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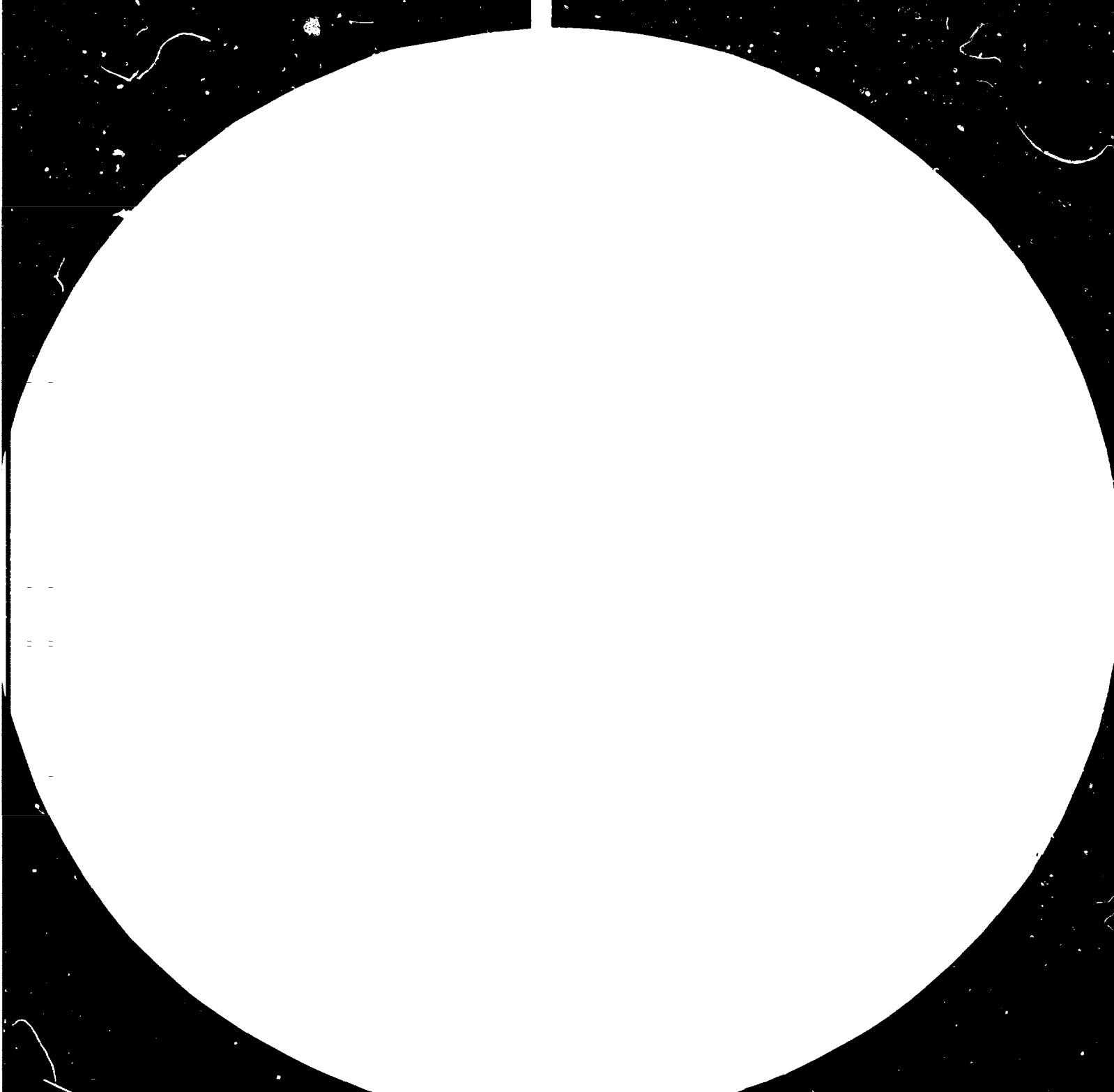

