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The First Arab Conference on
Perspective of Modern Biotechnologies
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PLANT BIOTECHNOLOGIES: PRESENT AND FUTURE, ESPECIALLY FOR DEVELOPING COUNTRIES

by

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ABSTRACT

At the heart of the newly developing biotechnology field are the technologies of isolating cells or tissues from plants and growing them under controlled conditions (in vitro). The purpose of these technologies is to carry out detailed studies aimed at selecting plants with desired properties and conducting genetic engineering to create new species or hybrids with improved characteristics (e.g. crop yield, resistance to environmental stresses and to pathogens, increased content of specific substances).

The possibility of growing cells, tissues and organs of plants in aseptic culture in defined media, and of using such cultured plants to study cellular processes was first envisaged by Haberlandt as early as 1902. But it was not until the 1950s that the ability of cultured plant cells to regenerate intact plants (totipotency) was demonstrated by Miller and Skoog (1953), Skoog and Miller (1956), and by Steward (1958). White (1934, 1939) and Gautheret (1939) demonstrated earlier that root and wound callus tissues could be indefinitely propagated in culture. Cocking (1960) developed a method to remove plant cell walls and to prepare and grow protoplasts.

Bourgin and Nitsch (1967) succeeded in producing haploid plants of Nicotiana from anther cultures. Carlson et al. (1972) obtained the successful fusion of protoplasts of two Nicotiana species and produced a somatic cell hybrid. Somatic hybridization, the possibility of introducing foreign DNA into protoplasts and of using the recombinant DNA techniques to transform them, as well as the obtention of haploid plants from anther cultures drew the attention of plant geneticists. These discoveries and technological innovations aroused a great interest from public and private sector research in the development, use and exploitation of plant cell, tissue and organ-culture techniques aiming at the genetic improvement of crop plants since the mid-1960s.

More sophisticated applications of tissue culture techniques and of plant-cell genetic engineering, during the next two decades, may lead to the production of new types of plants, e.g. haploid lines, nitrogen-fixing cultivars, or of plant cell lines synthesizing specific biochemicals.

Similarly to other areas of biotechnologies, plant biotechnology applications may have profound socio-economic implications for existing agricultural activities and policies.

Another implication is the change in the traditional free exchange of plant material and information among researchers, due to the rapid commercialization of the technology which is creating secrecy and restricts the free flow of knowledge. There is also a marked trend of privatization and monopolization by large multinational pharmaceutical, petrochemical and seed-marketing corporations of the know-how and of the results of plant biotechnology applications so that free access to the new plant varieties is becoming increasingly difficult for the developing countries.

There is also the risk to increase, through the selection of new crop plants or varieties, the limited range of plant genetic resources and diversity on which food and fibre production already relies. Consequently too little attention is paid to the conservation of the world's rapidly diminishing stock of natural genetic material, and the developing countries which cannot set up the adequate facilities for the preservation of their plant genetic resources will be at risk to lose them and to have to buy the needed genetic stock from the industrialized countries.

PLANT CELL, TISSUE AND ORGAN CULTURES

In 1952 and for the first time, a chrysanthemum was freed from its virus diseases through the growth of its meristem[1] by Morel and Martin in France. In 1955, the technique was extended by the two researchers to the potato. In 1963, meristem culture was applied by Morel to obtaining virus-free orchids, and the interest of this technique was realized for the rapid and consequently economic propagation of these high-value species, of which the natural vegetative multiplication is very slow (Martin, 1986-1987). Since 1963, in-vitro micropropagation has been growing very rapidly throughout the world, and especially in the industrialized countries. After having been applied to annual and herbaceous species, such as potato and ornamentals, in-vitro micropropagation was extented to medicinal plants and perennial woody species, including fruit and forest tree species.

The second "Green Revolution" which has been heralded since the mid 1970s will be the outcome of research aimed at the selection of high-yielding plant varieties more resistant to diseases, predators and drought, and which can grow with less fertilizer and pesticides. Such research will no longer be based on cross-breeding, cross-hybridization or cross-pollination techniques but will use cell, protoplast, tissue and organ cultures, as well as genetic recombination techniques, in order to propagate interesting cultivars very rapidly and to create new ones (Murashige, 1974; Vasil, 1984, 1985, 1986; Anon., 1985; Mantell et al., 1985; Zaitlin et al., 1986; Davies, 1987; Pierik, 1987; Zakri, 1988); they will therefore be based on the molecular and cellular mechanisms which are responsible for biological diversity (Collins, 1982; Kosuge et al., 1983; Tudge, 1983; Collins, 1984; Gelvin and Schilperoort, 1988; Zakri, 1988).

1. The meristem is a semi-spherical mass, of one-tenth millimetre diameter, containing a few hundred cells and located at the tip of the apical bud of the plant. The meristem gives rise to the main plant organs, stem, leaves and flowers. It has the unique property of being the only part of the plant which remains free of viruses, bacteria or fungi when the plant is contaminated or diseased.

The various plant cell, tissue and organ cultures and the corresponding areas of research may be linked to industrial applications in the following way:

- meristem cultures and regeneration of whole plants	micropropagation of cultivars and of virus-free plants
- cultures of protoplasts, of haploid tissues, selection of variants and mutants, protoplast fusion and regeneration of whole plants	improvement and micropropagation of cultivars
- genetic recombination techniques, gene transfer and regeneration of whole plants	improvement of cultivated plants
- large-scale plant cell cultures, selection of variants and mutants, culture and fusion of protoplasts, recombinant DNA techniques	production of useful substances
- cell or protoplast cultures, bioconversion.	synthesis of new substances.

The application time of these techniques enables their classification in three categories:

- short-term applications (in a three-year time span): in-vitro vegetative or clonal propagation, pathogen-free plant production, and germplasm[1] storage and exchange;
- medium-term applications (time span of three to eight years): somatic embryogenesis, somaclonal and gametoclonal variation, embryo rescue, in-vitro fertilization, anther cultures and haploid plant production;
- long-term applications (time span of eight to fifteen years): somatic hybridization, wide-cross hybridization, mutant cell lines, chromosome transfer, gene transfer, secondary metabolite production by in-vitro grown cells.

According to a forecast made by an international seed and plant-science consulting company, the world food production will rise by 5% to 10% in the next 25 years as a result of application of

1. Germplasm is a living resource, including all the species, subspecies, lines of determined genetic make-up, variants and mutants. Germplasm corresponds for each taxonomic entity to the organs (seeds, cuttings and tissues) which ensure its reproduction and propagation.

biotechnologies alone (see also Withers and Alderson, 1986; Zaitlin et al., 1986). Table 1 recapitulates for some cultivated plants probable marketing dates, as a result of the progress made in plant biotechnology and genetic engineering. Table 2 recapitulates the current and potential yields of selected cultivated species, whilst table 3 summarizes in the case of rice the results expected from the application of biotechnology to the improvement of rice varieties.

Table 1. Probable dates for marketing of new plant varieties (selected examples from L. William Teweles Co.'s report, 1983)

Crop species	Dates of genetic engineering work	Probable period of release of transformed plants	Probable period of routine growth of transformed plants
Maize	1983	Early 1990s	Mid-1990s
Wheat	1985-1987	Early 1990s	Mid-1990s
Rice	1985-1987	Late 1980s	Early 1990s
Soybeans	1983	Early 1990s	Mid-1990s
Tomato	1984-1986	1983-1985	1986-1988
Sugar-cane	1987-1989	Early 1990s	Mid-1990s
Cotton	1985-1987	Early 1990s	Mid-1990s

Table 2. Current and potential yields of selected agricultural and forestry species

	Actual yield tonnes/hectare	Potential yield tonnes/hectare
Sugar-cane	70-90	150-200
Cassava	60	100
Tomato	70-150*	150-200*
Oil palm (palm oil)	2.5	10-12
Groundnuts	1.6	4.0
Castor bean (castor oil)	0.6	2.5
Temperate conifers (wood)	6.8	20-30
Tropical conifers (wood)	12-20	40-60
Tropical broad-leaved trees (wood)	10-20	40-100
Bamboo	25	100

*Under cover or shelter

Table 3. Expected results from the application of plant cell and tissue culture techniques, and of genetic engineering to the improvement of rice varieties (after Swaminathan, 1982)

Technique	Expected results
Tissue and cell culture	
Induction and selection of mutants	Selection of rice varieties resistant to salinity, to aluminum toxicity and to pathogenic agents; selection of varieties having a low photorespiration rate; selection of varieties with high protein and lysine content in seeds.
Embryo culture	Intra- and interspecific hybridization.
Pollen grain culture	Decreasing time of selection.
Protoplast fusion	Interspecific and intergeneric hybridization. Improvement of hybrid rice. Improvement of Azolla.
Recombinant DNA techniques	Transfer of genes coding for atmospheric nitrogen fixation.

