

# Biotechnology and Food Production: Relevance to Developing Countries

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## I. AGRICULTURAL AND FOOD PRODUCTION IN DEVELOPING COUNTRIES: AN OVERVIEW

### A. Asia

By the year 2000, the per capita land availability of India's projected 1 billion inhabitants will have shrunk to 0.14 hectares (ha). For food grains alone, needs are estimated at 240 million tonnes for the year 2000.

With the exception of Brunei and Singapore, agriculture plays a prominent role throughout Southeast Asia. In Indonesia, it involved more than 60% of the population, and contributed 24% to the gross domestic product (GDP) and more than 60% to the value of nonoil exports by the early 1990s. Paddy production accounted for more than 40% of agricultural output, land use, and employment in 1990, with production increasing from 12 million tonnes in 1969 to 29 million tonnes in 1989, self-sufficiency in rice production having been reached in 1985 [Dart et al., 1991]. If between 1981 and 1991, 33 million were added to the Indonesian population, rice production doubled to 44 million tonnes [Margolin, 1993a].

In Malaysia, in 1990, agriculture and forestry represented 18.7% of the GDP; by the year 2000, they will not represent more than 13.4%, compared with 37% for industry [pomonti, 1991a]. Malaysia is also a leading exporter of cocoa.

By the early 1990s, in the Philippines, the poorest among the ASEAN (Association of Southeast Asian Nations) member states, coconut and sugarcane accounted for about 30% of the cultivated acreage and for about 50% of agricultural exports, on which about 15 million small holders depended [Pistorius and Smits, 1990].

In Thailand, agriculture still employed more than half of the active population and provides 32% of exports [Margolin, 1993b]. In 1990-1991, the production of major agricultural commodities was 18.4 million tonnes of rice, 25 million tonnes of cassava, 5 million tonnes of sugar, and 3.7 million tonnes of maize. Worldwide, Thailand has been the leading rice exporter

(49% of cultivated land) and the foremost exporter and second most important producer of cassava after Brazil [Bhumiratana, 1990].

In Vietnam, agriculture represented half of the GDP, one-third of exports, and occupied 75% of the active population in the early 1990s [Margolin, 1993b]. About 10.4 million farming households were restricted to only 0.1 ha of arable land per person. Sixty percent of land was privately owned in the South, whereas in the North, 95% of farms have been turned into collective farms [Pomonti, 1991b]. A prominent player on the international rice market since 1989, Vietnam exported 1.8-2 million tonnes of rice in 1993 [pomonti, 1993].

Southeast Asia also supplies the near totality of natural rubber, palm oil, copra, pepper, and a large proportion of cocoa, sugar, cassava, and coffee [Margolin, 1993b].

Agricultural productivity varies from one country to another: Cambodia has not yet recovered to 1960s levels, whereas in Laos, Myanmar, and the Philippines, per capita agricultural production has receded by at least 10% in a decade; in Vietnam, rice production doubled between 1990 and 1992, after it became the world's third-largest rice exporter in 1989. Malaysia imported 30% of its rice needs, the government supporting rice cultivation exclusively in the few irrigated areas; its powerful plantation sector (5 million ha, compared with 650,000 ha of paddy fields), as well as its industrial development, has enabled Malaysia to reduce its food bill from 10 to 5% of imports. Thailand has almost doubled its agricultural area in 30 years, but yields are still low: 2.1 tonnes of rice per hectare, compared with 3.1 tonnes per hectare in Vietnam, 4.4 tonnes per hectare in Indonesia, and 5.7 tonnes per hectare in China. On the island of Java or in Vietnam, there is a lack of cultivable land, whereas Malaysia is experiencing a lack of manpower or an increase in its cost [Margolin, 1993b].

In addition to necessary diversification or reconversion, an increase in efficiency and productivity (e.g., through lower production costs and improved quality), with the help of biotechnologies, could be an appropriate answer to the difficulties agriculture is facing in Southeast Asia.

## B. Latin America

Although agrarian reforms have eliminated the unequal dependence status and the contributions in kind made by farmers to their landlords, application of these reforms has been uneven. In Central America, land redistribution is still largely incomplete. In Brazil, only one-tenth of the program has been achieved and small holdings represent only 9% of total agricultural acreage. In Colombia, farms of more than 200 ha represent 40% of total cultivated land, compared with 46% before redistribution [Herzlich, 1992]. In Ecuador, land concentration has decreased significantly, although large farms exist in the Amazonian part of the country, over 4 million ha, in particular for the cultivation of oil palm and soybeans; agriculture represents 15% of the GDP and 40% of the total population live in rural areas [Niedergang, 1992].

To meet the needs of growing populations, many governments have blocked agricultural prices and have maintained export taxes which, paradoxically, has encouraged high-profit crops and livestock husbandry. As a result, landless farmers and rural dwellers have migrated to the slums of large cities [Herzlich, 1992]. Furthermore, instead of transforming all agriculture, successive governments have focused on a few crops and livestock husbandry geared toward exports. This was the case in Mexico, which nevertheless acknowledged exceptional agricultural growth in the 1970s [Baudin, 1992].

In Chile, in 1978, 60% of latifundia expropriated previously were more or less reconstituted, but they represented a mere 40% of cultivated lands. The nonrestituted land was bought by the wealthiest farmers, as well as by corporations, which introduced an intensive exploitation model, based on modern techniques and cheap labor. At the end of the 1980s,

the latifundium had completely lost its dominant position, whereas modern farms, called "Californian," often of a medium-size, were thriving and using intensive-cropping systems. Chilean agriculture had become the driving force behind national economic growth [Baudin, 1992].

In Brazil, during the 1980s, the country boasted a cultivated acreage of about 40 million ha: it produced 70 million tonnes of grains and more than 5 million tonnes of meat. Development of a capital and technology-intensive agriculture was not confined to the south and southeast; it had displaced traditional crops, such as coffee, soybeans, and wheat, to the cerrados, thus incorporating central Brazil into the new agricultural frontier. The cerrados region covers 180 million ha (21% of national territory), of which 50 million ha are potentially suitable for crop production.

Livestock husbandry had moved toward intensive management practices, in particular for swine and poultry. Once an importer of chicken meat, Brazil became the world's second largest exporter in the mid-1980s. The extension of citrus orchards has made Brazil the leading world exporter of concentrated orange juice, with an annual turnover of 1.5 billion dollars. Furthermore, Brazil has become the world's second-largest producer of soybeans and the leading exporter of soybean oil and meal.

Thus, in about 30 years, Brazil has built up its agroindustrial complex and agribusiness, which corresponds to more than one-third of GDP, to 40% of total employment and 60% of the trade balance. However, grain needs are estimated to be about 115 million tonnes in the year 2000. Consequently, in its proposal for a Programme for Agroindustrial Competitiveness (PCA), the Brazilian Government has stressed that the Brazilian Agricultural Research Enterprise (EMBRAPA) will emphasize the scientific and technological capability in such areas as biotechnologies and genetic engineering, informatics applied to agriculture and livestock husbandry, agroecology, and genetic resources.

