BIOTECHNOLOGY: OPPORTUNITIES FOR AGRICULTURAL RESEARCH IN LATIN AMERICA*

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INTRODUCTION

Biotechnology has existed for many years, the production of wines, beers, solvents and drugs through the manipulation of microorganisms and plant products are examples of this science. What sets the emerging technologies, or new biotechnology, apart is the use of knowledge about the interior of cells for directing or manipulating their products (1). Recent progress in cellular and molecular biology have application possibilities, unthinkable a decade ago, to all production activities; energy, industry, health, and food supply. In vitro technologies such as cell and tissue culture, monoclonal antibodies and recombinant DNA are beginning to be used in agricultural activities. The latest advances are possible because the basic process of replication, transcription and translation of genetic information are common to all life forms.

Emerging biotechnologies should be relevant to agriculture in developing countries. Numerous opportunities are directly related to the needs of developing agriculture: modifying crop plants to tolerate adverse environments, to increase disease resistant, and fix nitrogen more efficiently; increasing the nutritive value of agricultural products, carrying out quick disease diagnoses, producing useful compounds by bioconversion, etc. On the short or long-term, success will depend on the utilization of emerging technologies to reduce time, space, and costs in traditional crop improvement strategies, which necessarily requires a multidisciplinary approach. Breeders and agronomists must be the final recipients of these technologies, and the usefulness scope of their products must be demonstrated in the field.

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The purpose of this document is to discuss the opportunities offered by biotechnology for agricultural research and its application in national research programs and international agricultural research centers in Latin America. Cooperation among these institutions in research and technology transfer, will bring the benefits of biotechnology to the groups that are most in need. The current status of the most important technologies will be briefly analyzed, the situation in Latin America will be discussed, and possible areas of research and cooperation strategies, within and outside the region, will be suggested.

CURRENT STATUS OF BIOTECHNOLOGY

Biotechnologies with the greatest applicability potential in agriculture are: cell and tissue culture, recombinant DNA and gene transfer, monoclonal antibodies, and bioconversion.

CELL AND TISSUE CULTURE

Clonal propagation in vitro

Tissue culture is the route through which most forms of genetic manipulation should take in transition from the laboratory to the field. Therefore, the ability to regenerate plants by means of cell and tissue culture is necessary for the utilization of most biotechnologies.

Plant regeneration can take place by non-adventitious routes, e.g. enhancement of axillary shoots; or through adventitious routes, e.g. differentiation of organs directly from plant parts in vitro, or from cell masses or callus induced on plant parts; and, by means of somatic cell embryogenesis, which consists of the differentiation of embryo-like forms from individual cells of isolated plant parts or callus.

In 1968, there were approximately 30 species which were propagable in vitro, there were more than 300 in 1978; and a 10-fold increase is

expected over the next decade (2). Schemes for massive *in vitro* propagation are known. Somatic cell embryogenesis offers tremendous propagation potential, in addition to the possibility of producing "artificial seed" by encapsulating embryos in gels. In spite of the fact that *in vitro* propagation of difficult species (e.g., coniferous trees, mango trees, palm trees, rubber trees, etc.) is being developed, the control of regeneration in economically important plants is still one of the most important constraints for the effective utilization of the new biotechnologies. Advances in the metabolic and molecular regulation of cell differention should eventually allow the control of plant regeneration *in vitro*.

Disease elimination--Disease-free clones have been recovered from approximately 55 crop species by tissue culture. The technique is valid for all pathogens, but is especially useful for eliminating virus and viroids from vegetatively propagated plants. The most used method is culturing the shoot apical meristem plus one or two leaf primordia. Thermotherapy, or chemotherapy, is frequently applied to infected plants or to *in vitro* cultures (3). Since all plants regenerated *in vitro* are not necessarily virus-free, the use of virological techniques to demonstrate the absence of the viruses is important.

Tissue culture has been used to rehabilitate local cultivars which had lost their vigor and yield, (e.g. potato, strawberry, fruit trees, cassava, etc.). The yield of two cassava cultivars doubled and tripled, respectively, as a result of tissue culture from virus-infected plants (3). Yields of the variety "secundina" increased from 9 to 25 t/ha, and remained stable for three years; however, yield of a hybrid decreased after the third year of planting in the same region (Table 1). Interestingly, a 30% and 50% yield increase have been obtained with two cassava cultivars, respectively, following *in vitro* propagation of apparently healthy plants (3). These results demonstrate the potential of *in vitro* propagation for the production of basic "seed" in vegetatively propagated crops. Yield increase is often accompanied by changes in plant morphology which favor a more efficient distribution of

growth; this has been manifested in cassava by increases in the harvest index.

Germplasm conservation and exchange--Disease-free clones can be used for germplasm exchange by in vitro techniques, thereby minimizing the risk of disease and pest dissemination. Furthermore, germplasm collections can be stored in vitro, reducing cost of maintenance in the field, and loss of material due to diseases and pests, weather changes, and soil problems. A simple procedure of in vitro conservation consists of minimizing the growth rate of cultures by temperature or through modification of the culture medium. Ideally, although still experimental, storage of plant material in liquid nitrogen (-296°C) should provide the maximum statibility on the long term.