1. Short-term applications

1.1. In-vitro vegetative micropropagation

In-vitro micropropagation is an extension of conventional vegetative propagation and includes budding, cuttings, graftings and in-vitro plant tissue culture techniques, such as meristem (apical and axillary) cultures, embryo production (by somatic embryogenesis) and shoot production by organogenesis (Margara, 1982).

For instance, the utilization of tissue culture for the large-scale micropropagation of potato started in 1973. The technique consists of excising sprouts from tubers, sterilizing them and cutting them into fragments each comprising a node and a bud which are grown on a culture medium and produce a plantlet. The latter is thereafter divided into microcuttings which are also grown to regenerate plantlets or vitroplants. After a few micropropagation cycles in vitro, the plantlets are transferred into small cubes of soil which are stored in greenhouses where anti-aphid nets prevent them from being in contact with virus-bearing insects. Tubers collected on vigorous plantlets are thereafter planted in the field. Through this micropropagation

technique, it is possible to obtain about 2 million microcuttings from a single node in 8 months, taking into account the inevitable losses and various limiting factors; tubers produced by those plantlets can be used to plant over 40 hectares. With the conventional vegetative propagation technique, 7 to 8 years would be necessary to reach the same achievement, because one tuber produces, in temperate conditions, 10 tubers per year. The micropropagation technique has another advantage: it is carried out in the absence of viruses and therefore gives rise to disease-free plants. If the contamination of the starting plant material were to be overlooked, the plantlets regenerated in vitro or the tubers derived from them could be "cured" through thermotherapy, or the reculture of apices, or micrografting (Ducreux et al., 1986).

The overall advantages of in-vitro micropropagation of plants are :

a. Production of large numbers of plants or clones[1] in a short time and using small confined facilities.

b. Propagation of materials in an environment free of viruses and other pathogens, and under optimum conditions.

c. Ability to propagate plant species which are difficult to propagate vegetatively once they flower, or to propagate species only by in-vitro plant tissue culture techniques which are commercially superior to other conventional methods of propagation (e.g. tropical plants with high levels of tannins and phenols).

d. Ability to supply plants on a year-round rather than on a seasonal basis.

e. Maintenance of heterozygozity and cloning superior individuals (from the qualitative and quantitative viewpoints). This is particularly true for tree species the cloning of which can bypass all the time involved in out-crossing and selecting homozygous

1. The term "clone" (from the Greek klôn, meaning a slip or twig suitable for plant propagation) was suggested in 1903 by Webber of the United States Department of Agriculture to designate plants obtained by asexual reproduction; it is even applied to DNA multiplication (cloning of genes in bacteria). But, according to strict scientific usage, clone means an organism obtained from a single cell through mitotic divisions. Such a definition, however, does not imply phenotypic or genotypic identity between the clone and the individual which produced it; indeed, significant differences have been observed in several species between a given clone and its parent (see somaclonal variation).

seed donors ; heterozygozity and plasticity are also preserved in the cloned individuals and in the high-value plantations which are established from them. Fruit trees, oil, date and coco palms, species for lumber and pulp are good candidates for such in-vitro cloning and in fact outstanding results have been obtained with the palm species, with apple, cherry, rhododendron, eucalyptus, aspen and pine (Bonga and Durzan, 1982; Vidalie, 1983).

A major limitation of in-vitro micropropagation of plants is the difficulty to regenerate complete plants from tissue or cell cultures. Plant regeneration has been recorded as particularly difficult among the grasses, cereals and woody species.

From the economic viewpoint, the cost of labour involved in the replication of tissue cultures is the most important item in the ultimate cost of an in-vitro plantlet. Research is being carried out on the design of machines which would perform these repetitive tasks: in 1985, it was reported that a prototype made in Australia could replicate one plant per second. In 1988, a similar machine was functioning in Israel. Such devices or machines would considerably decrease the ultimate cost of the vitroplant (Martin, 1986-1987).

It should be emphasized that in-vitro micropropagation is not the only method for crop improvement, but it is rather used as an additional tool for breeding and other related research programmes. The release of plant clonal material should be preceded by tests in field conditions for at least one generation. Furthermore, once this has been the case, the clonal material could be further improved by using both conventional breeding techniques and long-term tissue-culture techniques. Consequently, a collaborative effort between plant breeders, phytopathologists, physiologists, food scientists and biotechnologists is imperative for the final improvement of a particular species.

1.2. Somaclonal variation

In the late 1960s, the research carried out in France by Lutz and in the Federal Republic of Germany by Melchers on tobacco showed that cultures on artificial media could lead to the regeneration of individuals which differ from their mother plant. See also Krishnamurthi and Tlaskal (1974); Krishnamurthi (1977). The Australian researchers Larkin and Scowcroft (1981) called this phenomenon somaclonal variation. Somaclonal variation is ubiquitous, occurring in rice, maize, wheat, barley, potato, alfalfa, rape and other species. Moreover, several cultivated

varieties are derived from somatic variants or sports: the pink grapefruit, the navel orange, the nectarine and several varieties of potato. In the sweet potato, these sports appear with a frequency that can be as high as 2% and as a result, the maintenance of varietal purity by using conventional cloning poses a real problem.

Although somaclonal variation must be controlled to maintain the genetic integrity of propagating material, especially if one deals with the clonal propagation of elite genotypes, the selection of somaclonal variants from adapted varieties may lead to new plant varieties, after checking in the field the stability of the new traits. Somaclonal variation concerns amino-acid overproduction, resistance to herbicides and to diseases. Sugar-cane, for instance, has developed resistance to eyespot disease, Fiji virus, downey mildew and smut (Heinz et al., 1977); potatoes to late and early blight; maize to Southern corn leaf blight; and rape to vitricular disease. Somaclonal variation may also provide genotypes suitable for tropical environments, i.e. able to tolerate heat or acidic soils containing harmful concentrations of aluminium and manganese.

1.3. Disease elimination, and germplasm exchange and storage

The techniques of production of virus-free and disease-indexed plant materials include:

- culture of the shoot-apex comprising the apical meristem and one or two leaf primordia;
- culture of the apical meristem, sometimes referred to as meristemming;
- grafting of the shoot-tip onto seedling rootstocks; and
- adventitious organogenesis or embryogenesis in special tissues such as nucellar tissue.

The last two techniques are applicable to crops, such as woody plants, for which shoot-tip culture is not possible. All the techniques are normally linked to thermotherapy and chemotherapy before or during culture.

As in the case of rapid in-vitro clonal micropropagation, virus-free plants have been a commercial reality since the mid-1970s and it is possible, by combining thermotherapy and meristemming, to free nearly all plant materials of nearly all viruses. This is achieved because virus replication is minimized by elevated temperatures and because meristematic growth outpaces virus proliferation (furthermore, vascular connections do not penetrate the meristematic dome of a shoot tip which

is therefore not easily reached by the virus). A virus-free plant can be regenerated from the smallest explant of a heat-treated meristem placed in culture. A virus-free plant production programme must be carried out in conjunction with a rigorous virus-testing programme and with plant protection measures.

Free-virus or disease plant materials provide a rapid and safe means of germplasm exchange accross international borders. In-vitro methods of plant propagation also provide a safe means of storing germplasm, either produced in vitro or collected in the wild and from less secure field gene-banks (clonal repositories, orchards, plantations, etc.). The conservation of vegetatively propagated and recalcitrant seed-producing crop plants include short to medium-term storage by slow growth for active collections and cryopreservation for base collections. See Withers (1980); Bajaj (1983).

2. Medium-term applications

2.1. Anther culture, production of haploids and gametoclonal variation

In 1964, two Indian researchers, Guha and Maheshwari, cultivated in an artificial medium Datura anthers, the pollen grains of which gave rise to embryos, then to haploid plants. Bourgin and Nitsch (1967) achieved the production of haploid plants of Nicotiana from immature pollen grains through in-vitro cultures of anthers.

