit most from long-term training of one to two years.

Second, UNIDO and other international agencies should help regulatory officials and scientists in developing countries access the results from international programs or projects that focus on biosafety, such as UNEP's Resource for the Release of Organisms Into the Environment and the UNIDO/UNEP/WHO/FAO Working Group on Biosafety. UNIDO should actively contact all governments of developing countries and present them with copies of the Voluntary Code of Conduct for the Release of Organisms Into the Environment. Further, UNIDO should continue to support courses in the Third World on risk assessment, risk management and constructing regulatory frameworks for activities, including research, the field testing of genetically engineered organisms, and manufacturing processes employing genetically engineered organisms. The short term courses offered during July 1991 by ICGEB on biosafety may be used as a model for courses offered elsewhere. In addition, UNIDO should continue its work to establish the International Biosafety Network and Advisory service according to the terms formulated by the UNIDO/UNEP/WHO/FAO Working Group and help governments access the network and use the advisory services once established.

Third, international agencies should assist the flow of scientific/technical information to the Third World. However, we must realize that the Third World researcher is most commonly prevented from accessing information sources because of substandard telecommunication lines, inefficient national mail services and a lack of computers. In addition, research institutions and researchers do not usually have the funds needed to make expensive international calls, to hook up to a data base, or to purchase journal subscriptions and books. The major barriers to the international flow of information usually have a systemic origin and are not peculiar to biotechnology. Nevertheless, Third World scientists require information about cell culture collection, the preservation of microbial germplasm, and on research material such as DNA sequences, gene probes and monoclonal antibodies. Collecting information, however, is no small task. Every year thousands of journal titles and hundreds of books are published devoted to every conceivable aspect of biotechnology. Much of this is collected in computerized data banks; there are about 30 data banks in industrialized countries devoted to biotechnology. A new phenomena, just beginning to make an impact, is electronic publishing, where each contribution is recorded only electronically. As the amount of information generated by biotechnology R&D expands exponentially every year, the number of journals, books and data banks also increases rapidly. Clearly, the problem with bioinformatics is not the lack of information, it is how to manage data, including making certain that it

reaches those who need it. UNIDO and other international agencies ought to do their best to collect scientific information and make it available to scientists in developing countries. As possible, they should assist governments overcome systemic barriers that hinder the dissemination of information to Third World researchers.

Fourth, UNIDO and other international agencies should develop mechanisms whereby equipment and supplies can be made available to Third World laboratories. Many imaginative possibilities exist for doing so. For example, functioning equipment is discarded by industries and universities in developed countries. An effective recycling mechanism could be set up under the appropriate UN agency's auspices, to collect, refurbish and disseminate used equipment to Third World institutes. Refurbishment centers, possibly set up on a regional basis, could have a dual function, to also train technicians to repair equipment and perform preventive maintenance. Also, UNIDO and WHO could be requested to develop a list containing the names of the 100 or 200 biochemicals vital to biotechnology R&D units. Then, agencies such as the World Bank could be requested to lend money to establish regional centers for manufacturing these biochemicals, which would be sold to Third World institutions at near cost.

Fifth, UNIDO should assist universities and research institutions in developing countries to set up technology transfer units to market their research according to the discussion on pages 11-12, above. Most important, UNIDO and other agencies can provide training on technology transfer to the staff of prospective units; providing training on intellectual property law to scientists and technology transfer personnel; and making available seed funding for setting up technology transfer units.

Sixth, UNIDO can act to help non-profit enterprises that seek to promote entrepreneurship in biotechnology for Third World development. An example is the Biofocus Foundation, incorporated in the Netherlands, and headed by professor C.G. Hedén from Sweden. One of its objectives is to identify commercially promising biotechnology projects in developing countries and assist local entrepreneurs undertake these projects with venture capital and business advice. UNIDO may support Biofocus and similar enterprises by helping them raise operating capital. Further, UNIDO should set up a fund for the purpose of providing seed funding and technical assistance to entrepreneurs so they can establish and operate biotechnology companies in developing countries. This is important because lessons from the short history of modern biotechnology indicates that small biotechnology companies will probably be the main vehicle for the development and commercialization of biotechnology products in developing countries.

Entrepreneurs may also be encouraged by indirect means. UNIDO may raise funds to provide venture capital to regional development banks so they can fund commercial biotechnology initiatives through lending or grants. For example, the Andean Development Corporation (Corporación Andina de Fomento) has a small biotechnology program that funds research at universities and commercial projects in industries. UNIDO should encourage other regional funding organizations and banks to establish biotechnology programs and provide capital for them.

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