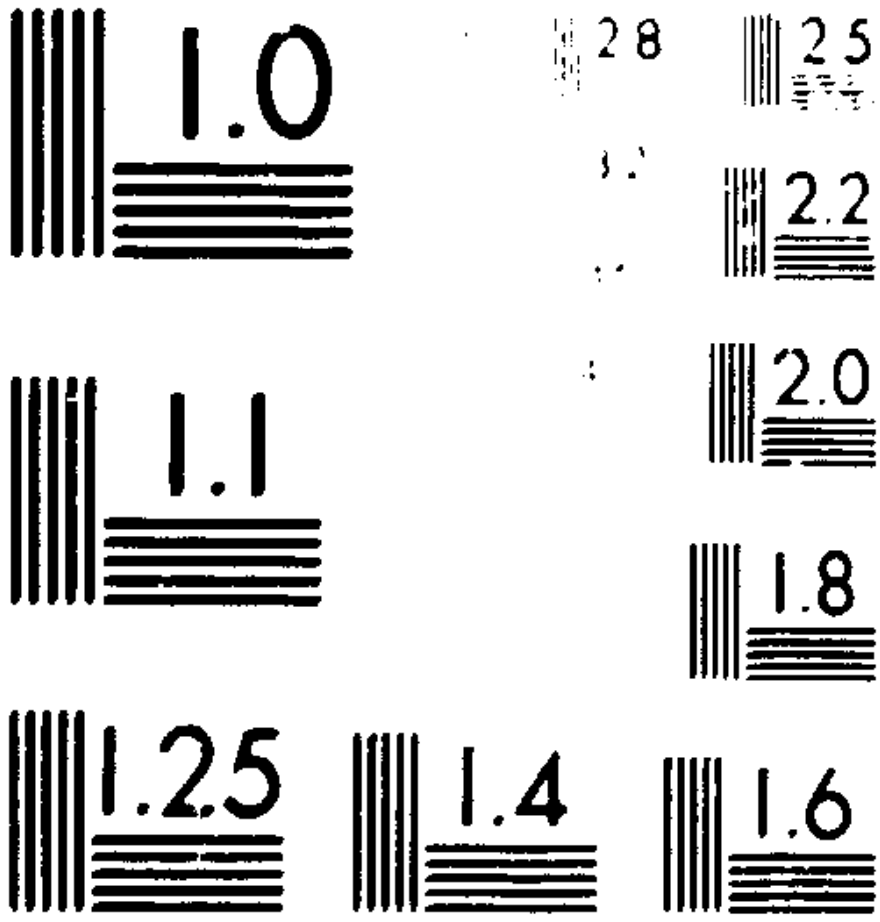
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IMPROVED AGRICULTURAL AND FOOD PRODUCTS THROUGH GENETIC ENGINEERING AND BIOTECHNOLOGY *

prepared by

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A. BACKGROUND AND JUSTIFICATION

Introduction

The technology of genetic engineering is now being applied to a variety of problems in the agricultural and food industries. In particular there are opportunities for the development of new strains of plants both for agriculture and forestry by combining the technology of genetic engineering with plant cell and protoplast tissue culture, for the use on a larger scale and by a different method of "natural" insecticides, and for the development of novel methods of food processing which will use alternative feedstocks for example cellulose, and will offer alternative uses for traditional feedstocks for example sugar. Another major effect of genetic engineering on agriculture and food will be the production of more effective vaccines for livestock.1/

Novel Methods of Plant Breeding

The breeding programmes of certain species and strains of important agricultural and forest plants is being revolutionised by the introduction of plant tissue culture and the regeneration of whole plants from tissue culture. Already the technology has greatly accelerated the rate at which plants can be cloned or vegetatively propagated, that is producing plants which are genetically identical to the single parent. For many plants this may not occur or may occur rarely or slowly by more or less natural methods.

For other plants it has been difficult to produce large number of plants by natural methods. Tissue culture methodology is leading to remarkable changes for example affecting the clonal production of plants as diverse as oil palms, conifers, orchids, citrus and asparagus amongst many others. The scale of the technology can be gauged by noting that "the number of trees (Douglas Firs) that can be grown from cells in 100 litres of media in 3 months are

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1/ See Application of Genetic Engineering and Biotechnology for the Production of Improved Human and Animal Vaccines with Particular Reference to Tropical Descases, ID/WG.382/2/Add.4.

enough to reforest roughly 120,000 acres (48,000 hectares) of land at 12x12 ft spacing". Thus in principle it is possible to identify a single superior forest tree, or oil palm, date palm etc. and use tissue cultures from this single plant to produce a very large number of genetically identical progeny in a relatively short time. The technique is also valuable because it offers a way of producing virus-free stocks. It has the potential disadvantage of establishing genetically homogeneous mococultures but this once recongised can be guarded against.