In-vitro production of haploids can be used to lower the ploidy level and to generate pure homozygous lines for hybrid production. Doubling the set of chromosomes of haploid cells or plants (e.g. with colchicine) results in homozygous lines that may be used as inbred lines for hybrid production, to stabilize unique germplasm in homozygous form (as in the case of trees with long sexual cycles) or to fix gene combinations at the end of a backcrossing scheme. Inbred lines can be used as varietal materials in inbred species or for the production of F1 hybrids in outcrossing species (Collins, 1982, 1984).

Anther culture technique has been improved and although it is not a routine operation for many crop species (Maheshwari et al., 1980), the production of haploid plants from anther cultures has been successful with maize, rice, rye, wheat, triticale, fescue, rape, grapes, tocacco, poplar and rubber (Collins, 1982, 1984; Collins and Genovesi, 1982). Anther and pollen grain culture has also become the main source of haploids in ornamental (petunia, geranium) and vegetable species

(asparagus, cabbage, capsicum, egg plant, potato, tomato; Dumas de Vaulx, 1983).

The availability of haploid plants is of special significance for the genetic improvement of fruit trees where breeding is made difficult by long generation intervals, by the highly heterozygous nature of most fruit plant species, by parthenocarpy and self-incompatibility. Haploid plantlets were regenerated from anther calli of custard apple (Annona squamosa) on a culture medium supplemented with 6-benzyl-aminopurine and naphtalene acetic acid (Nair et al., 1983). The Indian scientists have been trying to increase the percentage of haploid plantlets and their survival rate in natural conditions.

When the anther culture method is inefficient, the so-called bulbosum method developed by Kasha and Kao (1970) can be used. This method involves the pollination of cultivated barley (Hordeum vulgare) with Hordeum bulbosum pollen and rescuing the interspecific hybrids by in-vitro embryo culture. The subsequent elimination of the H. bulbosum chromosomes from the developing embryo results in barley haploids. Kasha and Reinbergs (1980) have used this method to develop a new barley variety within five years.

Wide crosses between two species result in the parthenogenetic development of an egg cell into a maternal haploid plant. When a tetraploid potato variety (mother plant) is crossbred with some diploid clones of Solanum phureja (father plant), there occurs a parthenogenetic development of the oosphere induced by the pollen of this species. The seeds produced therefore give rise to plants which have only the chromosome stock of the female gamete (oosphere), i.e. 24 of the 48 chromosomes of the mother plant, distributed in two sets of 12 chromosomes each. That is why these plants are called dihaploid (n=2x=24), i.e. "haploids from tetraploids". The in-vitro culture of anthers or ovules could also be followed by the regeneration of dihaploid plants (Rajnchapel-Messai, 1987).

The haplodiploidization process has the following advantages:
- it is easier to identify the desirable traits which are not hidden by the tetraploid state of most cultivated hybrid potato varieties;
- the study of transmission of desirable traits is easier because of the reduced number of offspring needed for that purpose;
- the breeding cycles are therefore less numerous and the whole selection process is accelerated;

- through this process, one has access to the vast pool of genetic resources and variation constituted by the diploid tuber-forming Solanaceae which can be crossed with the haplodiploid (because they have the same number of chromosomes).

2.2. Embryo rescue

Seed failure associated with embryo abortion and endosperm disintegration is common in intervarietal, interspecific and intergeneric crosses in plants, because of the various mechanisms of sexual incompatibility. Isolation and culture of immature embryos from defective seeds before embryo abortion sets in continue to be the primary means of obtaining viable seedlings from such crosses. This is accomplished by the aseptic removal of embryos and subsequent growth on appropriate culture media up to the stage of viable seedling. It is therefore crucial to remove the embryos in an uninjured state and to select the proper media for the rescue and growth of embryos to maturity.

Embryo rescue technique is used with orchids, tomato, cotton, beans, barley, banana and soft coconut.

3. Long-term applications

3.1. Somatic hybridization

Somatic hybridization involves the fusion of two somatic plant cells.

3.1.1. Plant protoplast culture

Cocking (1960) used fungal enzymes to digest cell walls in culture media of appropriate osmotic pressure. Later on, industrial production of "cellulases" and "pectinases" enabled the production of large numbers of viable protoplasts (Takebe et al., 1968; in Shepard, 1982). In 1971, the first regeneration of normal plants from tobacco protoplasts was obtained (Nitsch and Ohyama, 1971; Takebe et al., 1971; in Shepard, 1982).

The experiments on tobacco protoplasts showed that over 90% of the protoplast-derived clones, or protoclones, were remarkably similar to the parental lines, as regards both phenotype and genotype. Thus tobacco protoplasts made it possible to avoid the variations that characterized the other means of obtaining clones. Such a stability was confirmed for a few other plant species.

In the case of the potato, in 1977, Shepard and Totten (in Shepard et al., 1980) developed plant regeneration techniques, using cell protoplats of leaves from the Russet Burbank cultivar. Shepard (1982) observed that, unlike tobacco protoclones, those of the Russet Burbank cultivar were not identical with the parental line, nor were they very similar to one another. The phenotypic variations were caused by genetic modifications in somatic cells. Thus, Shepard and his co-workers were able to regenerate potato plants which were resistant to the late blight microfungus Phytophthora infestans and to the disease caused by another microfungus Alternaria solani (in Rajnchapel-Messaï, 1987).

Ducreux and Rossignol from the Morphogenesis Laboratory of the University of Paris (Orsay) observed differences among the clones thus obtained: increase of yield (tubers), early tuber formation and better tolerance to two parasitic fungi (Phytophthora infestans and Verticillium alboatrum). The clones were tested in field conditions between 1981 and 1984, and it was shown that the desirable traits were conserved for several generations, as in the case of the Russet Burkank-variety protoclones isolated by Shepard.

Protoplasts from embryonic explant tissue have also been used to regenerate intact plants of orange, sour orange, mandarin, lemon and grapefruit (Vardi et al., 1982).

Although plant regeneration from protoplast cultures of legumes proved difficult, it was reported for Medicago sativa, Trifolium repens, Trigonella foenum-graecum and Crotalaria juncea in 1980 and 1982 (in Krishnamurty et al., 1984). High yields of viable protoplasts were obtained from callus cultures derived from shoot apices of Vigna aconitifolia (moth bean) by Krishnamurty et al. (1984).

Cell and protoplast cultures leading to the induction of embryoids have also been used for the propagation of forest tree species, especially for conifers by Durzan and his collaborators at the Institute of Paper Chemistry, University of California, Davis, and by David at the Laboratory of Plant Physiology of the University of Bordeaux, France (in Boulay, 1980; Franclet, 1983). These techniques will enable the mass production of selected genotypes at a cost which would be competitive with the methods based on reproduction from seeds and clonal propagation.

3.1.2. Protoplast fusion

Protoplast fusion was first obtained by Carlson *et al.* (1972) in iso-osmotic solutions of sodium nitrate. The discovery of the powerful aggregation effect of polyethylene glycol by Kao and Michayluck and by Wallin *et al.* in 1974 was followed by the high rate of protoplast fusion.

Protoplast fusion can overcome barriers of sexual incompatibility between sexually distinct species. It allows transfer of cytoplasm between sexually distinct species and the crossing of sterile plants. It is a technique of choice for gene transfer when embryo rescue and *in-vitro* fertilization are not feasible. This promising technique does not depend on normal sexual reproduction which made it possible to create, with a certain amount of difficulty, hybrids of wheat and rye (triticale; Smith, 1983) and of turnip and cabbage (raphanobrassica).

When two different plant cells have fused, the resulting cell, an heterokaryocyte, consists of a mixture of cytoplasms in which are found chloroplasts, mitochondria and nuclei from both cells. Ideal fusion should lead to a somatic hybrid with only one nucleus from each parent cell. Random protoplast aggregation often leads, however, to multiple fusion products including various numbers of parental nuclei. Each somatic hybrid may undergo different nuclear and cytoplasmic genetic arrangements. So far parasexual plants have been essentially limited to genera and even species for which plant regeneration is easily achieved *in vitro*, including *Nicotiana*, *Petunia*, *Datura*, *Daucus* and, to a lesser extent, *Atropa*, *Solanum*, *Brassica* and *Medicago*, i.e. mainly genera of the Solanaceae family.

Among the examples of somatic hybridization which might lead to promising results, there is the creation of hybrids between a cultivated potato variety (2n=4x=48) and the wild species *Solanum chacoense* (2n=2x=24). These hybrids, which were obtained by Butenko and Kucho in the USSR, are resistant to late blight microfungus and various animal pests such as aphids. The resistance trait was inherited from the wild species, but they were associated with a high content of glycoalkaloids, and it seems difficult to dissociate both properties (Rajnchapel-Messaï, 1987).