Wide crossing

The excision and culture of immature embryos on defined culture media have been used to obtain viable plantlets from interspecific or intergeneric crosses, which otherwise might not succeed. Hybrids from more than 50 crosses have been obtained by using embryo, ovule, and ovary cultures (4). Other techniques such as ovule fertilization in vitro and embryo implantation in normal endosperms also can be used to obtain viable F_1 hybrids. Callus induction using the immature interspecific hybrid embryos, followed by plant regeneration can be a rapid way to produce hybrid plants with more stable genomes (5).

Haploidy

The incorporation of haploids into breeding programs is desirable to obtain rapid homozygosity, to incorporate and rapidly fix new genes after sexual recombination or after mutagenesis, to increase selection efficiency, and to minimize retention of deleterious genetic material; in addition, haploidy could pave the way to F_1 hybrid seed production in cross-pollinated and heterozygous species.

The most common method for producing haploids is the culture of anthers containing inmature pollen. Plant regeneration, via somatic cell embryogenesis or via organogenesis, depends on genotype, developmental stage of the microspores, culture medium, and anther or bud pretreatments. Callus formation can lead to changes in chromosome number of the regenerated plants. Anther culture has been used to produce haploids of 47 species including rice, oats, barley, wheat, rye, maize, triticale, pea, potato, etc.

Other systems for the production of haploids are: cromosome elimination during culture of interspecific hybrid embryos, e.g., barley with H. bulbosum; by parthenogenesis, e.g., in potatoes, following crossing with S. phureja; use of genetic markers, e.g., seed color in maize; genetic induction, e.g., ig gene in maize and hap gene in barley (2). In practice, haploids have been incorporated into potato, wheat, Brassica, and rice breeding programs. In Latin America, rice breeding for certain ecosystems where only one harvest per year is possible such as the upland rice areas of the savannas and the Southrn Cone countries, is a process which can take 10 years. Homozygous lines, produced by anther culture from F_1 or F_2 hybrid plants, can be evaluated rapidly by the breeders. This would reduced the breeding process by 4 or 5 generations with enormous savings in land, labor and inputs. Homozygous rice lines, generated through anther culture, with high tolerance to aluminum toxicity, dwarfness, precocity, and insect resistance is under evaluation at CIAT.

A requisite for the use of anther culture in breeding programs is the production of a very high number of lines. Regeneration depends to a great extent on genotype, but frequently F_1 or F_2 hybrids show a higher rate of regeneration than the parents. In the case of rice, the tissues of the anther wall most of the time do not take part in callus induction and, approximately 50% of the regenerated plants from microspore-derived callus become diploid spontaneously; therefore they are homozygous. One way of increasing callus production is culturing anthers which are floating on liquid medium (6). In this manner, each callus coming from one microspore can be isolated and cultured as a

genetically distinct line. Each callus can in turn give rise to phenotypically different plants; this type of variation, and the one that can occur in anther culture of pure lines has been called gametoclonal variation (Figure 1).

Somaclonal variation

Somaclonal variation is the increment of genetic variability in plants regenerated by tissue culture. Variation of monogenic and polygenic, as well as qualitative and quantitative characters, has been observed in regenerated plants, e.g., oats, wheat, sugar cane, maize, tomato, potato, rice, alfalfa, and tobacco. The phenotypic variation of somaclones can result from epigenetic or genetic changes. Epigenetic variation can be caused by alterations in gene expression which is not sexually transmitted, but may be amenable to vegetative maintenance. Such alterations can result from amplification or diminishing of gene copy, or from the movement of transposable elements to positions of the genome influencing its expression temporarily (5).

Genetic variation can be caused by changes in the primary structure of the DNA; insertion, deletion, or substitution phenomena in the nuclear or cytoplasmic genetic material; it can also be due to changes in chromosome structure such as inversion and translocation (7). Genetic variants must be in accordance with heredity laws; this analysis must be carried out at the first or second sexual progeny of the regenerated plants.

Fertile somaclones with T toxin resistance have been selected in maize, starting from cytoplasmatic sterile and toxin susceptible plants; this change has been associated with the loss of a specific fragment of the mitochondrial DNA; in the same way, somaclones have been selected in wheat and rice for plant height, precocity, size and number of grains, panicle size, etc. (5).

Somaclonal variation has the potential to generate useful variability in adapted cultivars without hybridization; also, somaclonal

variation can help to increase the introgression of genes through wide crossing.

Due to the current impossibility to controlling somaclonal variation it is necessary to regenerate a high number of somaclones for use in more conventional selection schemes.

Isolation of mutants

In vitro techniques make possible the selection of mutants which can condition useful agricultural changes. Compared to conventional selection, mutant isolation in vitro allows the application of very high selection intensities to a very large number of individuals, e.g., one flask with 100 ml of culture medium can contain 5×10^4 callus-forming cells, 5×10^6 cells in suspension, or one gram of leaf tissue can produce $2\text{-}4 \times 10^6$ protoplasts.

Up to date, 51 cell phenotypes from 20 species have been selected, but only 25 phenotypes from 8 species have been regenerated; and genetic analyses of the regenerated plants have been conducted in 9 of these (8).

This technique has been used to select resistance to pathotoxins, e.g., potato late blight, maize T toxin, and alternaria in tobacco; resistance to fungi growth, e.g., Phoma spores in Brassica; tolerance to salinity, e.g., rice, tobacco; tolerance to aluminum toxicity, e.g., tomato; resistance to herbicides, e.g., tobacco, potato; cold resistance, e.g., carrot and tobacco; increasing free aminoacids, e.g., lysine and threonine in maize grains due to elimination in enzyme feed-back inhibition; increasing aminoacids in vegetative tissues, e.g., tobacco, carrot, and barley; antibiotic resistance, e.g., tobacco, and carrot (7, 8).