In addition to the opportunity to carry out clonal reproduction on a large scale, tissue culture offers a variety of other opportunities for plant breeding.

(a) Plant cells which are resistant to toxins or other agents can be selected in tissue culture. Plants regenerated from such tissue culture may then be resistant, as for example in the case of maize regenerated from cells selected for resistance to Helminthosporium maydis mycotoxm.

(b) Intraspecific and interspecific hybrids may be produced by fusing protoplasts derived from the tissues of different plants, and then regenerating hybrid plants from the hybrid protoplasts. This technology has not so far been as successful as was once hoped for, in general only producing viable hybrids in systems in which the two parent hybridise naturally. But there is a great potential for using this method as another tool in plant breeding even within species, introducing genes for disease resistance into established varieties for example. Much work is being carried out in this area and as more experience is gained in the handling of protoplasts it is widely believed that this approach will give rise to novel valuable hybrids.

Genetic Engineering of Plants Using Recombinant DNA

The technology of recombinant DNA, commonly referred to as genetic engineering, was developed in bacteria and particularly in Eschericia coli, but is now in the process of being applied to plants, in conjunction with the techniques of tissue culture, protoplast preparation and the regeneration of plants from tissue culture. The combination of these methods is likely to lead to remarkable advances in plant breeding.

The principal technical objective is to transfer genes in the form of purified DNA from different strains and species into recipient plant cells or protoplasts in such a way that they become stably incorporated into the cells, replicating with the endogenous genes, and being expressed in the cells thus changing the character of the cells and of plants regenerated from the cells. The first stages of such a programme have been achieved now in several systems. The Ti plasmid of Agrobacterium tumefaciens is a highly promising vector into which exogenous DNA can be incorporated and which itself integrates into the DNA of the recipient cells. The DNA plants viruses including CaMV are also being developed as vectors. Studies are under way involving the transformation of plant cells with a variety of genes including ovalbumin, leghaemoglobin, sucrosesynthetase, zein, phaesolin, resistance to gentamycin G148, resistance to methotroxate. These genes are being tested when incorporated into vestors based on the Ti plasmid.

Many of these projects are now at the stage of refining the construction of the recombinant plasmid DNA molecules to obtain expression of the exogenous genes in plant cells.

This is a very active field of research and there are strong reasons for believing that it offers exceptional opporcunities for quite rapid progress when applied to the manupulation of simple genetic systems. These would include genes coding for herbicide

resistance, insect resistance and mycotoxin resistance. For example a bacterial genetic system has been assembled which codes for resistance to 2,4,5 T. If this system is incorporated into a field crop, 2,4,5 T could be used as a herbicide with that crop. The genetic system coding for resistance to a Helminthosporium myadis mycotoxin of maize has been identified as part of mitochondrial DNA. Hence it can be easily purified an important step in developing a genetic manipulation programme to introduce this system into different varieties.

Genetic engineering of plants by recombinant DNA involving the transfer of expression of simple model genetic systems into plants regenerated from tissue culture is likely to be achieved in many systems within a few years. Application of the technology to commercially important systems which will follow will depend on the rate at which a number of research objectives are attained. It is important to develop a range of expression vectors especially for use in monocotyledons, and reproducible methods are needed for preparing and transforming protoplasts from the commercially important plants and for regenerating whole plants from them. It will be necessary initially to concentrate on those commercially important traits which are controlled by single genes, and to develop the biochemical and immunological techniques needed to purify these genes. There are genes coding for disease resistance in a variety of species which would be analysed in this way as a preparation for introduction using recombinant DNA, and there are some genes already available including some bacterial genes which specify toxins which are pesticidal. These are discussed in the next section.