Another research team led by Binding in the USA was able to isolate plants which were resistant to the herbicide atrazine as a result of the fusion between protoplasts of dihaploid potatoes and protoplasts of *Solanum nigrum*, a diploid and resistant species. Helgeson, also in the

USA, succeeded in isolating somatic hybrids between a potato variety resistant to late blight microfungus and the wild diploid species Solanum brevidens (2n=2x=24), which is very resistant to a virus causing leaf roll but which does not form tubers. The somatic hybrids were tested in the field conditions and proved to be vigorous, to flower, to form tubers and to have inherited the resistance traits of both parents.

Protoplast fusion is also used as a means to create interspecific hybrids of tomato, such as that obtained between Lycopersicon esculentum (cultivated tomato) and Lycopersicon peruvianum by the Japanese company Kagome.

In the case of cybrids resulting from the fusion of two parental cell (protoplast) lines, only one parental cell line carries nuclear genes, while the other parental cell line is treated (X-rays, laser) to eliminate the nuclear genes. Such fusion therefore emphasizes the transfer from one species to another of only cytoplasmic genes which control many interesting agronomic traits, such as disease resistance, cytoplasmic male sterility, herbicide resistance, etc. These traits are coded by organelle genomes, i.e. by mitochondria and chloroplasts.

It should be emphasized that after the hybrid or cybrid plants are recovered, they have to be incorporated into a conventional breeding programme including the usual steps of crossing, backcrossing, selfing and selection in order to produce a new commercial plant variety. The time frame for such a programme would vary between 4 to 15 years depending on the plant species.

3.2. Mutant cell lines

In-vitro techniques now enable efficient recovery of mutant cell lines. The advantages of in-vitro mutant isolation over conventional field screening include the application of greater selection intensities and the substantial reduction in time, expense and resources use. The plant tissue culture specialist prefers to select the improved traits (such as disease resistance or herbicide and salinity tolerance) at the cellular level because of the size of the cell population (1 millilitre of a plant cell culture can contain about 1 million cells). After randomly recovering plants from the cell cultures, selection can also be made at the plant level, in the greenhouse or in the field.

Physical or chemical mutagens can be used to mutate the cells and they will increase the variability by 100 to 1,000 times and may favour the appearance of the desirable character. The application of this

technique enabled the recovery of maize plants with increased threonine content (33% to 59% higher threonine), tobacco plants with more lysine (10% to 15%), petunia plants resistant to mercuric chloride, tobacco plants resistant to salt and herbicides such as picloram and paraquat. At the International Rice Research Institute, in the Philippines, the researchers try to isolate rice mutant cell lines which could tolerate high concentrations of salts and aluminium, as well as varieties having higher protein and lysine contents in their seeds (Swaminathan, 1982).

Cell or tissue lines could also be helpful for the selection of plants resistant to pathogens. Such a technique was used to detect plant varieties which are resistant to toxins produced by fungi and bacteria. For instance, a maize cell line resistant to the toxin of the microfungus Helminthosporium maydis race T (Southern corn leaf blight) was isolated in 1975 and whole plants were regenerated from these cells. Similarly, cell lines resistant to Helminthosporium sacchari toxin were isolated from sugar-cane tissue cultures.

4. Some achievements

Plant tissue culture techniques have been successfully applied to several crop species playing an important role in the economy of developing countries and in the world trade of agricultural commodities. Vegetative or clonal propagation, using such techniques, is of particular interest in the case of tropical species which are highly heterozygous, are infected with viruses and are generally propagated through vegetative means. The following plant species can be micropropagated in vitro with a view to achieving their large-scale commercialization: artichoke, asparagus, beet, cassava, garlic, ginger, potato, raspberry, strawberry, sugar-cane, sweet potato, taro; agave, almond, apple, banana, citrus, coconut, cherry, kiwi, oil palm, papaya, peach, pear, pineapple, plantain, vine, walnut; bamboo, elm, eucalyptus, ficus; carnation, chrysanthemum, gerbera, iris, lily, nephrolepis, orchids, pelargonium, rhododendron, rose, saintpaulia. The following species are currently regenerated in laboratory conditions: avocado, cocoa, coffee, jojoba, rubber, date palm, tobacco, hazel-nut, carrot, endive, oilseed rape, corn, legumes, yam, soybeans. See Zimmerman et al. (1986); Ketchum et al., 1987; Pierik (1987); Dusing (1988).

Works edited by Conger (1981), Bonga and Durzan (1982, 1986), Fujiwara (1982), Rao (1982) and Crocomo et al. (1986), and the bibliography compiled by Dusing (1988) give detailed reports and lists

of references on the application of biotechnologies to the propagation of tropical species including food crops as well as commercial crops and forest tree species. The examples of oil palm, coconut, date palm, sugar-cane, coffee, and other food and commercial crop species (papaya, yam, cassava, potato; banana, cardamom; flower and forest tree species) constitute important achievements.

The example of date palm micropropagation is particularly important for Arab countries, and those of some forest tree species (Eucalyptus spp., Casuarina spp., Acacia spp.) as well.

A date palm (Phoenix dactylifera) lives for around 100 years but only gives between 30 and 40 shoots during this period. Vegetative micropropagation is therefore possible through the culture of meristems from the shoots (this is not the case for the oil palm which does not produce shoots). Furthermore, as with the oil palm, somatic embryogenesis can be applied to the date palm, i.e. embryoids can be obtained on cultures of various tissues, e.g. inflorescence tissue.

Plantlets have been obtained from date palm tissue cultures by several research teams including those working in Arab countries, which proves that date palm cloning is feasible. Nursery tests and field tests are carried out in order to observe the behavioural patterns of the clones.

It is important to create date-palm clones resistant to bayoud disease, caused by Fusarium oxysporum f. sp. albedinis and which provokes serious damage to the palm groves contaminated by this soil fungus. This disease, which is caused by the proliferation of the fungus in the vessels of the tree, has completely destroyed more than 10 million date palms over a century and the 4.5 million trees of the Moroccan groves are threatened by the spreading of the disease.

At the Laboratoire d'histophysiologie végétale of the University of Paris VI, Benbadis and his co-workers have succeeded in obtaining the in-vitro vegetative micropropagation of the date palm from axillary buds taken from shoots of female adult trees, or from inflorescence anlages. In this latter case, the cells from the inflorescence anlage which are specialized cells, dedifferentiate and give rise to a vegetative shoot. Such phenomenon can be observed in natural conditions. It was described at the beginning of the century for palm species from tropical humid forests. The inflorescence stem of these palms is so long (2 metres) that the tip of the inflorescence can be in contact with the soil; when

this happens, the development of the inflorescence stops and a vegetative development takes place (in Marty, 1986).

The sampling technique consists of taking at a very early stage a fragment of the inflorescence anlage and of growing it on the culture medium. The French researchers have been able to increase the micropropagation capacity of the date palm, with a view to applying the technique in field conditions. In the same laboratory, research has been pursued on the control of flowering in vitro. This has been achieved on plants obtained from germinated seeds or from excised embryos. Research also aimed at obtaining in-vitro fertilization, in order to shorten the period necessary for the improvement of the date-palm varieties (in Marty, 1986).

In October 1984, the French oil company, Total Compagnie française des pétroles, the Centre national de la recherche scientifique (National Scientific Research Centre) and the Institut national de la recherche agronomique (INRA, National Institute for Agricultural Research) signed an agreement aimed at promoting research and development on the date palm. The research programme of the new entity called Groupement de recherche français sur le palmier-dattier (GRFP, French Research Group on Date Palm) which is implemented at the Conservatoire botanique, located on the island of Porquerolles in the south of France, involves:
- the development of techniques for the in-vitro propagation of lines or varieties of date palm selected by INRA as higher-yielding ones and resistant to bayoud disease;
- the industrial production of young vitroplants;
- the nursery stage of these plants up to their marketing;
- the field trials of these plants in subtropical arid zones, i.e. in the natural environment of date palm.

At the end of 1987, it was estimated that the results obtained were satisfactory with regard to the multiplication rate of plantlets, the very low mortality during the nursing phase from the laboratory to the greenhouse, and the good growth of hundreds of trees (originating from in-vitro plantlets) introduced in several Middle-Eastern countries. However, it was felt necessary to await the first fruiting stages of the trees transplanted in their natural environment before envisaging the commercial development phase (in Europe Outremer, 683-684-685, December 1986-January 1987, p. 21-22).