In vitro mutant selection is limited to traits with simple genetic control, and is restricted to species with efficient regeneration from uniform cell or protoplast populations. In principle, the method can be

applied when the trait in question can be expressed at the cell culture level. Stress application to the plant, followed by culture and regeneration of putatively mutated cells, could be an alternative strategy.

In vitro selection of desired genetic recombinants can be accelerated by applying specific stresses during anther or microspore culture of F_1 hybrids; e.g., genotypes tolerant to aluminum toxity, to iron toxicity, to pathotoxins, to salinity, etc.

The application of temperature or other type of stresses at the time of pollen germination and growth could be an interesting technique to select pollen carrying the desired genes. These traits could be rapidly fixed through the culture of anthers of the F_1 plants.

Protoplast fusion

This is another mechanism for wide crossing, especially when sexual incompatibility prevents conventional crossing. In addition, it could permit nuclear and cytoplasmic gene recombination differing in this respect from sexual crossing.

Apparently there are no barriers to protoplast fusion; nevertheless the integration of parental genomes in the fused cells can be nill, partial or complete. It is necessary to have a vigorous selection scheme by which the isolation of fused hybrid cells in sufficient quantities for genetic analysis is possible.

Cytoplasmic fusion, followed by degeneration of one of the parental nuclei, can lead to the formation of cytoplasmic hybrids or cybrids. Elimination of parental nuclei can be performed by micromanipulation with laser rays or radiation. This is a promising mechanism for the transfer of cytoplasmatic traits such as herbicide tolerance, resistance to some diseases, male sterility, etc. Cybrid plants have been selected with resistance to atrazine and having male cytoplasmatic sterility, from fusions between Raphanus nucleus and Brassica cytoplasm (9).

Difficulty in regenerating plants from hybrid cells or colonies is an important limitation to the use of protoplast fusion. Up to now, only 5 fertile plants have been obtained from 13 regenerated intraspecific somatic hybrids, as well as 15 fertile plants from 28 interspecific ones, most of them belong to the family Solanaceas (10). An important somatic hybridization between S. tuberosum and S. brevidens has recently been achieved (11). While these potato species are sexually incompatible, the latter has genes for resistance to commercially important viruses.

RECOMBINANT DNA

It is possible to purity, characterize, and even synthesize specific DNA segments through current in vitro techniques for DNA manipulation; thus the posibility of directed genetic modification of plants now exists. The process is called genetic engineering.

Genetic engineering, which includes recombinant DNA techniques, involves several interrelated steps: gene characterization; isolation of specific DNA sequences; DNA cloning; transfer of DNA to an appropriate receptor; plant regeneration from the transformed receptors cells, protoplasts, callus; gene expression in the mature plant and sexual transmission of the trait (1).

Gene characterization

Ideally, the trait chosen for manipulation must be translated into a specific product, (an enzyme, for example) and be controlled by one or few genes. Currently, it is not possible to manipulate multigenic traits. Among the important genes that have been isolated and characterized are: the enzyme ribulose -1,5-biphosphate carboxilase, oxygenase, important in photosynthesis; the enzyme: alcohol dehydrogenase from maize; seed storage proteins of cereals and legumes (phaseoline, lectin, zein, etc.); several genes for N fixation (Nif. and Hup); three nitrogenase genes from Anabaena; nodulation genes from Rhizobium. The lack of well-characterized genes of economic importance

is one of the strongest constraints on genetic engineering; nevertheless progress in molecular biology is so fast that other gene sequences will gradually become available. In order to characterize genes, it is necessary to identify the portions of the DNA responsible for coding the trait. The insertion of transposable elements into plant genomes can help to isolate and characterize the DNA sequences underlying an altered phenotype (2).

Isolation of DNA sequences

The general procedure for isolating DNA sequences coding for a trait, consists of isolating the messenger RNA (mRNA) from tissues that synthesizes large quantities of the gene product, then, building a complementary DNA (cDNA) to the mRNA by means of reverse transcription. Another strategy consists of determining the partial amino acid sequence of the protein produced by the gene in question and translating this sequence into a DNA sequence. These steps can be carried out using special machines, with time and cost savings.

DNA cloning

Treating nuclear or cytoplasmic genomes with restriction enzymes, yields specific DNA fragments of variable size. These fragments can be spliced to bacterial plasmids for storage to form the so-called gene libraries. The cDNA, built as above, can be used as probes to detect and isolate specific DNA sequences from structural genes. For this, the DNA fragments of the gene library are separated electrophoretically and immobilized in a matrix to which a radioactive cDNA probe is applied. Complementation of the nucleotide sequences of the probe and the structural gene is visualized by autoradiography. The cDNA requires amplification which is accomplished by splicing to plasmids before the hybridization step.

Plant DNA cloning can be a routine procedure, provided that genes have been fully characterized.

Gene transfer

DNA must be introduced in a plant in such a way that genetic information can be expressed; to achieve this, DNA must pass successfully through all the cell's surveillance systems to reach the nucleus. Several transfer strategies exist: direct microinjection to cell or protoplast nuclei, co-culture with haploid or diploid cells or protoplasts, and use DNA-containing virus and bacterial plasmids as vectors.