#### c. Arab States

Near Eastern countries have become large importers of foodstuffs owing to their high population growth rate, the stagnation of agricultural production, and the decline in food output. The value of agricultural imports for the Near Eastern countries reached 17 billion dollars in 1990, whereas the agricultural exports amounted to about 2.5 billion dollars, with a resulting deficit of 14.5 billion dollars [Salamé, 1993].

Land reclamation has been instrumental in the development of nontraditional agriculture in Egypt. Over the 1980–1990 decade, some 26,400 ha of desert land were reclaimed, and in 1992, reclamation was adding 17,600 ha of cultivable land annually [Mackie, 1992].

The 1977–1987 decade witnessed a major agricultural expansion in Saudi Arabia. In 1992, Saudi Arabia was entirely self-sufficient relative to bakery products and was the sixth world wheat exporter: 2 million tonnes out of the 4 million tonnes produced. A major problem was that of water resources.

Agricultural land covers about 7.5 million ha in Algeria, 8.8 million in Morocco and 4.8 million in Tunisia [Daaloul et al., 1990]. In Morocco, more than 57% of the total population lives in rural areas; 40% of the total active population belong to the agricultural sector and, in rural areas, 68% of the population derive their income from agriculture.

Agricultural production needs to be supplemented by imports of cereals, sugar, vegetable oils, milk, and meat, although Morocco had achieved self-sufficiency to a large extent (10 million tonnes of cereals in 1994, a record after 2 years of drought and mediocre harvests; e.g., 3 million tonnes in 1993).

Several programs concerning crop breeding, agricultural techniques, and plant protection have led to important results; however, there has been a need for further research to enhance productivity and to meet national needs.

#### D. Sub-Saharan Africa

The United Nations Economic Commission for Africa has been prompted to state that the 1980s was a lost decade for the development of sub-Saharan Africa because production of agricultural foodstuffs per capita dropped by 20% down to 200 kg/person in 1990; a cash crops' recession (except tea) and marked decrease in export earnings; subsidized food in the cities, but decreasing remuneration of farmers; failure of many agricultural development projects; and ineffectiveness of agricultural-assistance projects. The 1990s could be another lost decade for the development of Africa, judging from the pessimism of forecasts for agricultural and food production, industrialization, and participation in international trade.

If food security were to be ensured everywhere, cereal needs were estimated at 410 million tonnes of maize, or its equivalent, for the year 2020, whereas production would reach only 165 million tonnes; the resulting deficit of 245 million tonnes would have to be met by imports [Fottorino, 1992].

Among the major causes of this situation was the lack of sufficient attention given to the farmers' situation; they had to feed cities in exchange for low prices for their commodities, while political leaders focused on costly industrial projects; in the absence of incentives and adequate agricultural policies, compounded by the lack of appropriate techniques and inputs, the African farming communities tended to revert to self-subsistence agriculture [Fottorino, 1992]. Sub-Saharan Africa also remained, to a large extent, on the outer rim of the "green revolution" spreading throughout Asia and benefiting from hundreds of years of knowledge of irrigation techniques and the presence of major rivers. The green revolution played a role only in Kenya and Zimbabwe, where it contributed to extending and improving maize crops [Gherardi, 1993].

However, in 1990, agriculture still represented 32% of the GDP in sub-Saharan Africa [Chalmin, 1992].

## II. AGRICULTURAL BIOTECHNOLOGIES: ACHIEVEMENTS, COOPERATION, AND PROSPECTS

### A. Asia

#### 1. China

Anther culture was being practiced in about 1000 institutions throughout China. A high number of microspore-derived plant species were obtained by Chinese scientists, a world record. These species included wheat (*Triticum aestivum*), maize, hot pepper (*Capsicum annuum*), and sugar-beet (*Beta vulgaris*). The acreage of haploid paddy rice and wheat was extended to millions of hectares, with outstanding economic profit [Zhaoxiang and Yongchun, 1990]. The use of embryo rescue technique and in vitro culture of a hybrid between *Triticum aestivum* and *Agropyron elongatum* led to a new wheat variety "Xiaoyan no. 6," which, in 1990, was grown on 38 million ha, with a yield of 16 million tonnes.

It became possible to propagate more than 100 crop species, by in vitro tissue culture. In vitro micropropagation is also used for the clonal multiplication of banana: in the Guangdong province, 3–4 million banana plantlets are produced annually by tissue culture, and 1 million plantlets have been exported. Meristem culture is also being carried out to produce virus-free

seedlings of potato, strawberry, garlic, and asparagus (*Asparagus cochinchiensis*). Seedlings of virus-free potato have been commercialized and cultivated on about 300,000 ha (i.e., 10% of the total acreage) in the early 1990s. A propagation and extension system for virus-free potato production has been established in several provinces, and yields have been increased by up to 100 or 200% [Guang-Nan Wu, in a paper submitted to the workshop on "Assessment of biotechnology for food production in Vietnam," Hanoi, 9–12 December 1991].

In the Guangxi province, the sugarcane cultivar "Gueitang 11," with a high content of sucrose, has been mass-propagated and grown on more than 30,000 ha in 4 years, instead of the 10 years generally needed when conventional propagation is used.

At the Shanghai Institute of Biochemistry, plant genetic engineering uses the introduction of alien DNA by the pollen tube after pollination, with a view to developing resistance to diseases in rice, wheat, soybeans, and cotton. This simple technique of genetic transformation is an indication of the unsophisticated approach chosen in some Chinese laboratories to obtain good results in crop breeding. At the National Laboratory of Protein Engineering and Plant Genetic Engineering of the University of Beijing (Beida), research on transgenic tomato and rice are being carried out with a view to transferring genes coding for resistance to fungal blast and rice rust, respectively [Borry, 1992].

At the Laboratory of Plant Cell Engineering of the Beijing Academy of Agricultural Sciences, research projects are being carried out on

Anther culture aimed at selecting new winter wheat and rice varieties

Culture of protoplasts and cell fusion (a winter wheat variety has been regenerated from protoplasts)

Somaclonal variation and embryo cultures in maize and rice

Interspecific crosses to breed disease resistance in winter wheat

Chinese strains of *Bacillus thuringiensis* and *Bacillus sphaericus* were applied successfully to vegetables, maize, rice fields, and pine trees, covering over 18 million ha. For more detailed information, see Sasson [1993].

The need to increase China's present grain yields can be met by using both conventional breeding and plant biotechnologies. The rice genome project, supported by the National Commission of Sciences and Technology, is an important, long-term research project, with major potential applications. The Rockefeller Foundation also supports about 15 laboratories for rice biotechnology research.

## 2. India

It is anticipated that over the 1993–2000 period, about 8 billion rupees would be invested in health care, 2 billion rupees in agriculture, and some 4 billion rupees in other areas, mainly in most conventional biotechnology-derived products, investments in recombinant products being rather small [Ghosh, 1993].

It is anticipated that the consumption of hybrid seeds would rise from 90,000 tonnes in 1992 to 115,000 tonnes in 1995, and about 165,000 tonnes by 2000. Increasing quantities would be produced locally, to exploit the potential of hybrid vigor in paddy, wheat, rapeseed, and mustard [Ghosh, 1993].

Up to 1993, 22 approvals had been granted giving a capacity of 110 million tissue culture-derived plantlets per year for the whole country [Ghosh, 1993].