At the end of 1986, according to the estimates made by Twyford Plant Laboratories Ltd. (TPL Ltd., Baltonsborough, Glastonbury, Somerset, United Kingdom), 10,000 plantlets of date palm should have been sold at a price of $20 each. These plantlets are obtained through somatic embryogenesis and their roots are inoculated with mycorrhizae (Glomus); this inoculation stimulates leaf growth and development. DNA probes have been developed to check that the genome of the plantlets is identical to that of the mother plant. Concerns have been raised about the normality of the clones, and it seems that the main customers of Twyford Plant Laboratories Ltd. (Saudi Arabia, Oman and the Islamic Republic of Iran) requested their supplier to insert in the delivery contracts a safeguarding clause on the normality of the clones derived from the vitroplants. The English company was also negotiating with a Japanese commercial firm (Nissho Iwai) for the establishment of an experimental farm and of a research institute at Shisuoka or Chiba (in McGraw-Hill's Biotechnology Newswatch, 16 June 1986).[1]

1. In addition to a new genetic engineering laboratory located at the Cambridge Science Park where Nickersons and the Agricultural Genetics Company are also located, TPL Ltd. has built important laboratories outside the United Kingdom. In California, TPL Inc. runs the largest tissue culture laboratory in North America; with British American Tobacco, TPL Ltd. set up a similar laboratory in Malaysia and may do so in Kenya.

GENE TRANSFER AND GENETIC ENGINEERING IN PLANTS

Gene transfer and genetic engineering require gene identification and isolation, and especially of those genes or groups of genes which control economically desirable traits; gene cloning and sequencing; development of appropriate gene vectors for plant cells ; successful gene transfer, gene expression, gene stability and gene transmission through subsequent sexual reproduction (Cocking et al., 1981; Davies, 1981).

The success of genetic engineering with higher plants will depend on the research progress achieved in all the above mentioned areas. Although the tools of genetic engineering are available, much better knowledge of plant molecular biology is needed, and the recovery of improved crop species by successful execution of genetic engineering will be the result of a team effort involving at least molecular and cell biologists, plant tissue culture specialists and plant breeders.

Single genes to be used for transformation must be sought, and DNA sequences underlying multigenic traits should be identified and isolated. A promising approach for both is insertional mutagenesis using transposable elements. The transposable elements identified in maize by Barbara McClintock in 1951 have already proved of paramount importance for this cereal crop. These elements change their position in the chromosomes and when one of them inserts itself into a dominant single gene, its effect on the plant phenotype can be observed. The molecular biologist can identify the gene by isolating the transposable element from its position and characterizing the DNA adjacent to the element. The transfer of transposable elements is readily possible for maize with conventional breeding methods. The mobilization of maize transposable elements into dicotyledonous plants is being explored.

A detailed knowledge of the chromosome map of a plant is useful for the identification and subsequent isolation of genes. Many chromosome markers can be generated as a consequence of polymorphism in DNA sequences throughout the chromosomes. These polymorphisms are easily detected by digestion of DNA with restriction enzymes (endonucleases) and are known as restriction fragment length polymorphisms (RFLP). RFLP mapping is a useful tool for the screening of desirable traits once a RFLP has been located close to the desirable gene.

The establishment of vector systems for plant gene transfer has acknowledged significant progress in recent years. The most promising

candidates up to now are the plasmids of Agrobacterium tumefaciens, synthetic plasmids or naked DNA (encapsulated in liposomes), E. coli plasmid fused with chloroplast DNA, cauliflower mosaic virus (CMV) or other plant viruses.

1. Structure and function of Agrobacterium plasmids

Agrobacterium tumefaciens is a soil bacterium which infects a broad range of dicotyledonous plants after they have been wounded. As a result of this infection, the wound tissue begins to proliferate as a neoplastic growth commonly referred to as a crown gall tumour. The crown gall disease causes heavy dammage not only to fruit and vegetable crops but also to ornamental species (Expert et al., 1980).

In 1947, Braun of the Rockefeller Institute for Medical Research succeeded for the first time in cultivating crown gall tissue, free from bacteria, on a medium containing sucrose and mineral salts. The cultivated tissue had the properties of a tumour tissue, i.e. it could grow rapidly and axenically in the absence of any plant hormone which is necessary for the growth of normal plant tissues; the tumour tissue continues to proliferate in the absence of bacteria when transferred to a fresh culture medium (in Chilton, 1983).

In 1960, Morel and his co-workers of the Institut national de la recherche agronomique, France, discovered that the crown gall cells synthesized compounds which do not exist in the normal healthy plant cells. These opines are derivatives of amino-acids and ketonic acids. Two opines were studied by Morel: octopine, derivative of arginine and pyruvic acid, and nopaline, derivative of arginine and alpha-ketoglutaric acid.

In 1974, Schell of the Max-Planck Institute for Plant Breeding, Cologne, Federal Republic of Germany, and Van Montagu of the State University of Ghent, Belgium, and their coworkers discovered large plasmids in all virulent or oncogenic strains, but not in the non-virulent strains of A. tumefaciens. They showed that the strain C lost its virulence when grown at 37°C, because of the denaturation of its plasmid. The introduction into the harmless strain of this plasmid either by transformation or by conjugation, gave back to the strain its tumour-inducing ability. These plasmids of 140 to 235 kilobases were called Ti (for tumour-inducing) because they are responsible for tumour induction in the plant tissues infected by A. tumefaciens.

Chilton and her co-workers of Washington University observed in 1977 that 3% of the Ti plasmid DNA were present in several fragments in the cells of tumoral tobacco tissue. These fragments were called T-DNA (for transferred DNA) and the American researchers put forward the hypothesis that the T-DNA was integrated to the plant genome (in Chilton, 1983).

Infection by A. tumefaciens is a natural genetic engineering process whereby a segment of DNA (T-DNA) from a bacterial (prokaryotic) plasmid (Ti) is integrated to the plant cell (eukaryotic) genome (Expert et al., 1980). T-DNA can transform isolated plant cells or protoplasts. This was obtained by fusing protoplasts and bacterial spheroplasts (i.e. bacterial cells of which the walls are removed enzymatically), by introducing intact bacteria into protoplasts with walls partially regenerated, and by introducing into protoplats intact Ti plasmids in the presence of fusing agents such as polyethylene glycol and calcium (Schilperoort et al., 1979, 1982, Draper et al., 1982, in Ream and Gordon, 1982).

Agrobacterium rhizogenes induces in many dicotyledonous plants a disease consisting in root proliferation and referred to as hairy root. Virulent strains of this bacterial species contain a large plasmid of 230 kilobases on which are located the genes inducing rhizogenesis. These plasmids, called Ri (root-inciting), contain a segment of DNA, comparable to the T-DNA of the Ti-plasmid, which is integrated to the host plant DNA. Ti and Ri plasmids show some homology for some DNA sequences which are involved in the determination of the virulence of strains. These genes seem to have been conserved during the evolution of bacteria (in Ream and Gordon, 1982).

2. Utilization of Agrobacterium plasmids as gene vectors

Using the Ti-plasmid to introduce a foreign gene into a plant cell starts by making from a plasmid which can be cloned in E. coli cells a recombinant plasmid in which a particular segment of T-DNA has been inserted. This recombinant plasmid is thereafter cleaved by an endonuclease at a site within T-DNA and the foreign DNA is inserted into the slot thus made. This foreign DNA is associated with a genetic marker, such as the gene for resistance to kanamycine. The hybrid plasmid is recloned into a larger plasmid and is introduced into a strain of A. tumefaciens having a non-modified Ti plasmid. When the bacteria multiply, a recombination may take place and may give rise to a

clone of bacteria of which the T-DNA is associated to the foreign DNA. This clone is detected on a culture medium containing kanamycine to which cells are resistant. Infection of plant cells by these bacteria transfers the foreign gene with the T-DNA.

Ri plasmids could also serve as gene vectors, as the cells transformed by these plasmids can regenerate healthy plants. Tempé and his co-workers of the Institut national de la recherche agronomique, France, showed that the cells of roots of which the proliferation was induced by these plasmids, synthesized an opine close to agropine, contained several copies of T-DNA and regenerated whole and fertile plants as in the case of cells transformed by mutant rooty Ti-nopaline plasmids (Tepfer and Tempé, 1981; Chilton et al., 1982; Gordon et al., 1982; in Ream and Gordon, 1982 and in Chilton, 1983).