Plasmids Ti and Ri of Agrobacterium are the preferred vectors to transfer genes to dicotyledons. The DNA sequence to be transferred is spliced to the T-DNA region of plasmids and, after the plant cells have been infected with bacteria, the T-DNA covalently joins the nuclear DNA. Recently it has been possible to mutate the T-DNA and eliminate its oncogenic effect (12).

Selection of transformed cells

Since the frequency of transformation is generally low, it is necessary to treat large cell populations and use efficient selection systems. Availability of genetic markers is therefore very important, e.g., identification of opines synthesized by transformed cells (12), differential resistance to antibiotics, etc.

Plant regeneration and gene expression

Plant regeneration from transformed cells is of crucial importance for the practical utilization of genetic engineering. It is not enough to demonstrate transformation at the cellular level; the transferred trait must be the expressed in the mature plant and in its sexual progeny. Furthermore, gene expression must occur developmentally in the correct plant tissue or organ; for example, expression in roots of genes coding for inhibition in leaf respiration could kill the plant.

OPPORTUNITIES FOR CROP GENETIC ENGINEERING

Improvement of crop varieties by recombinant DNA techniques is not too far off, at least in the case of traits with simple genetic control. Until now, most work has dealt with model systems (e.g., tobacco, and carrot). Gene transfer for antibiotic tolerance, using Ti-plasmids as vectors, through tissue infection or co-culture with protoplasts, has resulted in the expression and sexual transmission of the trait (13). Also, introduction and expression of genes encoding the bean seed protein phaseoline to tobacco plants was accomplished, using the Ti plasmid as vector.

The following are some of the plant genetic engineering subjects requiring more study and development: identification and characterization of economically important genes; developmental regulation of gene expression; manipulation of weakly linked structural genes or genes with independent segregation; manipulation of traits controlled by nuclear and cytoplasmic genes simultaneously; transfer of polygenic traits; development gene vectors for monocotyledonous plants; efficient plant regeneration from transformed cells.

A great challenge to genetic engineering is achieve the subtle combination of a large number of genes, each with small effects, into a single genotype, as has been the aim of traditional breeding. The introduction of specific genes to a selected cultivar without disturbing the adapted genetic background of the cultivar might be a useful application of modern techniques.

Among the traits amenable to recombinant DNA manipulation, the storage of seed or tuber proteins may be worth considering. Short fragments of DNA, encoding proteins with improved levels of essential aminoacids could be synthesized in vitro and linked to Agrobacterium plasmids. Following plant or cell infection, regeneration of plants is a necessary step.This is a possibility in the case of bean phaseoline whose genes have been well characterized, and are known to be transmitted as a single Mendelian block (7). Similarly, the

manipulation of protein quality and quantity in potato, sweet potatc, barley, wheat, and soybean are interesting possibilities, as is the introduction of protein-encoding genes to cassava. In cases where regeneration techniques are not available, other strategies such as DNA microinjection to germinating pollen grains, or developing ovules, could be utilized. Soaking germinating pollen in solution of small DNA fragments, before pollination, is another interesting transformation technique.

Other traits with potential for manipulation are: tolerance to heavy metals and/or salinity; resistance to herbicides, pathotoxins, and viruses. Manipulating genes responsible for nitrogen fixation is a long-term task because of the complex genetic regulation of the process.

Finally, yield potential is a very difficult trait to improve by traditional breeding methods. Biotechnology can offer some strategies for gene transfer from wild species: in a shorter term, hybrid embryo culture and anther culture from F_1 hybrid plants and, in a longer term, gene transfer from distant relatives using recombinant DNA techniques.

Other Uses of Recombinant DNA

It is possible to construct a cDNA using a virus RNA as template. The cDNA can be used as molecular probes of high specificity and sensitivity to detect virus or viroid nucleic acids through the formation of RNA-DNA hybrids (14). This spot hybridization technique can be used to select germplasm resistant to viruses, or for detection of virus-free plants. Once the virus has been purified, the development of this technique is rather fast. A current limitation is the requirement of a radioactive label for the probe; nevertheless there is progress in the development of non-radioactive molecular probes.

In a similar manner, DNA probes can be built from DNA fragments encoding specific traits. Such probes can be used to select germplasm with genes that have been introduced by wide crossing (15).

MONOCLONAL ANTIBODIES

Hybridoma cultures, resulting from the fusion of cancerous cells and antibody cells that produce a single type of antibody, have the ability of secreting highly specific antibodies *in vitro*, indefinitely (16). Monoclonal antibodies can be used to detect pathogens, particularly virus, when serology is not specific enough, or serum is not available in sufficient quantities. Monoclonal antibodies can also be used for rapid and low-cost disease diagnosis, in seed certification programs or quarantine work.

BIOCONVERSION AND PRODUCTION OF USEFUL METABOLITES

Total utilization of the plant, and not only fruits or grains, is an interesting possibility. Plant biomass separation in cellulose, hemicellulose and lignin fractions could provide material for bioconversion. Microbial protein, solvents, and chemicals are possible bioconversion products. Research should be done on lignin utilization in agriculture as cellulose fermentation. These technologies have received great attention in some developed countries such as Japan.