During 1988–1989, the Department of Biotechnology (DBT) of the Indian Ministry of Science and Technology launched the Oil Palm Demonstration Projects, with a view to reducing edible oil imports, which accounted for 104 million dollars in 1990–1991, and providing a

regular source of income to the farmer, as this crop species is perennial. Many state governments, private entrepreneurs, and financial institutions have drawn up ambitious expansion programs for oil palm in Indian [Kumar et al., 1993].

A program for tissue culture of coconut was launched in 1986–1987. Large-scale plantations using in vitro plants are anticipated after evaluating field performance. In 1995–2008, massive-scale production units will be set up to supply high numbers of plantlets of high-yielding coconut varieties to replace traditional low-yielding ones [Kumar et al., 1993].

In 1984–1985, the A. V. Thomas group (AVT) initiated commercial production of cardamom through tissue culture. On average, tissue culture-derived plantlets show an increase of 63% in yield, the mean estimated yield being 360 kg/ha. AVT has also applied tissue culture to tea, for it owns 2000 ha of tea plantations; the company has been successful in increasing tea yield to 2520 kg/ha, compared with the average South Indian tea yield of 2,060 kg/ha [Kumar et al., 1993].

Unicorn Biotek is a recent example of a tissue culture-based company moving modern biotechnologies from laboratory to marketplace. In addition to a project on the large-scale production of virus-free banana plantlets, supported by the Department of Biotechnology, Unicorn Biotek, produced and exported 120,000 *Spatiphyllum* (a foliage ornamental) and 2000 rose plantlets over 12 months at competitive prices in the world market. In addition to selling to clients in Belgium, The Netherlands, Denmark, and the United States, the company is also present on the local market: farmers in Andhra Pradesh, Karnataka, and Maharashtra bought 20,000 disease-free strawberry plantlets from Unicorn Biotek; they can buy higher numbers of high-yielding and disease-free plantlets from the company, which has improved productivity and lowered the price of tissue culture-derived plantlets. Although Unicorn Biotek is a good illustration of efforts by India's more than 40 tissue-culture companies (in 1993) to bring commercially viable products to the market place, it faces competition from major Southeast Asian companies, and also from other Indian companies [Knudsen, 1993].

Together with the Biotech Consortium of India, Ltd., the Department of Biotechnology (DBT) has assisted in setting up two pilot plants for the mass production of biocontrol agents. The DBT plans to promote small production units in villages by providing training and credits to entrepreneurs. The major focus is to control one of the world's most damaging pests, the cotton bollworm, recorded in 96 crop species and 61 weeds and wild species in India [Kumar, 1992].

Sandoz India, Ltd. produced a *Bacillus thuringiensis* Berliner-based biopesticide in September 1992. Similarly, Hindustan Lever, Ltd., the Indian subsidiary of Unilever NV, produced a *Bacillus thuringiensis israelensis*-based biopesticide using molasses as a culture medium. It has been successfully tested against insects attacking cabbage, pigeonpea, maize, safflower, and cotton. The insecticide is also active against black flies and mosquitoes and therefore, can be used in malaria vector control.

The biopesticides extracted from neem (*Azadirachta indica*) of the Meliaceae family, are attracting increasing attention from scientists and companies throughout the world. Neem extracts reportedly control more than 200 species of insects, mites, and nematodes, and major pests such as locusts, rice, and maize borers, pulse beetles, and rice weevils [Khanna, 1992].

In 1993, about 1000 tonnes of *Rhizobium* inoculants were produced, but it was forecast that, by the year 2000, the consumption of *Rhizobium* fertilizers may rise to 8,000–10,000 tonnes/yr, covering 50–60% of the 30 million ha of land used for leguminous crops. For the blue-green algae (BGA), used for rice cultivation, more than 400 production ponds have been set up for growing BGA for field trials and nearly 1100 field demonstrations have been organized in different parts of India to popularize the use of BGA fertilizers. The latter increase rice yield by 7–9%, enabling a reduction in chemical fertilizer use of up to 30%. Large-scale use of

BGA, which is considerably cheaper than chemical fertilizers, could have a substantial influence, because about 40 million ha of land have been devoted to paddy cultivation [Ghosh, 1993].

Promising results have been obtained in conventional research and development for increased production of sugarcane, wheat, and rice, but more attention has to be directed to oil seeds, pulses, and coarse grain cereals. Despite shortcomings in packaging, storage, and transportation of plantlets, India has the potential of becoming a major international trading partner of horticultural and floricultural products. For more detailed information, see Sasson [1993,1994].

### 3. *Indonesia*

The Agency for Agricultural Research and Development—Badan Litbangtan (AARD)—supports the main program on agricultural biotechnologies in Indonesia, and has set up a National Center for Agricultural Biotechnology at the Bogor Central Research Institute for Food Crops (BORIF), nominated as a national center of excellence in biotechnologies by the Ministry for Research and Technology.

Initial research emphasis at the National Center is placed on seed technology, and, as part of the Rockefeller Foundation's International Rice Biotechnology Program, cooperation has been established with the International Rice Research Institute (IRRI, Los Baños, Philippines), concerning the regeneration of plants from calli of javanica rice. This regeneration is a prerequisite for any selection based on somaclonal variation, protoplast fusion, or plant transformation [Dart et al., 1991].

The Australian Center for International Agricultural Research (ACIAR) scheme for collaboration between laboratories in Australia and Indonesia on projects of mutual benefit became highly productive in terms of both scientific achievements and technology transfer. An ACIAR project involving the New South Wales Department of Agriculture concerns the selection of *Rhizobium* strains for soybean inoculation, the legume species that is grown after rice and in acid soils in newly developed fields in transmigration areas. Another project sponsored by the ACIAR concerns the improved diagnosis and control of groundnut stripe virus. This project aims to protect groundnut from the virus by transforming the legume species with the viral coat protein gene using microprojectiles, followed by the regeneration of transformed groundnut calli [Dart et al., 1991].

The Dutch Government supports the setting up of the Lembang Horticulture Research Institute (LEHRI). Biotechnologies are being used at the LEHRI for the major commercial crop species, garlic, potato, and asparagus; the research agenda for garlic and shallot (*Allium* spp.) includes virus elimination, diagnostic kits for virus identification in plant tissue; cabbage and tomato are also being studied (virus-free seeds, multiplication of elite clones, somaclonal variation). A new research institute in Solok is expected to focus on citrus, papaya, mango, and banana (i.e., on the production of disease-free planting material, varietal improvement, postharvest activities and marketing) [Dart et al., 1991]. For more detailed information, see Sasson [1993].

The first oil palm clones derived from somatic embryogenesis were outplanted in the field in 1987. About 1 million plants were sold in 1993. Although clonal plants are sold at five times the price of seedlings, there is a large market in Indonesia trying to catch up with Malaysia in overall palm oil production [Dart et al., 1991].

Some 3 million ha of coconut are under production, with generally low yields. Hybrids are being produced and their cloning by tissue culture has been hindered by difficulties in regenerating plantlets from the cultures. There are commercial incentives to develop clones of the

Kopyor mutant coconut tree which produces a nut with special flesh, used in ice cream, confectionery products and preserves, and is worth 10–15 times more than the ordinary nut [Dart et al., 1991]. Two coconut projects in the Lampung province of southern Sumatra involve researchers from the French International Cooperation Centre for Agricultural Research for Development-CIRAD's Department of Perennial Crops: one government project for small plantations funded by the World Bank and one private project run with the help of the Multi Agro Corporation on coconut and cocoa.