2.1. Examples of gene transfer

Complete expression of bacterial genes for resistance to certain antibiotics, transferred by Ti plasmids, was obtained in plant cells by three groups of researchers of Washington University, St Louis, Missouri (Chilton et al.), of the Monsanto company, also at St Louis (Horsch et al.), of the Max-Planck Institute for Plant Breeding, Cologne (Schell et al.) and of the State University of Ghent (Van Montagu et al.). The bacterial genes were those for resistance to kanamycine, transferred by Horsch, by Chilton and by Bevan of the Plant Breeding Institute, Cambridge, United Kingdom, who made experiments in Chilton's laboratory; for resistance to methotrexate, kanamycine and chloramphenicol, transferred by Schell and by Van Montagu. The promoters of the genes coding for opine synthase which are like typical eukaryotic promoters were used for that purpose.

Each one of the resistance genes was stitched to the regulatory sequences (promoters) of the gene for nopaline synthase. Each recombinant gene was thereafter inserted into a Ti plasmid which was the vector. The complete expression of the transferred gene in the plant cells was shown as the acquisition of drug resistance. The transformed plant cells have thus become resistant to a concentration of kanamycine which would have killed non-resistant cells (Chilton, 1983). Complete plants were not obtained from the transformed cells where the transferred genes were fully expressed, because the Ti plasmids, used as gene vectors, tumourized the cells. Chilton, Schell, Van Montagu and

their respective co-workers used other non-oncogenic Ti plasmids to transfer bacterial genes for resistance to antibiotics.

In July 1983, Calgene Inc., Davis, California, announced that its researchers were able to clone the gene aroA for tolerance to glyphosate - a herbicide produced by Monsanto and marketed under the name of "Round-Up". In 1985, the gene was transferred into tobacco plants and expressed in them; the gene was also transferred into cell cultures of soybean, rape and tomato. Calgene Inc. signed with Phytogen an agreement aimed at developing glyphosate-tolerant cotton plants. A similar agreement was signed with the U.S. Forestry Service in order to transfer the gene for tolerance to glyphosate to tree species used in the paper-making industry (in Biofutur, April 1985, p. 66).

Research work carried out since 1984 by Schell and his collaborators at the Max-Planck Institute in Cologne showed that it was possible to transfer genes coding for the resistance to antibodies and herbicides into potato plants. The transformation is stable and the transferred genes are transmitted to the offspring. In the case of resistance to herbicides, the stage of application of research results was reached: in Belgium, the scientists of the company Plant Genetic Systems NV (Van Montagu and his co-workers) succeeded in introducing the gene coding for the resistance to Basta, a herbicide marketed by Hoechst AG, into potato plants.

In February 1985 and for the first time, a bacterial gene of agronomic interest was introduced into a cultivated plant. The gene coding for the toxin of Bacillus thuringiensis was introduced into tobacco plants by a team of 16 scientists of the Belgian genetic engineering company Plant Genetic Systems NV, led by Zabeau and collaborating with Van Montagu of the University of Ghent who is the scientific director of this company (Yanchinski, 1985). The same scientists were also trying to introduce into the potato the gene for the toxin of some strains of Bacillus thuringiensis which could protect this crop against Colorado beetle and other similar pests (Rajnchapel-Messaï, 1987).

Among the plant genes which could be transferred, genes coding for the synthesis of known proteins were isolated. The genes for seed storage proteins are, for instance, easy to study, because they are very active in maturing seeds; they can be detected by the great quantities of their messenger RNAs.

3. Prospects and limitations for the use of Agrobacterium plasmids

It can be stated that the experiments made by several groups of researchers (especially those of Washington University and of Monsanto, St Louis, Missouri, of the University of Ghent and the Max-Planck Institute, Cologne, of the University of Wisconsin and Agrigenetics Advanced Research Laboratory, Madison, Wisconsin, and of Ciba-Geigy Inc., Greeboro, North Carolina) showed that the Ti plasmid of A. tumefaciens could be used as an effective acceptor and vector for any foreign DNA sequence and that plant cells transformed with this plasmid were fully capable of growth and differentiation. The design of improved Ti plasmid vectors can be improved not only for a more effective transfer of foreign genes, but also to study the expression and regulation of the transferred genes (in Marx, 1982).

The genes for resistance to environmental stresses and to pathogens or those controlling productivity are most probably clusters of genes which are not yet possible to fish out by the known techniques of genetic recombination. The magnitude of the task to be accomplished to better know the genetic constitution of plants can be appreciated, when it is realized that 5 million genes or so are contained in the nucleus of most plant cells. It is not technically difficult to pick up a segment of DNA in a plant cell nucleus, but it is not easy to know which DNA segments are worth picking.

The host range of A. tumefaciens includes most dicotyledons and some gymnosperms. To date, the major hosts for Ti-mediated DNA transfer experiments have been various species of Nicotiana. Potatoes, carrots and flax were used to a lesser extent (in Caplan et al., 1983). On the other hand, although significant progress were made in the culture of several dicotyledonous crop species (e.g. Brassica and Solanun), many important crop plants such as legumes, alfalfa, soybean and cereals cannot yet be propagated easily and rapidly in vitro (Green et al., 1974; Bingham et al., 1975).

Moreover, the cereal food crops which are monocotyledons lie outside the normal host range of A. tumefaciens. The barrier to infection could be because Ti plasmid DNA cannot enter the cell, or because if fails to be integrated into plant DNA, or because the infected cells do not respond in a tumourous fashion (i.e. cell division is not disrupted by the oncogenic genes as it is in dicotyledons). The progress in monocot cell culture and the development of alternative methods of gene

introduction (other than natural infection by A. tumefaciens), coupled with the availability of selectable markers and monocot transposable elements (e.g. those of maize), will help solve in the near future the problem concerning the efficient transfer and integration of DNA into monocot species.

Although the transfer of genes into plant cells or protoplasts, using Ti plasmids or other means of DNA transfer, is still in the development stages, there are some potential applications for tropical crop species, such as modified invertase in sugar-cane, cyanide elimination in cassava, haemagglutinin in soybean, and inhibition of oxidizing enzymatic systems in oil palm fruits (in Sawyer, 1984a). See also Hollaender et al. (1983); Sharp et al. (1984); Zakri (1988).

4. Other means of DNA transfer

Micro-injection of DNA into the nucleus of protoplasts immobilized on a glass slide after a treatment with polylysine or by succion on a holding pipette can be done rather effectively. Facciotti et al. (1985) of Calgene Inc., Davis, California, used tobacco and rape mesophyll protoplasts which were synthesizing a new cell wall and could be manipulated more easily than freshly prepared protoplasts. The slight thickness of their wall did not prevent the micro-injection of DNA into the cell nucleus. The micro-injected protoplasts showed a survival rate of about 90%.

In 1985, Calgene's researchers micro-injected the bacterial gene for an enzyme that determines resistance to an antibiotic into rape cells (Brassica napus), and complete plants were regenerated from the transformed cells (in Biofutur, April 1985, p. 66).

Embryos could be micro-injected. Embryos of cereal seeds having 10 to 20 cells could be isolated and micro-injected. Their regeneration into complete plants will condition the success of the micro-injection and transformation technique.

Once the effectiveness of the micro-injection of non-cloned DNA is established, it could become a promising technique for the transfer of groups of genes that code for interesting agricultural characters, and the pollen grain can be the best natural vector for this transfer.

Electroporation is another means to transfer alien DNA through the pores created in the cell membrane of protoplasts or cells when the latter a resubmitted to an electric shock.

SOCIO-ECONOMIC IMPACT OF PLANT BIOTECHNOLOGIES

1. Impact on agricultural and food-processing sectors

The agricultural and food-processing sectors in developing countries are more likely to suffer from the commercialization of bio-industrial products, which will compete with foodstuffs produced by the developing countries where they represent the most important source of export income (Sasson, 1988). Such competition is bound to be increasingly harsher, because the international character of agriculture favours the predominance on the world main commodity markets of multinational giant firms which also play a leading rôle in the marketing of fertilizers, seeds, biocides and veterinary drugs (Herdhuin, 1983; Kenney and Vellutini, 1985). About twenty of these multinationals occupying a leading position in the production and commercialization of petrochemicals have contributed to a great extent towards increasing agricultural production as a result of advances in the field of plant breeding. They are also on the vanguard of "biotechnological revolution". See Sasson (1988).