Novel Biological Pesticides by Genetic Engineering

Genetic engineering will have a substantial impact on the products and use of proteinaceous pesticides such as the pesticidal spore proteins of varieties of Bacillus thuringiensis. These are used

as commercial pesticides and are active against a variety of crop pests. It has also been reported that some strains of Bacilli produce toxins which are active against the vestors of yellow fever, filariasis, malaria and river blindness so that the value of this genus is not confined to agriculture. These are many other species of bacteria and fungi which have potential as insecticides, and their value may be increased by applying genetic engineering. The gene coding for the toxin from one variety of B. thuringiensis has been isolated and transferred to another bacterial species where it has been expressed. It will be possible to transfer it into plants and so to test whether they can be made directly toxic for insects which feed on them.

Nitrogen fixation, photosynthesis and other complex processes of plants

It was widely suggested that genetic engineering may be valuable in producing novel strains of plants which fix nitrogen or which photosynthesise more efficiently. It is possible that either or both of these may be realized but it is now generally accepted that such objectives are likely to be difficult to attain and they will probably take many years. It is however certain that genetic engineering has already led to remarkable advances in our understanding of both of these processes especially nitrogen fixation which are complex genetic traits controlled by a large number of different genes, and there are some related projects which appear to be more hopeful because they are directed at manipulating single genes. Some of these are detailed under the work programme on production of fertilizer using biological systems. For example, it seems reasonable to try to manipulate the genes of free-living nitrogen fixing bacteria to cause them to adhere to the roots of important crops which absorb fixed nitrogen from the soil. In principle the concentration of fixed nitrogen should be increased in the immediate neighbourhood of the roots.

It is important for developing countries to have a keen appreciation of the genetic engineering studies being carried out on nitrogen fixation. The plant genetic engineering and the fertilizer programmes to be undertaken at the ICGEB will provide a source of expert advice on this area.

Genetic Engineering and Food Processing

The advent of genetic engineering has greatly increased in the application of biotechnology to the food industry. It is being used in a great variety of ways of which perhaps the most far reaching is in the application of enzymes in food processing. The use of enzymes is a long and well-established tradition in a great many societies- an obvious example is the use of chymosin (rennet) in cheese manufacture and of course enzymatic activities are essential in baking, brewing, distilling and many other ancient and widespread food processes. In modern times the uses of enzymes have become more sophisticated and diversified as more enzymes have become available in partially purified form in large quantities. No about 20 different enzymes are used on what amounts to industrial scale, including amylases, proteases, amyloglucesidase, chymosin, lipases, glucose oxidase, pectinase, glucose isomerase etc. The total market in these enzymes is approaching $ 300 million and is expanding rapidly. They are used to add value to food for example by improving storage properties as in the conversion of milk to cheese or fruit to fruit juices or wine,or by improving the nutrient value as in the conversion of cellulose to glucose. Some enzymes have become extremely important in the manufacture of new foods or food additives. The best known example of this is use of a series of enzymatic activities especially glucose isomerase to produce high fructose syrup from maize. This new sweetener now accounts for about half of the United States sweetener market displacing cane and beet sugar and seriously affecting both the United States and foreign sugar production. There are substantial other

opportunities for innovations of this kind which could have very serious consequences for those developing countries which are primary producers. On the other hand developing countries can use the same general approach to innovate for their own purposes. One example is the use of a series of enzymes to convert cellulose to glucose, using grass, seaweed, algae, straw and other plant wastes such as sunflower and cotton residues as the basic feedstock. Many of the enzymes valuable in the food processing of this kind, converting starch, cellulose or hemicullose into glucose or xylose including β-amylases, amyloglucosidase, pullulanase, β-glucanases and exlanase and ligninases will also be valuable in the development of biofuels.

Genetic engineering will affect the food processing industry by making a much larger range of enzymes available on a larger scale at reduced cost. It will also affect the production of a number of proteinaceous food additives for example the sweeteners thaumatin and monellin. These proteins are over one thousand times sweeter than sugar, but because they are proteins they have a lower calorific value than sugar. They are produced as part of the fruits of some West African areas but in relatively small amounts. The genes for these proteins could be transferred to bacteria to direct the synthesis of the protein sweeteners in large scale fermentations. If the taste of the proteins is not acceptable as a sugar substitute or if the physical properties are not suitable for incorporation in some foods, site-specific mutagenesis could be used to generate many derivatives which may have more desirable properties. Proteinaceous sweeteners, such as the very small synthetic protein Aspartame, are becoming more widely used: if the proteins are large (greater than say about 30 amino acids like monellin and thaumatin) it is likely to be more economic

to make them by genetic engineering than by chemical synthesis. The implication for the developing countries is however clear. The new technology of genetic engineering permits developed countries to produce food materials previously confined to the tropics.