A facility at the Faculty of Agriculture of the Gadjah Mada University in Yogyakarta produces *Rhizobium* inoculants (with the trade name Legin) for government-sponsored crop improvement projects. The production capacity is approximately 20 tonnes/yr, an amount sufficient to inoculate about 111,000 ha of soybeans. The full capacity of the facility is probably five times this production. Rhizogin is the trade name of another *Rhizobium* inoculant prepared and marketed by the private company, Rhizogin-Indonesia, the production capacity of which is sufficient to inoculate approximately 440,000 ha/yr with the recommended dose of 180 kg inoculant per 65 kg of soybean seeds. It is supplying about 70% of the inoculum needed for soybean cultivation in the country. With additional labor and investment in bioreactor capacity, the factory could double or treble its output and unit costs could be reduced markedly [Saono, 1991].

Integrated pest management and biological control of plant pests are likely to have a high return on investment. Between 1986 and 1989, the government phased out pesticide subsidies and banned 57 broad-spectrum formulations of insecticides. A national integrated pest management program was set up, coordinated by the National Development Planning Board; as a result, the spraying frequency per field dropped from 2.2 to 0.8 times per season, resulting in a 50% decline in insecticide consumption, while rice production increased by 12%. The Lembang Horticulture Research Institute (LEHRI) and the Indonesian Government's extension service teamed up with the Swiss chemical company Ciba-Geigy AG in a pilot project on reducing pesticide consumption in cabbage cultivation; farmers have found that three to six applications of pesticide are adequate, without any loss of yield, compared with the 15 sprays normally applied. The project also relies on the use of natural predators: for cabbage, a parasitoid wasp *Diadegma eucerophaga* was chosen, which lays eggs in the diamond backmoth larvae, preventing them from reaching maturity. Encouraged by the promising results, Ciba-Geigy AG has extended the program to potatoes, then to onion and tomato cultivation [Kumar, 1992]. The Dutch Government is supporting an extension program of integrated pest management to complement that of the FAO on rice.

Indonesia gives a high priority to the development of biotechnologies, with direct support from the Ministry of State for Research and Technology and the Departments of Agriculture and Education. Plant tissue culture and micropropagation techniques are well established in several laboratories, and large-scale commercial production of planting material has been achieved for oil palm [Research Institute for Oil Palm at Marihat]. Similar developments for other plantation crop species are likely in the mid-1990s. The use of the same technique to eradicate viruses is also likely to support commercial production of planting material of potato and several horticultural crop species, and selected strains of mycorrhizae are expected to become available for commercial inoculant production [Dart et al., 1991].

#### 4. Malaysia

One of the major areas of research and development is the clonal propagation of high-yielding, oil palm varieties, carried out by the Palm Oil Research Institute of Malaysia (PORIM) and the Federal Land Development Authority. The market is sizeable, as more than 1.6 million ha of



plantations existed in the early 1990s. The problem of abnormal flowering has been solved and cloned superior oil palms has been marketed since 1992. All Malaysian laboratories working in this area, including the PORIM, are producing such clones. They were also increasingly involved in the mass propagation of other crop species, with a view to diversifying production and meeting local needs.

The Malaysian Agricultural Research and Development Institute (MARDI) have established a Biotechnology Center devoted to the in vitro culture of the following crop species: coconut (cloning of selected plants, in vitro germination of embryos, callogenesis, and somatic embryogenesis); cocoa (organ culture); papaya (cloning of selected varieties); anther culture of rice hybrids and somaclonal variation of rice; pepper (production of pathogen-free plants to control *Phytophthora* disease); strawberry (meristem culture); and pineapple (culture of axillary buds to propagate the crosses of Sarawak and Singapore Spanish varieties, suitable for the canning industry) [Bordier, 1990]. A cocoa clone derived from in vitro micropropagation was released at the MARDI Cocoa Research Station near Teluk Intan, Perak [Zakri, 1991]. For more detailed information see Sasson [1993].

The Southeast Asian lowland swamps are home to the sago palm (*Metroxylon sagu*). The three leading world producers—Malaysia, Indonesia, and Papua New Guinea—are interested in cooperation aimed at developing sago plantations and using sago starch extensively. Japan is also supporting this cooperation through the creation of a Sago Research Fund.

In the early 1990s, Sarawak had a total area of 19,720 ha of sago palm, including both semiwild and cultivated stands. Sago flour production increased by about 60% between 1984 and 1990 to 84,991 tonnes. Since 1984, there has been an upturn in the export of sago starch: from 3,406 to 27,502 tonnes, earning the State of Sarawak 11.4 million dollars in revenue [Zulpilip et al., 1991].

A potential area for sago plantation exists in Malaysia, which has 2.4 million ha of peat land, of which 1.66 million ha are in Sarawak. Because of its natural adaptation to peat soils of low nutritional value and high acidity, sago seems to be the only crop species that could grow on these soils without reclamation. Furthermore, sago being little prone to natural disasters, such as drought, pest and disease infestations, and flooding, is considered by farmers a minimal-risk crop species. A langorous cycle makes its cultivation ideally suited to a part-time activity, leaving the farmer time between planting and harvesting to seek other income-earning opportunities [Zulpilip et al., 1991].

With the increased demand from industry for its products and a 9- to 15-year growth cycle, quality varieties or clones of sago palm will need to be planted on a large scale. Although sago palms can be propagated from suckers, the number is limited; therefore, clonal propagation through in vitro techniques is the most suitable means both for producing the vast amount of planting material required for extensive plantations and for improving the quality and vigor of palms [Alang et al., 1993].

Since 1983, research has been conducted at the Department of Biotechnology, Faculty of Food Science and Biotechnology, Universiti Pertanian Malaysia, Serdang, Selangor, toward clonal propagation of sago palm. Several hundred clonal plantlets were produced over a period of 18–24 months from culture initiation; the protocol for inducing embryogenesis from explants and developing embryoids into plantlets was being improved [Alang et al., 1993].

To sum up, with more than 10 multimillion dollar modern sago factories in the early 1990s, a 200-ha Sago Research Station, and 16,000 ha at the planning stage, Sarawak became the world leader in sago development, research, and the exploitation of the palm. There is a need for market promotion, a standardized grading system, and quality regulation of sago flour, because the prospects for Sarawak's sago industry in the international market depends not only

on price competition, but also on consistent quality and a reliable supply of sago flour [Zulpilip et al., 1991].

## 5. Philippines

The most active tissue culture work in the Philippines is being carried out at the Institute of Plant Breeding in micropropagation, germplasm conservation, disease elimination, and crop improvement. The laboratory works on vegetables (tomato and white potato), fruit species (rambutan, pineapple, papaya, citrus, banana, and durian), plantation crops (sugarcane, rattan, bamboo, ramie), cereals (corn, wheat), legumes, and ornamentals (*Anthurium*, orchids, *Mussaenda*). The tissue culture program of the Institute of Plant Breeding was expanded in 1989–1990 under a new Cellular and Molecular Plant Biology Programme [Zamora and Barba, 1990].