In addition to taking over seed-producing companies, the large petrochemical and agro-industrial firms invested huge sums in the field of biotechnological research applied to crops. By so doing, these firms are perfectly conscious of the economic stakes, as their investments not only involved small venture-capital companies engaged in advanced research, but also larger companies engaged in research and development. Many chemical and pharmaceutical corporations have even created their own research laboratories in plant biology and physiology in order to carry out investigations in areas which would be most profitable to them (Kenney and Vellutini, 1985).

Plant genetic resources required for continuous improvement of crop varieties tend to decrease, as improved varieties marketed by multinationals replace hardy cultivars in developing countries on ever increasing acreages and, in so doing, homogenize the genetic make up of the crops, leading to their increased susceptibility to pathogens and parasites. The harsh competition among seed-producing companies in their quest for markets in developing countries in order to sell a restricted number of improved crop varieties, may contribute to the shrinkage of plant genetic resources.

In another area, that of plant cell or tissue cultures for producing a wide range of substances, once technical hurdles are overcome and economic advantages established, systematic utilization of these

cultures in the years ahead could seriously threaten the traditional methods of culture or harvesting of those substances. Developing countries exporting the latter will therefore face a significant drop in their income and foreign currency earnings. These plant substances indeed play an important role in the local economy and foreign trade of these countries (Kenney, 1983; Kenney et al., 1983; Crocomo et al., 1981, 1986). Regarding natural (calorie and low-calorie) and artificial sweeteners, biotechnology is to contribute largely to increasing the variety of sweeteners and, in this respect, would be responsible for the negative impact on the economy of the sugar-producing developing countries.

2. Other socio-economic effects

The replacement of old or traditional cultivars by new ones can result in unemployment or underemployment in at least part of agricultural labour, if the new varieties necessitate less work. For example, the replacement of banana by oil palm has caused unemployment in Costa Rica, because the maintenance of the oil-palm plantations need about one-third less manpower (Izurieta, 1984). However, the overall fall in employment in the agricultural sector could be offset in part by increased earnings and capital accumulation which by stimulating secondary and tertiary sector activities would create enough jobs (Watanabe, 1984).

According to the British group Imperial Chemical Industries Ltd., production of single-cell proteins require only 10% of the labour force necessary for producing an equal amount of protein from soybean cultivation. Therefore production of such proteins from n-paraffins, methanol, wastes or by-products of agro-industries would not only compete with soybeans and their derivatives used in livestock feeding, but would also have repercussions on employment.

Applications of biotechnologies to improvement and selection of crop varieties tend to favour crops almost exclusively destined for export markets. Such crops as banana, coconut or oil palm do also play an important role in the national economy, as they generally have a wide range of end uses, not only in food and cooking, but also in the household economy. Given the overwhelming influence of the major food and fruit groups which control the markets of such products, research carried out to improve the current varieties, using plant cell and tissue culture, tends to cater for the needs of the international

markets rather than for domestic needs: the dessert banana may be favoured at the expense of plantain[1]; coconut improvement may select for clones yielding higher quantities of oil for the production of margarine, soap and lubricants, sold on the world market, rather than focus on the multipurpose uses of coconut in the local economy; fuel-alcohol production programmes from plant biomass also aim at substituting commercial crops to food crops, except in cases where large acreage of land is available.

Benefits reaped from the application of biotechnologies to crops, as for instance the selection of higher-yielding clones or in-vitro vegetative propagation of cultivars of desirable agronomics characteristics, may in all likelihood strengthen the large agricultural estates rather than improve the standard of living of small landholders. This is because the large agricultural estates dispose of operational skills, financial resources and market experience, all of which enable them to take full advantage of the latest know-how and technological applications. Small landholders and landless farmers run the risk of being displaced by further expansion of large agricultural estates, unless appropriate national policies ensure equitable distribution of benefits derived from biotechnologies in favour of small farmers. Selection of crops being able to use optimally water and fertilizers and which necessitate minimum treatment with biocides should, however, benefit farmers with low means and who cannot buy all the expensive agricultural inputs.

1. It should be mentioned, however, that efforts are made at the international level and within bilateral agreements with tropical countries in order to improve the plantain banana and to select new varieties. The International Network of Investigation on Banana and Plantain (INIBAP) aims at developing new varieties which are resistant to pathogenic micro-organisms, such as the microfungus Mycosphaerella fijiensis var. difformis which causes a very serious disease (black cercosporiosis or sigatoka). INIBAP also aims at establishing in vitro banana and plantain germplasm collections which will be made available to all. In France, the Centre de coopération internationale en recherche agronomique pour le développement (CIRAD, In Vitro Culture Laboratory, Montpellier) has been involved since 1982 in a long-term research programme on the genetic improvement of plantain and similar staple-food banana varieties, using tissue culture techniques.

3. Plant breeders' rights and farmers' rights

Measures taken by technologically-advanced countries to protect the products of increasingly expensive plant genetic research and to make this research more profitable entail, for developing countries, the payment of fees for the seeds of crop varieties bred in industrialized countries. The latter also tend to use their germplasm collections for commercial exploitation, and the private sector is playing an increasing role in the collection, conservation and utilization of germplasm.

Furthermore, developing countries which do not always have the financial and technical means to set up seed banks, nor to maintain them in satisfactory conditions, have no other choice but to buy any new cultivar, bred from cultivated varieties or from wild relatives existing in their own regions. This is the case, for instance, with varieties grown and improved for many generations by farmers in the developing countries and which were later on cross-bred with other varieties in industrialized countries and thereafter resold eventually in their countries of origin as "new and different" varieties. The award of plant breeders' rights and more generally of property rights on crop plants and agricultural products to those who have recently improved the genetic make-up of these plants, neglects the endeavour of all those who have been improving them over a long period of time, but who have not reaped any profit whatsoever (Hobbelink, 1987).

For the first time in agricultural history, there is a confrontation between the breeders' rights and the farmers' rights. The latter were promoted at the international level with a view to conferring on farmers in the developing countries benefits similar to those given to plant breeders in industrialized countries under the PBR legislation. The rationale behind such promotion is that farmers' efforts, in conscious and unconscious breeding and maintenance of genetic diversity, have led to the availability of valuable cultivars which the breeders of industrialized countries used in their breeding schemes. M.S. Swaminathan, former Director-General of the International Rice Research Institute and first laureate of the World Food Prize in 1987, explained that "the trend toward privatization of plant breeding research in developed countries and the ever-widening scope of patents rights also cause concern in developing countries. From such a concern arises the recent move in FAO to promote, in developing countries, rich in crop genetic resources, the concept of farmers' rights to compensate for breeders' rights prevalent in developed nations."

"The North is rich in seeds, the South in genes": plant genetic resources of most crops are found in developing countries, especially in tropical ones, whereas breeding and improvement of crop species and varieties are carried out mainly in industrialized countries. Developing countries which have become conscious of the economic importance of their plant genetic resources intend to protect them by, for instance, prohibiting export of any reproductive plant part, i.e. its germplasm. They claim that the price for re-acquiring the varieties selected and improved from their own plant genetic resources is too high and that it is unfair to be thus condemned to indirectly purchase back part of their own plant genetic heritage (Hobbelink, 1987). Developing countries intend also to promote the national production of improved seeds.

4. Search for an international agreement on the conservation and utilization of plant genetic resources

The alarming decrease in genetic diversity or "genetic erosion", together with the restrictions imposed on the distribution of plant material needed for crop improvement and creation of new cultivars, led to the search for an international agreement aimed at conserving plant genetic resources, considered to be part of the common heritage of humankind, and at ensuring their equitable utilization, rather than leaving their exploitation under national jurisdiction alone. If industrialized countries wish to gain access to the plant genetic resources of developing countries and use their hardy cultivars, developing countries want, for their part, to benefit from the services offered by gene banks of industrialized nations and, at the same time, claim national sovereignty over their crop species and varieties. Thus, the problems of conservation of plant genetic resources and of access to these resources have become a geopolitical issue within the context of discussions relating to the exploitation of global resources for the benefit of all nations (Dahlberg, 1983).

In a letter dated 30 October 1987, and circulated among potential donors, the Director-General of the FAO confirmed the establishment of an International Fund for Plant Genetic Resources, in which donor governments, intergovernmental organizations, non-governmental agencies, private industry and other bodies were invited to participate. In November 1987, at the 24th session of the Conference of the FAO, Canada,

Japan and the USA (among others) did not join the Voluntary International Undertaking on Plant Genetic Resources. On the other hand, the network of gene banks under the auspices or jurisdiction of the FAO, which was established to store the duplicata of accessions of the main plant species and to guarantee their free circulation, was not wide enough and had a vague legal status.