Production of specific plant substances using modern biotechnology may be a more interesting possibility for developing countries. Extraction of certain compounds, (pigments, alkaloids, antimicrobial substances, drugs, insecticides, etc.) that occur naturally in native tropical species have occupied scientists' attention for many years. It now is possible to culture cell lines, that can produce high yields of a given compound, on a large scale using bioreactors. Product extraction can be made more efficient through cell inmobilization techniques. This alternative can be useful mainly when the species grows in distant places, in small populations, or when field propagation is difficult. In contrast with recombinant DNA biotechnologies, plant regeneration is not necessary in this case; genetic modification for producing different chemical structures is a possibility for the future. Nevertheless, one must remember that plant cells, in comparison to microbes, grow more slowly; conservation of specific cellular lines can be difficult,

although cryogenic methods may offer solutions in the future. For these reasons, plant cell culture could be used mainly for the biosynthesis of high unit-value products.

AGRICULTURAL BIOTECHNOLOGY IN LATIN AMERICA

NATIONAL INSTITUTES

Table 2 shows the number of national institutes in Latin America where various kinds of biotechnological research of current or potential relevance for agriculture is conducted. This information was drawn from 70 institutes in 11 countries, giving a clear, though not complete, idea of the present situation in Latin America. A Latinamerican directory of biotechnology is being prepared which will include persons being trained at institutes in developed countries.

Clonal propagation in vitro, a technology which has reached practical application, is being used at the national agricultural research institutes (e.g., INTA in Argentina, INIPA in Peru, etc.) and at higher education institutes (e.g., universities) for crop disease elimination in vegetatively propagated crops and fruit and forest trees. As can be predicted, research in more modern technologies, (somaclonal variation, protoplast culture, recombinant DNA), besides basic studies on biochemistry and morphogenesis, is concentrated in the basic research institutes of the region (e.g., SENA in Brazil, CENIC in Cuba, etc.) and in higher education institutes. Nevertheless, very useful technologies, such as embryo rescue and haploidy, have not been sufficiently adapted in the national agricultural research institutes.

From the above, the following division of labor emerges: the basic research institutes and higher education institutes conduct work with emergent, and thus less developed, technologies; national agricultural research institutes are mostly engaged in those technologies which have reached practical application. Exception occur in two which conduct recombinant DNA-related research.

Three basis research institutes and one higher education institute can be identified in the field of molecular research, with potential to conduct research in plant recombinant DNA technologies. Collaborative research projects between national agricultural research institutes, basic research institutes, and higher education institutes are lacking; such collaborative projects could help direct the basic studies of the latter toward relevant agricultural problems of the region.

INTERNATIONAL AGRICULTURAL RESEARCH CENTERS

The national agricultural research institutes and the international agricultural research centers make up a system for research in agriculture. They cooperate to achieve increased productivity of basic food crops and cattle production in Latin America. This cooperation should continue in the field of biotechnology.

Table 3 shows the state of development of several biotechnologies in the Latinamerican international centers: CIAT, CIMMYT, CIP, and CATIE. Technologies with potential to reduce time, space, and costs of specific processes have been developed in conjunction with institutes in developed countries. The objective of the international centers is the integration of the new techniques into current breeding strategies. In this fashion, clonal propagation by tissue culture techniques was the first to be adopted (Table 3). The complete implementation of these techniques has facilitated the flow of germplasm between the international centers and national agricultural research institutes, as well as the development of germplasm banks of vegetatively propagated crops. Other technologies used at these centers help to achieve interspecific and intergeneric transfer of valuable traits; thanks to anther culture, a final product can be obtained in the laboratory which is similar to the product obtained in the field, but saving time and costs. Advanced molecular technologies are used to increase the efficiency of virus detection. Soon it will be possible to select cell variants in the laboratory that can be extrapolated to plants with new attributes.

The international centers should continue acting as a bridge to transfer the available biotechnological advances from institutes in developed countries to tropical agriculture. To accomplish this, the centers have the comparative advantage of the multidisciplinary approach of their research programs, as well as knowledge of the factors that limit progress in their research areas. World germplasm collections at these centers are another important asset.

DEVELOPED INSTITUTES

Most investment in biotechnology is concentrated on human and animal health, (interferon, insulin, growth hormone, hemophilia treatment; in vitro disease diagnosis, vaccines, etc.) and plant bioprocessing. Including basic research companies (e.g., Biogen, Genetech, Genex, etc.) and multinational companies (e.g., Monsanto, du Pont, etc.), at least 500 biotechnology companies operate in developed countries such as the United States of America, Japan, Germany, France, England, and Switzerland. In the United States more than 35 basic research companies are active in the agriculture, with an investment close to 3 billion dollars (17).

Basic research for the development of biotechnologies is carried out in primer research institutes and universities. Multinational companies invest in start-up research companies which in turn consider the multinationals as a means of commercializing their products rapidly. Especially noteworthy is the increasing participation of start-up and multinational companies in basic research projects carried out by universities. The former invest in the universities, or scientists from the universities are hired by private companies. This phenomenon tends to the privatization of biotechnological research in the developed countries (18).

COOPERATION IN BIOTECHNOLOGICAL RESEARCH

To develop biotechnological research capability in Latin America, it is neccessary first to have adequate access to information on basic

aspects and techniques. Traditionally, universities in developed countries have been the main source of scientific training for Latin America, it is desirable that this relationship continue in biotechnology. In view of the current tendency to privatization of biotechnological research, cooperation between national and international institutes and universities in developed countries should be strengthened.