Genetic engineering is being applied in the developed countries to novel agricultural systems and new methods of food production and processing in many different ways. The scale of the effects may be very large as for example in the case of the switch from cane and beet sugar to high syrups caused by the development of a new biotechnological process using enzymes. The gasohol programme of the United States and similar programmes elsewhere may have even more drastic consequences as starch in the form of maize or cassava, and cane and beet sugar are diverted from food to fuel consumption. In effect the economic cast of primary foodstuffs will be affected by the cost of oil. If effective processes are developed for converting cellulose to glucose and therefore to alcohol then the price of cellulose in the form of wood, straw, seaweed, grass etc. will also be affected by the price of oil. It is impossible at this stage to see all the ramifications of these new biotechnologies but it is plain that they are already very large - there are new strong economic pressures to channel food surpluses especially maize to fuel using biotechnology.

At the same time the new biotechnology may be used by developing countries for their own purposes, for example in their own biofuel programmes as in Brazil. Sugar producers can institute their own programmes to manufacture high fructose syrup from cane or beet sugar, one response to the steady fall in world sugar prices. If cellulose becomes an important source of biofuel it can also become an important source of glucose which is an intermediate in biofuel production.

It is easy to see, therefore, that genetic engineering and biotechnology are going to have a very large impact on developing countries. The purpose of this part of the research programme of the ICGEB is to undertake research projects which will help the developing countries to adapt and apply the new technology to relevant aspects of agriculture, forestry and food production. This will mean emphasising research on plants, and on the processing and preservation of food products which are important and which are not being carried out in developing countries. Some research will be concentrated on novel methods of breeding plants including clonal propagation from tissue culture and genetic engineering using recombinant DNA. Other research will be directed towards applications of genetic engineering for the production of enzymes for the food processing industry, and this will entail the establishments of cloning systems in yeast and Bacillus subtilis. These are essential for producing materials to be used in the food industry (because they are generally acceptable under food regulations which prohibit Escherichia Coli Systems) but they are not readily available because of patenting.

The ICGEB should identify a number of species of plants which are important in developing countries and establish breeding goals for these plants which are unlikely to be addressed in developed countries and which are expected to be achieved most effectively by using a combination of plant tissue culture and genetic engineering. These plants chosen should include both monocotyledons and dicotyledons. Presently these techniques are best developed for dicotyledons but it is essential that the ICGEB actively participate in developing the new technology for use in monocotyledons a group of plants which includes all the major grains.

The main emphasis of the programme will be on primary food plants but one element should be directed towards forestry with the same general objective that is to apply the new technology of genetic engineering towards breeding better strains rapidly and on a large scale to assist in the reafforestation to protect soil coverings, to supply timber for local requirements and export, and as a part of energy farming programmes.

The ICGEB programme in food processing will be concentrated on the production of enzymes especially those which catalyse the hydrolysis of the polysaccharides starch, cellulose, hemicellulose and pectin, and the hydrolysis of proteins and lignin. It will be necessary to develop a series of cloning vectors for expression of exogenous genes in yeast and B. subtilis. Such vectors are not readily available, partly because they are extremely important to industry in the developed countries, but they are essential not only for the research programmes at the ICGEB but also for national research programmes in developing countries. The immediate objective of this part of the programme is to develop vectors for both yeast and B. subtilis and apply them to the production of a small number of enzymes for use in food processing. These enzymes will be chosen to take into account the obvious overlap of interest between biofuel and food processing programmes.

B. ACTIVITIES

Research and Development Components

The research in this area will be conducted by two teams, one responsible for the application of genetic engineering to plant breeding and the other to its application to the enzymology of food processing.