Yams are micropropagated by culturing shoot tips and nodal segments. Micropropagated bananas (cultivars Lakatan, Saba, and Bungulan) are evaluated for plant and yield traits, and are similar to sucker-derived bananas. However, growth is faster among micropropagated bananas than sucker-derived bananas. Flowering also occurs earlier among micropropagated plants [Zamora et al., 1989]. Elimination of mosaic and banana bunchy top viruses by isolation of meristems from heat-pretreated plants and subsequent *in vitro* culture became possible [Zamora and Ramos, 1989].

From the shoot tip-grafting technique developed by Spanish researchers [Navarro et al., 1975], the meristem-budding technique has been adapted to suit locally available citrus and to produce plants free of tristeza and leaf-mottling diseases [Zamora et al., 1988]. The technique has been extended to the Bureau of Plant Industry for the production of disease-free citrus plants.

At the National Institute of Biotechnology and Applied Microbiology, research activities include producing monoclonal antibodies to detect citrus tristeza virus, banana mosaic virus, banana bract mosaic virus, banana bunchy top virus, and papaya ringspot virus [Zamora and Barba, 1990].

There are several research and commercial tissue culture laboratories in the Philippines. These research laboratories have developed tissue culture techniques for disease elimination and micropropagation in ornamentals and in food crop species, ranging from vegetables to plantation crops. Most commercial laboratories are carrying out orchid tissue culture, very few of them have gone into plantation and food crop species. The disparity in cost of micropropagated plants versus conventional propagules and the resources available to small farmers limit the use of micropropagated plants [Zamora and Barba, 1990]. For more detailed information, see Sasson [1993].

Scientists at the International Rice Research Institute (IRRI) estimated that the tiny nitrogen-fixing fern *Azolla* was used on less than 2% of the world's total rice cultivating area of 150 million ha, whereas its beneficial effect has been demonstrated. *Azolla* can double its biomass every 2–5 days and supply nitrogen to the paddy field. IRRI experiments showed that it could reduce the total weed mass by 72%, thereby diminishing the need for applying expensive herbicides. *Azolla* contains between 22 and 37% protein and can be consumed as animal feed, but also directly by humans, as in omelettes and burgers [Komen, 1990].

## 6. Thailand

Thailand has led the world in ornamental flowers, such as orchids, and in many processed fruits and vegetables, such as canned pineapple, fruit juice, and concentrates, and canned baby corn.

Plant tissue culture was introduced into the Thai orchid industry in 1964, and the continuity of research and development has kept Thai orchids popular worldwide. Thailand also continues to be the world leading exporter of rice and cassava. It is also very strong in frozen products, particularly those arising from aquaculture (shrimps and prawns), but also new vegetable varieties such as green soybean and okra.

Current research initiatives under the leadership of the National Center for Genetic Engineering and Biotechnology are aimed to foster the development of biotechnologies and accelerate the transfer of these technologies from local as well as overseas sources. Recent achievements included:

1. The setting up of companies to produce biofertilizers from algae and *Rhizobium*
2. The establishment of a new firm for the production of phycocyanin and shrimp larvae feed from *Spirulina*
3. The commercial production of virus-free potato tubers for the supply of fast-food chains and snack producers who were spreading over Asian countries
4. The development of a heat-tolerant shiitake mushroom, now produced by small farmers in the north and northeast of Thailand

The National Corn and Sorghum Research Program was organized in 1966 as a cooperative effort involving Kasetsart University, the Ministry of Agriculture and Cooperatives, and the Rockefeller Foundation. In 1983, the International Center for the Improvement of Maize and Wheat replaced the Rockefeller Foundation in this venture. This program provided Thai farmers with improved maize varieties and crop-culture practices, resulting in a 100-fold increase in maize production within two decades. In addition to conventional plant breeding, Thai researchers, in particular at Kasetsart University, have applied biotechnologies to the improvement of crop species.

This is particularly true in horticulture: tissue culture, used for genetic preservation of outstanding cultivars, rapid propagation, and mutation breeding, has been applied to several fruit crops.

The Department of Agriculture in the Ministry of Agriculture and Cooperatives, together with staff from several departments at Kasetsart University, have contributed to the development and extension of straw mushroom (*Volvariella volvacea*) production technologies throughout Thailand. According to the survey conducted by the Department of Agricultural Extension from 1965 to 1968, the ten largest spawn makers in Bangkok altogether produced an average of 832,250 cans of straw mushroom spawn per year. Straw mushroom cultivation has become an important biotechnology-based industry and a supplementary source of income for many farmers. Shiitake mushrooms (*Lentinus edodes*) also represent a major food item, but they are mostly sold in a dried form. The development of systems for growth in simple indoor facilities can create a worldwide market for fresh mushrooms. For more detailed information, see Sasson [1993].

The King of Thailand, Bhumibhol Adulyadej (Rama IX), has been a promoter of rural development in his country and over 1000 so-called Royal Projects testify to his prominent role in this respect. Some of these contained a biotechnological component. The King's goal was to curtail poppy-growing and bring legitimate livelihood to Thailand's hilly tribes through crop substitution. These crop species would lure farmers away from opium production and also arrest the destruction of forests and watersheds.

Public and private agencies assisted in research and development as well as in the transfer of crop production and processing technologies. Contributions of the National Science and Technology Development Agency included variety selection, massive propagation, and variety improvement of cut flowers by tissue culture, development of virus-free potato tubers and

strawberry runners, heat-tolerant shiitake mushrooms, and high-efficiency wastewater treatment for the food factories.

Necessary infrastructure was not omitted (e.g., village roads, electricity grid, and small irrigation devices and systems). Foreign assistance came from the United Nations agencies, New Zealand, United States, and Taiwan. The project buys the farmers' produce, then grades, packages, and markets it. It has turned a profit for the villagers, also by processing their jams and wines, frozen strawberries, canned vegetables, dried fruits, and flowers for export. Poppy cultivation has declined by 85% as the farmers became vegetable, fruit, coffee, and flower growers.

The Thai Department of Agriculture, in Bangkok, produces legume inoculants distributed by the Thai Department of Agriculture Extension and dealers in the private sector. There had been a considerable growth in the use of these inoculants: from 3.36 tonnes in 1977 to about 200 tonnes in 1992.

## 7. *Vietnam*

Rice breeding deserves the highest priority. Anther culture and the derived haploid lines have been used in Vietnam since 1980 in the institutes of the Ministry of Agriculture and Food Industries. Cooperation with China, where millions of hectares have been planted with hybrid rice cultivars derived from haploid breeding, could help Vietnam catch up.