Figure 2 shows the current relationships and proposed new areas of cooperation. It is essential that the national institutes for agricultural research in Latin America prepare themselves to use the most developed biotechnologies, however with few exceptions, this is not the case. The international centers can select technologies with higher potential for transfer to the national institutes in view of the comparative advantages previously mentioned.

National agricultural research instititutes should also establish research links to basic research institutes and higher education institutes with a good level of basic preparation. The establishment of research relations between international centers and national institutes and the basic research companies in developed countries is an alternative. The basic research companies develop technologies mostly oriented to highly develop markets; besides, the crops or varieties which are subject of improvement at those companies generally are not basic to tropical agriculture. For these reasons, it is likely that universities in developed countries will continue to be the main source of biotechnology training for Latin America.

In the case of several tropical crops, which have received very little attention in industrialized countries, developing the basic knowledge on genome organization and genetic regulation, which can eventually lead to the manipulation of important traits, is fundamental.

Biotechnology research and development progress rapidly. Besides the well-known journals on cell and tissue culture, molecular biology,

molecular and applied genetics, etc., the following are possible sources of critical information on biotechnology:

1. International conferences: International Association for Plant Tissue Culture (every 4 years); International Plant Molecular Biology Association (every 2 years).

2. General news: Nature (monthly) and Science (monthly).

3. Information on Activities of institutes, conferences, methodologies, and bibliography: IAPTC Bulleting (quarterly), Plant Molecular Biology Newsletter (every 2 months).

4. Summaries of new discoveries: Agricell Report (monthly), Agricultural Biotechnology News (every 2 months), Genetic Engineering News (8 per year), Mc Graw Hill's Biotechnology News Watch (every 2 weeks).

5. Research summaries: Molecular Biology Reporter, Biotechnology Research Abstracts (monthly), Bio/Technology Telegen Reporter (monthly), Telegen Reporter Review, Index and Abstract (annual), Telegenline (data base), Telegen Alert (hot line every 7 days).

6. Technology identification and evaluation: ATAS Bulletin of the Science and Technology Development Center, United Nations (bi-annual).

7. Short training courses in Latin America:
   Subjects: Selected techniques on tissue culture,
   General methodologies on tissue culture,
   Molecular biology and genetic engineering techniques.

   Institutes: International Agricultural Research Centers,
   ICRO/UNESCO.

National Institutes: Campomar Foundation, Argentina; Pontificia Universidad Católica, Chile; SENA, Brazil; IDEA, Venezuela; National Autonomous University, Mexico.

Another source of collaboration in biotechnology for developing countries will be the UNIDO program which has created an International Center of Genetic Engineering and Biotechnology. This Center has been situated in Trieste, Italy and Dehli, India.

RESEARCH AREAS

Undoubtedly, progress of Latin American agriculture will continue to depend primarily on crop breeding. Therefore, the new technologies must be oriented, directly and indirectly, toward this objective. Some opportunies for short, medium and long term research in biotechnology are:

SHORT-TERM

1. Rehabilitation of local clonal cultivars to increase in yield and quality of crops.

2. Basic "seed" production in clonal crops for later mass propagation through conventional and unconventional techniques.

3. Massive propagation of new food and industrial species, (American tropical palms, fruit trees, forest trees, industrial fruits, etc.)

4. Improving of yield potential of crops and increased tolerance to adverse environments (heavy metals, salinity, drought stress, low temperature, etc.) through interspecific or intergeneric crosses, or through in vitro manipulations before or after pollination.

MEDIUM-TERM

1. Shortening traditional breeding processes through fixation of traits in homozygous lines produced by haploidy (anther culture, parthenogenesis, chromosome elimination, etc.).

2. Rapid disease diagnosis using molecular probes and monoclonal antibodies.

3. Production of varieties with desirable agricultural traits through somaclonal selection. This is especially useful for improving adapted varieties without sexual crossing.

4. Selection of variants at the cellular level, with tolerance to salts, aluminum, herbicides, pathotoxins, and other stresses; and increase of nutritional value.

LONG-TERM

1. Increase hybrid vigor in cross-pollinated and heterozygous crops, through rapid homozygosis followed by sexual crossing.

2. Transfer of cytoplasmatic male sterility, herbicide resistance and disease tolerance through protoplast fusion and organell transfer.

3. Genetic engineering transformation of crops. Introduction of DNA sequences encoding traits of economic importance: tolerance to adverse environments; improvement of grain, tuber, and root protein quality; increase of yield potential and total biomass yield of crops; increase of photosynthetic efficiency and nitrogen fixation.

This classification is relative to current and future (next decade) development, of the national agricultural research institutes in the region.

CONCLUSION

Biotechnology now offers a wide range of new technologies that can increase the efficiency of specific processes in crop improvement. Choice of the appropriate technologies is essential to success in applying them. This requires that national research programs give priority to training personnel in emerging technologies; therefore, access to biotechnical information is of critical importance. Cooperation among national agricultural research institutes, international research centers, and universities and institutes in developed countries is indispensable to develop biotechnological capabilities in Latin America.