In the near future, therefore, genetic information necessary for crop improvement is likely to become a commercial commodity, subject to competition among seed companies, among nations and between these companies and the countries concerned. It is nonetheless important to find a compromise between the legitimate wish to reward human ingenuity by awarding rights to breeders in industrialized countries, and the necessity for developing countries to acquire improved varieties at a cost compatible with their limited means and their agricultural development needs. Furthermore, from an ethical viewpoint, the toil of generations of farmers in developing countries in improving their crop varieties should also be taken into consideration in the process of selling back to them improved varieties of their major crop species (Dahlberg, 1983; Mooney, 1984; Hermitte, 1985, 1986; Alaux, 1987; Hobbelink, 1987; Juma, 1989).

It is, by no means, urgent and indispensable to collect and conserve the plant genetic resources which include the primitive cultivars, as well as the wild relatives of cultivated plants, so as to prevent their irremediable loss and to guarantee the future of breeding research. The difficulties and constraints of tomorrow's agriculture are not known: for instance, climatic variations and the appearance of new pathogens and pests. Farmers, agronomists and livestock breeders of tomorrow should have the tools with which to solve these new difficulties or constraints. They cannot do so unless they have at their disposal the largest genetic pool, i.e. the most varied genetic resources. That is why the in-situ and ex-situ conservation of genetic resources is a major responsibility for humankind, whatever the geopolitical and economic stakes might be. See also Sasson (1989).

ADVANTAGES OF PLANT BIOTECHNOLOGIES FOR DEVELOPING COUNTRIES

Plant tissue culture offers several advantages equally valid for industrialized and developing countries (United Nations Centre for Science and Technology for Development, 1984; Crocomo et al., 1986; Crocomo and Cidade de Araújo, 1988; Zakri, 1988). Production can be continuous and is not limited by season or climate. The quality of products obtained is more reliable; they are generally free from pathogens and contaminants. Such a production can therefore complement that of agricultural foodstuffs, the quality of which varies with environmental conditions, climate and farming practices. The facilities needed for plant tissue culture do not necessarily have a size which should be adapted to seasonal harvest peaks, nor do they have special storage requirements to prevent harvested products from losing their quality before being converted or processed. Such facilities can operate throughout the year and be sited near markets and places of consumption rather than near the cultivation sites.

The application of biotechnologies to plants can indeed contribute both to crop improvement and protection of threatened species. Meristem cultures can lead to the development of tissue banks for crop varieties, especially for those which run the risk of being eliminated by intensive agriculture.

If one deals with plant cell culture for the production of metabolites such as pharmaceuticals, dyes, food additives, sweeteners, flavours and taste enhancers, the fermentors used can also be utilized for the production of several categories of substances; production costs are lower and much time is saved. Although the inventory of tropical and subtropical plants needs yet to be completed, numerous floristic studies already carried out together with the indigenous pharmacopoeia indicate that a wide range of useful substances are synthesized by a wide spectrum of plant species, mainly the arboreal ones. Most of these secondary metabolites could be obtained by cell suspensions in bioreactors. This would be an interesting perspective for many developing countries, especially for those where cell and plant tissue cultures are widely utilized. This option should not, however, prevent the selection through somaclonal variation of new varieties rich in secondary metabolites (in Sawyer, 1984).

PRIORITIES, CONSTRAINTS AND APPROPRIATE STRATEGIES

Significant results have been obtained in the vegetative micropropagation of tropical crop species and of virus-free lines; cell culture is also considered a promising technique for the production of useful substances. However, plant biotechnologies are not the only techniques or the only means of improving crop species; they in fact complement plant breeding methods and agricultural practices aimed at increasing agricultural production (IRRI, 1984). The success of these biotechnologies in developing countries depends, to a large extent, on their close association with conventional crop breeding, as well as with agricultural extension services, on the establishment of incentive price policies for agricultural products, and the existence of an efficient marketing network. In all likelihood, advanced regions or districts with intensive agricultural systems will benefit more rapidly from the application of plant biotechnologies.

Plant cell and tissue culture techniques as well as genetic engineering methods are not solutions, but merely tools. For many developing countries, it should be preferable to take advantage of simpler techniques of plant tissue, meristem and organ culture in order to achieve rapidly the vegetative propagation of valuable cultivars and the release of virus-free lines. It is also worth adopting and scaling up particular plant biotechnology methods which are less costly, well established and easily transferable and adaptable to local conditions (in BOSTID, 1982; Collins, 1982, 1984; Marin, 1984; Panchamukhi, 1988).

Such a choice does not at all imply that one must accept or tolerate an international division of biotechnologies: that is for some, the technologically advanced countries, the sophisticated biotechnologies, whereas for others, the developing countries, the outdated ones. In fact, a range of biotechnologies, of variable complexity or sophistication, should exist in each given situation; any national scientific or technological community should be capable of handling most sophisticated techniques or adapting them to their development projects in order to meet local needs and keep abreast with new trends in biotechnology. It is therefore necessary for each country to determine its priorities with a view to identifying its economic objectives and drawing maximum advantage of available resources. Biotechnological processes which can have socio-economic benefits must be identified. Inventories of local resources should be implemented for this purpose, and research-and-development priorities in biotechnology set up.

These priorities must take into account the availability of capital, the mode of payment with foreign currency and possible investments, the critical mass of qualified staff and technicians as well as the size of local, regional or international markets for the end products of biotechnologies. Special management measures are also needed to ensure transition from traditional to more complex biotechnologies, requiring different know-how (Ventura, 1983; Bhalla et al., 1984; Panchamukhi, 1988). The establishment or strengthening of public or private research centres should also be considered just as creating or defining better co-operation links between relevant institutions (in Sasson, 1984, 1988).

In addition to priority setting in research and development in plant biotechnologies, measures should be taken to mitigate the adverse socio-economic effects resulting from the production by industrialized countries of biotechnological substitutes for agricultural commodities exported by developing countries, from the increasing privatization of biotechnological know-how and research data, and from the obstacles to technology transfer (Kenney, 1983; Kenney et al., 1983; Quintero Ramírez, 1985; Yanchinski, 1987).

Whatever be the option adopted towards developing plant biotechnologies and improving or adapting them to local needs, higher education and training will play an important role. The very nature of these biotechnologies which are related to several disciplines of plant sciences (genetics, biochemistry and physiology) and of engineering (fermentation technology, automatization of production techniques, industrial chemistry and microbiology) necessitates multidisciplinary training programmes and an integrated approach. In many developing countries, training in biotechnology is given at postgraduate level. It should instead be introduced as from the third year in university courses, as part of classical biological disciplines such as microbiology, biochemistry, plant physiology and genetics.

Shortage of biotechnology researchers and technicians is manifest in developing countries: for 23,000 researchers in the USA, 12,000 in the USSR, 8,000 in Japan, there were, in 1983, only 3,400 in Asia (excluding Japan), 1,900 in Latin America and 400 in Africa (in Langley-Danysz, 1983). One must not, however, underestimate employment problems which are directly related to economic infrastructure and development. Economic difficulties faced by many developing countries are the cause of increasing unemployment of scientists and technicians.

The local constraints or difficulties that can hamper the smooth operation of laboratory research in biotechnology include, for instance, the absolute availability of continuous power supply, in order to maintain temperature, humidity and light conditions in plant tissue culture laboratories. A power cut of only three hours suffices to destroy these cultures and the respective germplasm, thus annihilating several years of labour by researchers and technicians, as well as the investments made in the equipment of the facilities (which was estimated at $50,000-250,000 for a cell and tissue culture research laboratory in 1984). Another constraint lies in the regular and quick supply of high-grade chemicals and reagents which must be kept in cold stores. Two days' deposit in a hot environment, as for example at the customs, can result in irreversible deterioration of these chemicals.

Other constraints are external and include existing competition of products from industrialized countries, protection by patents, or by industrial or trade secrets which make access to specific technology or to germplasm banks difficult, if not impossible, and availability of foreign capital (in Sasson, 1984, 1988). That is why preservation and conservation of germplasm and, in general, of plant genetic resources constitute a priority for developing countries who want to prevent the disappearance of their semi-domesticated crop varieties and their wild relatives, and to implement a programme of genetic improvement of their cultivated varieties through plant cell, tissue and organ culture techniques (in National Academy of Sciences, 1978; Sasson, 1988, 1989).

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