Potato has become a major food crop in Vietnam since the 1970s, after the new, higher-yielding rice varieties of the green revolution had enabled the Vietnamese farmer to grow a winter crop between two rice crops. A rapid propagation procedure was developed through the collaborative efforts between scientists of the National Council for Scientific Research (NCSR) Institute of Biotechnology in Ho Chi Minh City and farmers in the Dalat region. The so-called flash out system (FOS) combines *in vitro* techniques (the starting point was potato plantlets of a selected variety grown on an artificial medium cut into microcuttings that give rise to plantlets) and conventional vegetative propagation (apical and axillary bud cuttings that give rise to new plantlets). The subsequent large-scale commercial production of potato planting material could progressively replace the conventional tuber planting and save thousands of tonnes of "seed" tubers, which could be consumed instead of being stored. Furthermore, in 2 years, at Dalat, potato yield doubled from 9 to 18 tonnes/ha, owing to the quality of the planting material, which means that imports of European potato tubers can be avoided. Many farming families are involved in this profitable agribusiness, while contributing to the rapid dissemination of the most appropriate potato varieties. One farming family of four can produce and sell 500,000–1 million potato plantlets per year [Nguyen Van Uyen, 1991, personal communication]. The objective is to raise annual potato production from 500,000 tonnes in 1991 to 1 million tonnes for both domestic consumption and export [Commandeur and Pistorius, 1992]. The flash out system could be extended to other tuber or root crop species, such as cassava, sweet potato, and vegetables.

A cooperative research program on banana was designed by Vietnamese and French partners. The objective of this 5-year (1992–1996) research program is to provide Vietnamese producers with healthy clonal planting material, belonging to higher-yielding and more resistant banana cultivars, at a reasonable cost.

A private joint venture to set up a plant tissue culture laboratory and greenhouses for plant propagation at Thu Duc, near Ho Chi Minh City, has been established between a Taiwanese company, Pan Viet Co., and the NCSR Institute of Biotechnology. Since beginning operations in May 1991, the laboratory has produced 20,000 banana plantlets per day. The medium-term goal is to plant 20,000 ha on land leased by the company to the Government of Vietnam, to

export between 800,000 and 1 million tonnes of bananas annually. Estimated at about 60 million dollars, the joint venture will lead to exports worth about 200 million dollars/yr. This kind of successful development could be extended throughout the Mekong River Delta, where there is a great potential for large-scale banana plantations for export, as well as for other crop species.

Sugarcane was the first monocotyledonous crop species to be multiplied through the flash out system. In this case, the tillers are cut into three to five portions that are used (instead of apical buds in the case of potato) to propagate the plant. The technique is used to accelerate the release of new varieties in about 2 years. For more detailed information, see Sasson [1993].

At the Institute of Agricultural Sciences, Microbiology Section, Hanoi, Nguyen Kim Vu and his co-workers selected efficient and effective strains of *Rhizobium* for soybean and groundnut inoculation. Field experiments carried out for several years in various regions of Vietnam have shown that inoculation with these strains could increase seed yields from 40 to 290 kg/ha. Since 1988, the same group has been testing a preparation made of *Azospirillum* (called azogin) in eight northern provinces of Vietnam and has demonstrated that it could replace one-third of the inorganic nitrogen fertilizer applied. At the Institute of Biology of the National Center for Scientific Research, Hanoi, blue-green algae has been under study since the early 1980s. Rice plants inoculated with *Anabaena sphaerica* and *Nostoc muscorum* result in a yield increase of 44 and 29%, respectively, compared with plants that received no blue-green algal fertilizer [Commandeur and Pistorius, 1992].

Six different institutions are working on the inoculation of cereal crop species (rice and maize), to stimulate the growth of the plants through enrichment of their rhizosphere with useful bacteria.

#### 8. *Asia Network for Small-Scale Agricultural Biotechnology* (Appropriate Technology International)

Appropriate Technology International (ATI) is a private, non-profit-making development assistance organization, headquartered in Washington DC, with projects in Asia, Africa, Latin America, and the Caribbean. The ATI, in cooperation with the NIFTAL Project (Nitrogen Fixation by Tropical Agricultural Legumes, College of Tropical Agriculture, University of Hawaii), SATE (Small Enterprise Development and Appropriate Technology Europe), and the Department of Biology and Society, Free University, Amsterdam, has developed a biotechnology network as part of a program referred to as the Lab to Land Program. The network focuses on small-scale agricultural biotechnologies, according resource-poor farmer the double role of beneficiary and participant.

The Asia Network for Small-Scale Agricultural Biotechnology (ANSAB), with its headquarters in Kathmandu, Nepal, is the nucleus of the Lab to Land Program, initially linking Bangladesh, China, India, Indonesia, Nepal, the Philippines, Sri Lanka, Thailand, and Vietnam. It was established during the first ANSAB workshop (30 March–1 April 1992), held in Kathmandu and attended by 70 participants from the nine Asian countries.

The ANSAB has a multifaceted membership, including farmers, nongovernmental organizations, researchers and biotechnologists from both the public and private sectors, private sector businesses, financial institutions, and policy makers. The unifying factor of this diverse membership is the concern for resource-poor farmers.

The ANSAB workshop identified the following mature agricultural biotechnologies in the nine participating countries (Table 1).

The biotechnologies initially selected for the program were in various stages of commercialization in different countries. Barriers to commercialization efforts often existed, such as a

**Table 1** Biotechnologically Mature Agricultural Countries as Identified by ANSAB

Country	Mature agricultural	Biotechnologies	Priority-wise
Bangladesh	Biofertilizer	Mushroom cultivation	Bamboo propagation
China	Mushroom cultivation	Biofertilizer	Plant tissue culture
India	Biofertilizer	Mushroom cultivation	Seedling raising (trees and vegetables)
Indonesia	Biofertilizer (blue-green algae)	Biofertilizers ( <i>Rhizobium</i> and mycorrhizae)	Biopesticide
Nepal	Plant tissue culture	Mushroom cultivation	Biofertilizer ( <i>Rhizobium</i> )
Philippines	Biofertilizer	Biopesticide ( <i>Trichoderma</i> )	Biofertilizers ( <i>Rhizobium</i> , <i>Azospirillum</i> , mycorrhizae)
Sri Lanka	Biofertilizer (soybean <i>Rhizobium</i> )	Biofertilizer ( <i>Azolla</i> )	Plant tissue culture (coconut, fruit trees)
Thailand	Plant tissue culture (asparagus and strawberry)	Plant tissue culture (for reforestation)	Biofertilizers ( <i>Rhizobium</i> , blue-green algae, mycorrhizae)
Vietnam	Biofertilizer	Biopesticide	Plant tissue culture

lack of research and development support or agricultural extension, poor technology-transfer mechanisms, lack of entrepreneurial and technical skills, lack of venture capital, high product cost or poor product quality, and subsidies for alternative products (such as chemical fertilizer). By providing a regional context for considering these issues, it was expected that the sharing of experience, technology implementation, and regional transfer would be significantly improved.

Vietnam has been a pioneer in developing low-cost, simple methods for the rapid micro-propagation of disease-free potato varieties (see Sec. II.A.7). The Vietnamese example illustrates the practicality of small-scale operations in tissue culture with direct benefit for resource-poor farmers. It also points to the need for close cooperation between research centers, farmers, and sources of capital.

Soils from South and Southeast Asia that frequently tested low for *Rhizobium* and legume seed would benefit from inoculation (although phosphate fertilizer application might also be needed, and low soil pH had to be corrected, for good response). Legume yield increases of 10–90% following inoculation were reported in South Asia. In the Philippines, inoculation trials gave an average yield increase of 124% for soybeans, 29% for mungbeans, and 37% for groundnuts; in spite of this potential, use of inoculant is very low.