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TABLE 1. Yield of cassava (cv. Secundina) in the north coast of Colombia following elimination of a viral mosaic disease by tissue culture and comparison wih the hybrid CM 342-170.*

Clone	Cycle	Fresh roots (t/ha)	Starch (t/ha)	Stem cuttings (No./plant)
Secundina	1st year	25.1 a	7.1 a	10 a
	2nd year	23.0 a	6.8 a	10 a
	3rd year	22.0 a	5.6 a	9 a
	Control**	8.9 b	2.1 b	3 b
CM 342-170	1st year	34.8 a	7.9 a	14 b
	2nd year	36.2 a	8.4 a	10 ab
	3rd year	15.1 b	3.1 b	6 b

* Adapted from: Annual Report, CIAT, Cassava Pathology, 1984.
** Conventional planting material, without tissue culture.

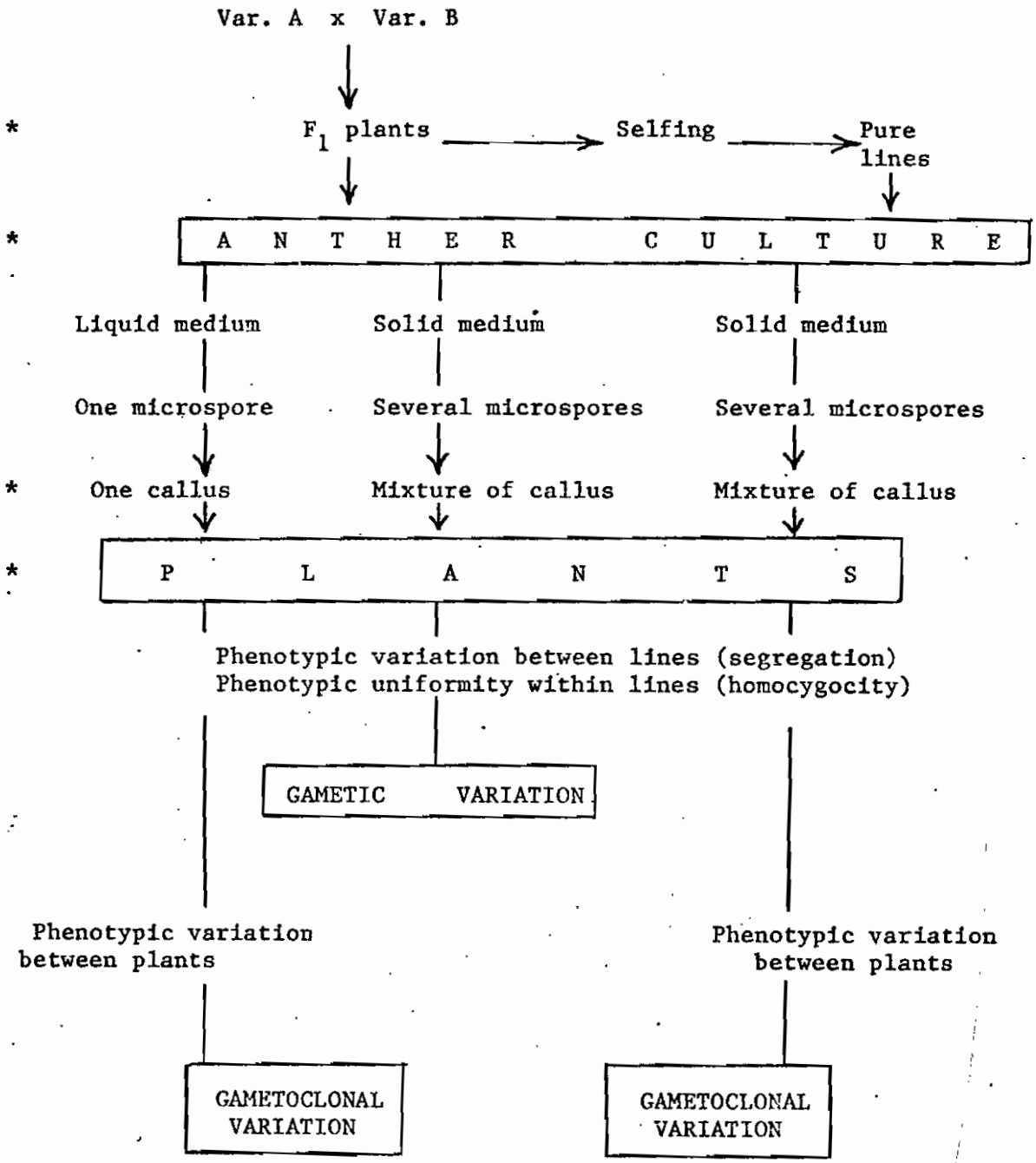

Figure. 1. Anther culture as a source of genetic variation: gametic (genetic recombination) and gametoclonal (genetic recombinacion + clonal variation); ej. arroz.

* Possible sites for stress, ej. aluminum, to select tolerance in vitro.

TABLE 2. Research in agricultural biotechnology in the National Institutes of 11 Latin American Countries (August, 1984).

Technologies	No. of countries	No. of institutes*		
		INIA	IES	IIB
Clonal propagation *in vitro*	11	21	24	6
Wide crossing (embryo rescue)	2	2	1	1
Haploidy (anther culture)	3	-	3	-
Somaclonal variation and *in vitro* selection	5	1	3	7
Protoplast culture and fusion	4	-	2	3
Recombinant DNA	7	2	2	5
Biosynthesis (cell cultures)	2	-	2	2
Biochemical studies (cell cultures)	5	-	4	6
Morphogenesis *in vitro*	4	-	4	3

* INIA = National Agricultural Research Institutes
IES = Institutes of Higher Education
IIB = Institutes of Basic Research

TABLE 3. Biotechnological research in the International Agricultural Research Centers of Latin America (August, 1984).