The plant genetic engineering team will develop DNA vectors for use in plant of economic importance in developing countries and will use these to introduce exogenous genes into these plants, by for example preparing and transforming protoplasts and regenerating whole plants from them. Single exogenous genes will be chosen which confer pest or desease resistance, or which enhance the nutritional properties, or which confer resistance to herbicides, or which specify other valuable characteristics. There will be active discussions with the Food and Agriculture Organization of the United Nations (FAO), the International Rice Research Institute (IRRI) and other international agencies to coordinate this programme, and to ensure that the plant species and exogenous genes are chosen to the best advantage. Where possible projects will be chosen in association with national research programmes in the developing countries. The focus of this group will be the development and use of plant DNA vectors, based on plasmid and plant DNA viruses, and the isolation and genetic engineering of single genes of potential economic importance. It will concentrate in part on the development of the new technology for monocotyledons because these include the most important food crops, the grains, and because the Ti vectors currently available have been reported not to be effective in this group of plants.

The food processing group will establish expression vector systems for B. subtilis and yeast. These will be used to obtain expression of exogenous genes coding for a series of enzymes used in food processing. The objective in each case will be to engineer strains of B. subtilis or yeast to overproduce these enzymes first on a laboratory scale and then on pilot plant scale in conjunction with the Advanced Biotechnology Group at ICGEB. The enzymes will be chosen in consultation with enzyme producers or users in the developing countries, and the Biofuel Group at ICGEB. Particular attention may be paid to the enzymology of lignocellulose degradation by tropical insects, including gut flora and by tropical bacteria and fungi.

The group will contribute to an annual workshop on the technology of genetic engineering. It will have special responsibility for providing experimental training in the design and use of plant vectors. It will organize a series of expert group meetings to facilitate the application of genetic engineering to problems of plant breeding and food processing in the developing countries. These will include meetings covering different topics such as Plant Vectors, Economic Effects and Biochemistry of Major Genes in Plants, Enzymology of Salt Tolerance, Biochemistry and Genetics of Nitrogen Fixation, Polypeptide Pesticides, all emphasing aspects related to developing countries.

The group will maintain and make available collections of plants, yeast and B. subtilis vectors, bacteria, and plant tissue cultures. These collections will be well catalogued and well publicised so that scientists in developing countries have ready access to them. The group will produce manuals of experimental protocols which will be made available to scientists in developing countries and will be available to consult and collaborate on projects being undertaken in developing countries.

The following results are expected from this work programme:

Application of Recombinant DNA to Plant Breeding

- Plant DNA vectors which can be used to introduce exogenous genes into plants which are important in developing countries, especially food plants including monocotyledons and forest trees, so that these genes are expressed. The vectors are to be distributed to scientists and institutes from developing countries;

- Novel strains of plants which have been vegetatively propagated by tissue culture and genetically engineered by recombinant DNA. The methods and strains are to be distributed to scientists and institutes in developing countries;

- Scientists and technicians trained in this field of research;

- Manuals of experimental procedures;

- Proceedings of conferences and workshops.

Application of Recombinant DNA to Food Processing

DNA vectors to permit the expression at a high level of genes cloned into B. subtilis and yeast. The methods and vectors are to be distributed to scientists, industries and institutes in developing countries;

- Strains of B. subtilis and yeast which overproduced enzymes which degrade polysaccharides such as starch cellulose, hemicellulose and pectin, and the lignin components of lignocellulose. The methods and strains are to be made available to scientists, industries and institutes in developing countries;

- Scientists and technicians trained in this field of research;

- Manuals of experimental procedures;

- Proceedings of conferences and workshops.

C. WORKPLAN

Genetic Engineering of Plants and Food Processing Systems

Year 1:

- Research priorities established by the Council of Scientific Advisers;
- Establishment of plant tissue cultures and regeneration systems for selected plant species;
- Establishment of "current technology" of cloning systems for plants, yeast and B. subtilis.

Year 2:

- Biochemical studies on genetic systems in which single genes code for major plant characters;
- Isolation by cloning of genes which code for major plant characters;
- Biochemical studies on enzymes which hydrolyse polysaccharides and lignocellulose;
- Isolation by cloning of genes which code for these enzymes;
- Development of cloning vectors to allow the stable expression of exogenous genes in plants including monocotyledons;

- Development of cloning vectors to allow inducible high level expression of exogenous genes in B. subtilis and yeast;

- Further studies of plant tissue culture and regeneration.