The ATI is seeking to develop the commercial small-scale and decentralized production of *Rhizobium* inoculants to increase soybean yields. Launched in 1987, the project was designed to benefit about 10,000 small holders in the northern provinces of Thailand, which produce about 70% of the total soybean harvest. The expected benefits were an increase in income of 17–42%, because of higher yields, together with a reduction in the use of chemical nitrogen fertilizers; a decrease in soybean and nitrogen fertilizer imports; and improved soil fertility through the nitrogen compounds remaining in the soil after harvest [Pistorius and Smits, 1990]. In collaboration with the Thailand Institute for Scientific and Technological Research and the Thailand Rural Reconstruction Movement, the ATI sponsored trials to demonstrate the use and commercial viability of small-scale, decentralized production of a new blue-green alga inoculant. Yield increases in initial field trials during 1988–1989 for both off-season and in-season rice farming ranged from 16 to 39% [ANSAB *Newslett.*, 1992–1993].

The Bangladesh company, Biolink, in collaboration with the Department of Soil Science of the Bangladesh Agricultural University, has been producing *Rhizobium* inoculants since 1991. The company produces about 7 tonnes of different kinds of inoculants annually, the annual capacity being 10 tonnes. Agricultural extension programs might further enlarge the market [Haque, 1993].

In Nepal, ANSAB assisted with field trials of the high-yielding, late blight-resistant potato variety MS 42.3. This variety was received in 1986 from the International Potato Center, Lima, Peru, in the form of a test-tube plantlet. MS 42.3 yields an average 32.4 tonnes/ha (i.e., a 22% higher yield over the average yield of Kufri Jyoti and Cardinal, the two most popular commercial improved varieties). Furthermore, MS 42.3 being resistant to late blight disease, does not require spraying, which has cost Nepalese farmers 90–105 dollars/ha [ANSAB Newslett. 1992–1993].

These trials represent a further step toward improved potato “seed” production and supply in Nepal, where potatoes—the fourth staple crop species after rice, maize, and wheat—are cultivated on an estimated 86,000 ha. However, the average yield of 8.5 tonnes/ha is one of the lowest in the world. The multiplication of the MS 42.3 variety, called sand rooting, bypasses rooting of microshoots in sterile media; instead, microshoots are directly rooted in clean but nonsterile sand, with more than a 90% survival rate. Minituber production from sand-rooted potato plantlets results in significantly lower costs: cultivation costs for minituber production average 20 dollars/ha, compared with normal potato tuber production (1.5 tonnes) cost of 96 dollars/ha. Storage costs of minitubers are also much lower [ANSAB Newslett. 1992–1993].

In June 1992, the ATI financed a study of the mushroom market in Nepal, Bangladesh, and India by S. T. Chang, professor in the Department of Biology at the Chinese University of Hong Kong and president of the International Mushroom Society for the Tropics. Nepal has developed a fairly good technology for the production of white button mushrooms (*Agaricus bisporus*) and oyster or abalone mushrooms (*Pleurotus* spp.). However, the current yield of 200 kg/tonne of substrate for *Agaricus* and 400 kg/tonne for *Pleurotus* can be raised to 250 and 500 kg/tonne, respectively, through the improvement of sterility and cropping techniques. The spawn production system, primarily a governmental activity currently, is expected to be developed privately. The present domestic market in Nepal for button mushrooms is estimated at 260–500 tonnes/yr, and growth potential is good owing to increasing tourism. The estimated production of fresh button mushrooms in 1991 was 80–100 tonnes, so that Nepal imports the balance as canned mushrooms from both China and India. The low cost of skilled labor, the favorable climate, the abundance of substrate, and the presence of direct air service to potential importing countries provides a good basis for a mushroom export industry in Nepal [ANSAB Newslett. 1992–1993].

In Bangladesh, the Department of Agriculture Extension has developed cultivation methods for oyster mushrooms, straw mushrooms (*Volvarellia volvacea*), and *Auricularia* sp., as well as the procedures for spawn production of these species on a commercial scale. A 1991 economic study of straw mushroom production concluded that rural landless women were encouraged and attracted by mushroom cultivation as a part-time work activity. A domestic market has been developing and one company has planned to set up a modern facility for export of button mushrooms [ANSAB Newslett. 1992–1993].

In 1991, the total production of all types of mushrooms in India approached 7000 tonnes. Button mushrooms accounted for 80–85% of total production, followed by oyster mushrooms (9%) and straw mushrooms. Production was also moving to the plains owing to the availability of cheap substrate materials and ready access to markets. Average yields ranged from 60 to 80 kg/tonne of substrate in 6–8 weeks cropping periods. In modern medium-to-large cultivation

operations using environmentally controlled conditions, the average yield is 150–160 kg/tonne of compost [ANSAB *Newslett.* 1992–1993].

## B. Latin America and the Caribbean

Almost 73% of total biotechnology research has been directed toward in vitro tissue culture, micropropagation, and clonal multiplication of crop species. Projects using genetic engineering account for about 9% of the research endeavors [Bijman et al., 1990]. By 1990, 40% of research and development projects concerned root and tuber crop species, in particular, potato, cassava, and sweet potato projects being carried out in international agricultural research centers, such as the International Potato Center (CIP, Lima, Peru), the International Center of Tropical Agriculture (CIAT, Cali, Colombia), and the Tropical Agricultural Research and Training Center (CATIE, Turrialba, Costa Rica), which play a leading role in the dissemination, development, and transfer of these biotechnologies.

Research on plantation crop species is of lesser importance and largely concerns coffee. The use of tissue culture for micropropagation of banana and plantain is a routine in most countries of the region. Research and development activities concerning ornamentals and fruit species have shown a fast growth rate [Bijman et al., 1990; Buttel, 1986; Sorj et al., 1989].

Genome mapping; gene identification, isolation, and transfer; anther culture, and haploid breeding are less frequent and often at an experimental stage. About 15% of laboratories are classified as highly efficient institutions, developing and applying advanced biotechnologies in priority areas for the genetic improvement of crop species. Conversely, 25% of them lack the funds and trained personnel to carry out frontline research.

### I. Mexico

The Research Center for Genetic Engineering and Biotechnology of the National Autonomous University of Mexico was created in April 1982 at Cuernavaca, State of Morelos; by 1986, research activities were in full swing. Basic research covered the following areas:

1. Molecular biology of nucleic acids
2. Biochemistry of proteins and peptides
3. Genetic improvement of microbial strains of basic and industrial interest
4. Fermentation, scaling-up, and bioengineering of processes (pilot-plant studies)
5. Enzyme engineering

In plant biotechnologies, the Center for Research and Advanced Studies of the National Polytechnic Institute (CINVESTAV-IPN) at Irapuato, has been the only center working on plant genetic engineering and molecular genetics of biological nitrogen fixation. In addition to work on chili pepper, beans, and other food crop species, a major research project on the transformation and regeneration of amaranth is being carried out. In addition to their role in improving the nutritional quality of amaranth, the genes coding for proteins of amaranth can be transferred into other grain crop species [Komen, 1992].