Technologies	Status of technologies per crop*		
	Initiating	Developing	In use
Clonal propagation *in vitro*	Beans	Coffee, plantain	Potato, cassava pastures (Gram.)
Wide crossing	–	Maize, potato	Wheat
Haploidy	–	–	Rice, potato
Gametoclonal variation	–	Rice	–
Somatic embryogenesis	Cassava	Cassava	–
Somaclonal variation	Rice,potato	Pastures (Legum.) wheat, potato	–
Selection *in vitro*	–	–	–
Protoplast culture	–	Potato, cassava	–
Transformation (DNA absorption)	–	Maize	–
Recombinant DNA	–	–	Potato
viroid probes	Potato	–	–
protein improvement	–	Cassava	–
Cryogenics	Beans	Cassava, Pastures	Potato
Genotypic markers			

* CIAT (cassava, rice, beans, tropical pastures); CIMMYT (maize, wheat); CIP (potato); CATIE (coffee, plantain).

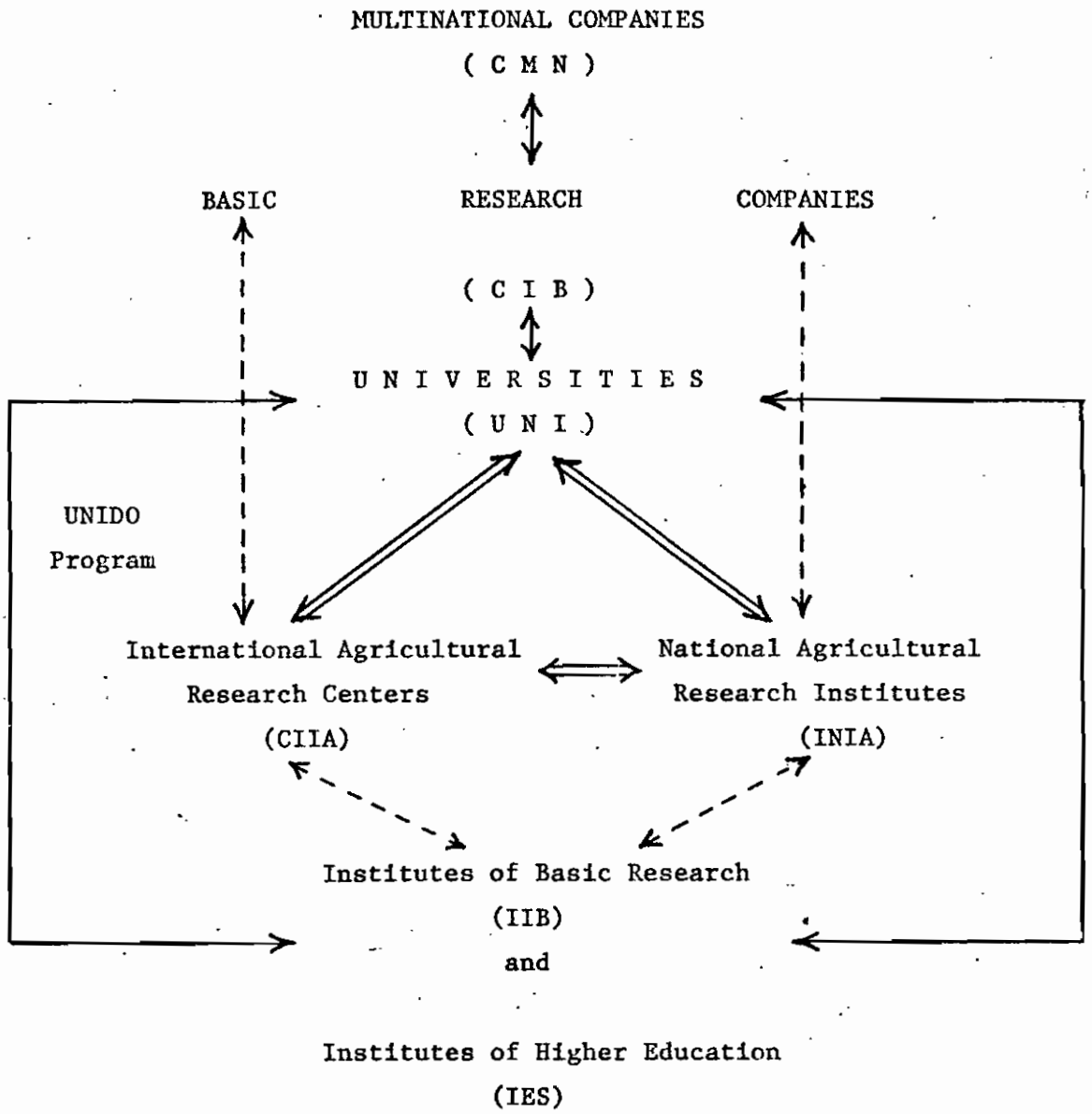

FIGURE 2. Collaboration in agricultural biotechnology among national research institutes (INIA, IIB, IES), international agricultural research centers (CIIA) of Latin America, and developed country universities (UNI); and their relationship with developed biotechnology companies (CIB, CMN).

current relationships ——————
proposed new relationships - - - - - - -
relationships to be strengthened ═══════