Year 3:

- Introduction of major exogenous genes and analysis of expression of these genes in plant cells in tissue culture;

- Production of strains of yeast and B. subtilis which expresses genes coding for polysaccharide degradation and lignocellulose degradation at a high level;

- Continuation of biochemical studies on enzymes which degrade polysaccharides and lignocellulose;

- Continuation of biochemical studies on single genes coding for major effects in plants;

- Continuation of studies on plant tissue culture and regeneration.

Year 4:

- Regeneration of plants from tissue culture in which major exogenous genes have been cloned and expressed;

- Pilot scale testing of yeast and B. subtiis strains;

- Application of the technology to other genetic systems in plant breeding and the enzymology of food processing.

Year 5:

- Application of the technology to other systems;
- Field testing of novel plant strains;
- Pilot scale testing of yeast and B. subtilis strains.

D. COLLABORATION WITH OTHER INSTITUTES

- Co-ordination with the Food and Agriculture Organization (FAO), the International Rice Research Institute (IRRI), UNDP and UNESCO;
- Co-ordination with national research programmes in developing countries.

E. PREREQUISITES

A major prerequisite is the appropriate academic qualifications of the trainees from developing countries. Such trainees must have very good background in microbiology and plant physiology and genetics with considerable knowledge in analytical techniques.

F. FINANCIAL REQUIREMENTS

Under the supervision of a senior scientist, the following two teams will be conducting research:

The Genetic Engineering of Plants

The four scientists in this area will be comprised of one expert in plant tissue culture and one expert in plant DNA vectors, and two others with appropriate complementary experience in such areas as the biochemistry of major plant genes affecting economically important traits, polypeptide, pesticides, tissue culture and regeneration of monocotyledons, tissue culture and regeneration of forest trees and other plants species of economic importance in developing countries. The team should include two post-doctoral fellows, four technicians and six trainees.

The Application of Genetic Engineering and Food Processing

The four scientists in this area will include one expert in the biochemistry and enzymology of food processing and one expert in the molecular genetics of B. subtilis (or yeast). There will be two other scientists - one with expertise in the molecular genetics of yeast (or B. subtilis) and the other with expertise in the biochemistry of enzymology of polysaccharide degradation. One of these four scientists must have experience in DNA cloning and one should have worked in lignocellulose degradation. Other components of the team will be one post-doctoral fellow, two technicians and four trainees.

Five-Year Budget

Staff		(US$ thousands)
(first year 40 per cent, second year 60 per cent of full operation)		
Senior scientist	8 man years	300
Junior scientist	32 man years	1440
Post-doctoral scientist	12 man years	288
Technicians	18 man years	408
Subtotal		2436
Management of the Centre and supporting personnel		570
Total Staff		3006

Operational Activities		
Visiting scientists	40 man months	320
Expert group meetings	4	100
Advisory services	30 man months	300
Training	40 man years	900
Information material	30	30
Purchase of chemicals etc.	76 man unit years	760
Associateship		150
Miscellaneous (travel, telephone)		84
Total operational activities		2644
Total Work Programme		5650

ANNEX I

Equipment Requirements

Centralized Facilities

The groups will need access to centralized facilities including:

4 ultracentrifuges
2 scintillation counters
1 electron microscope
media preparation facilities
dish washing facilities
sterilizing facilities
Oligonucleotide synthesis facilities
DNA sequencing facilities
Monoclonal antibody preparation facilities
containment laboratories

Special Facilities for Plant Tissue Culture, Plant Regeneration and Plant Growth

Plant tissue culture rooms;
Plant tissue culture cabinets with controlled temperatures and lighting systems;
Laminar flow hoods;
Plant culture facilities with control systems for light and heat;
Costs are difficult to estimate. Set aside $150,000 as contingency.

Major Equipment

4 Medium speed centrifuges, e.g. Sorvall RC5B
15 Eppendorf type centrifuges
1 Ice machine
2 Spectrophotometers
2 Cold rooms 4°C
10 Orbital Incubators
10 Water baths
20 Power supplies
Electrophoresis equipemnt
4 Fraction collectors

2 Vaccuum ovens

8 Incubators

2 Microbalances

2 Top loading balances

2 pH meters

5 Microscopes

8 - $20^{o}C$ Freezers

4 - $70^{o}C$ Freezers

10 Close up domestic refrigerators

Autoradiography equipment

Polaroid camera

U.V. light boxes

Total cost of the major equipment will be about $250,000.