At the Fruticulture Center, Postgraduate College, Chapingo, temperate, subtropical, and tropical fruit crop species are being micropropagated, with a view to producing plantlets on a large scale for plantations. In addition, micropropagation is used for the multiplication of rootstocks that are tolerant to adverse soil conditions (drought, salinity) for apple, or for pathogen-free plant production (blueberry, raspberry, pineapple, banana, citrus, and grapes). Peach plants with high tolerance to cold and unseeded grape plants have been obtained from immature embryos. A procedure was developed to rescue hybrid embryos of *Carica* spp. tolerant to papaya ring spot virus and plantlets have been obtained.



In Mexico, about 2.6 million people depend on coffee cultivation and production. Coffee rust affects 90% of coffee plantations. The Mexican Government has taken measures to facilitate the distribution of disease-free in vitro micropropagated plantlets to the needy planters to protect labor in this sector and, at the same time, maintain export earnings.

There are several modern businesses with a total annual production of more than 1.2 million micropropagated plants, and a few of these are planning to direct their activities toward foreign markets. For more detailed information, see Sasson [1993].

## 2. *Costa Rica*

In Costa Rica, the world's second-largest banana producer after Ecuador and leading supplier to the European Union, banana has become the top export item. Costa Rica undertook a vast program aimed at increasing the banana area from 32,000 to 45,000 ha, or even more, in 1995, so that annual production should reach 1.8 million instead of 1.5 million tonnes. In its laboratories located at Guapiles, amidst the plantations, the national banana corporation, Corbana, a semipublic company, is carrying out research on organic fertilizers and the large-scale production of nematode-free plantlets. This biotechnological approach has eliminated the need for nematocide use in the soil of Corbana's plantations since 1987–1988. The combination of a fallow period and use of nematode-free plantlets could lead to the eradication of the soil-living and root-invading worms. The control of the fungal disease, black cercosporiosis, is a high research priority and cooperation has been initiated with the French International Co-operation Center for Agricultural Research for Development-CIRAD [Bertrand de la Grange, 1992].

The Research Center for Cellular and Molecular Biology of the University of Costa Rica, in San José, has broad experience in molecular biology and diagnosis of plant viruses. Research also focuses on virus resistance in beans, the expression of viral proteins in bacteria, and molecular biology of *Rhizobium*. The Tropical Agricultural Research and Training Center (CATIE), Turrialba, is considered the most important center in agricultural higher education in Central America and the Caribbean. The Tropical Crops Improvement Programme concentrated on coffee, cocoa, and Musaceae (plantain, sweet and cooking bananas). Research is also carried out on cassava, sweet potato, cocoyam, taro, and other crop species of local importance.

The American Cocoa Research Institute (ACRI), the research arm of the Chocolate Manufacturers' Association, provides financial support to biotechnological research in countries of Central and South America, particularly at CATIE: yield enhancement, plant resistance and integrated pest control, and cocoa processing. Work to develop nucleic acid probes for fungal diseases of cocoa is being carried out by Penn State University scientists, in collaboration with researchers at CATIE, under the Cocoa Molecular Biology Program, set up by the ACRI [Brenner, 1992].

## 3. *Colombia*

Research on plant tissue culture forms part of the Agro-Food Plan of the Universidad Nacional, carried out by the Faculties of Sciences and Agronomy. In addition to micropropagation and somatic embryogenesis of banana and plantain varieties, in particular of those varieties resistant to black cercosporiosis, radioactive methods are used to induce mutants. Vegetative propagation was the only means of multiplying the babaco (*Carica pentagona*, Caricaceae), a palatable papaya hybrid. Micropropagation is being studied as the successor to conventional, fastidious means of propagation. The Biotechnology Institute is leading research in somatic embryogenesis of cocoa and plantain [Luz Marina Dueñas, 1990].

At the Colombian Institute for Agricultural Research, three projects are progressing aimed to incorporate biotechnologies into conventional agricultural research programs: micropropa-

gation and cloning of fruit species (e.g., citrus, mango, maracuja-passion fruit tree, guava, papaya, and pineapple); improvement of food and plantation species (potato, rice, banana, and cocoa); and molecular diagnosis of plant pathogens (in potato, garlic, sweet potato, citrus, guava, and papaya).

In 1932, the National Federation of Coffee-Growers of Columbia was set up. The Federation supported the National Coffee Research Center (CENICAFE), which developed a rust-resistant coffee variety after 20 years of investigation. Promising results were also obtained in somatic embryogenesis, regeneration of plantlets, culture of protoplasts, and biochemical studies on cell cultures. Efforts are being made to achieve the genetic transformation of coffee, with a view to incorporating the gene(s) for *Bacillus thuringiensis* toxin(s) to make coffee plants pest-resistant. Somatic hybridization is also meant to overcome the difficulties concerning conventional hybridization between wild and cultivated coffee species, owing to the incompatibility between diploid and tetraploid species.

At the Faculty of Agriculture of Caldas University, Manizales, a program has developed on the propagation and breeding of Musaceae. An interinstitutional plantain research group was created following a meeting at this Faculty on 18 June 1993. The French International Cooperation Center for Agricultural Research for Development-CIRAD's Department of Fruit and Horticultural Crops was requested to provide technical support and to seek funding sources [CIRAD News, October 1993, 6]. For more detailed information, see Sasson (1993).

#### 4. Brazil

By the late 1980s, the National Center for Genetic Resources and Biotechnology (CENARGEN) research teams were able to master the techniques for gene isolation, cloning, transfer, and expression in bacteria and plant cells. An agreement was concluded with the American biotechnology corporation Agracetus to transfer and express the gene for methionine (from the Brazil nut, *Bertholletia excelsa*) in common beans (*Phaseolus vulgaris*). CENARGEN has strengthened its capability in plant tissue culture and regeneration. The center also focused its activities on the biological control of pests by baculoviruses and *Bacillus sphaericus*, and such fungi as *Metarhizium*, *Beauveria*, and *Nomuraea*.

In addition, in plant tissue culture, the EMBRAPA's National Research Center for Temperate Climate Fruit Species and National Research Center for Vegetables has obtained significant results with the production of virus-free plantlets of strawberry, potato, and other species. The National Research Center for Wheat has obtained double-haploid lines of wheat derived from anther cultures and has tested the productivity of these lines in different regions. Resistance to disease has also been transferred to wheat from wild relatives. At the support unit for the National Research Programme on Soil Biology, highly efficient *Rhizobium* strains have been isolated for the inoculation of common beans and soybeans; equally important are the research activities of this unit on atmospheric nitrogen fixation by various strains of *Azospirillum* and *Acetobacter* in the rhizosphere of grasses.

Brazil also has established a strong cocoa research program that, until 1981, was funded by a levy of 10% on exports of cocoa beans and products. This country has the largest national cocoa germplasm collection in the world and plant collection is under way.

The CIRAD collaborated with the EMBRAPA on the production of high-quality oil palm seeds at the Rio Uruba oil palm research station. Launched in 1992 with a special grant from the French Ministry of Foreign Affairs, this activity aimed to produce between 500,000 and 1 million seeds in 1993. This project would augment oil palm cultivation not only in Brazil, which imports 100,000 tonnes of palm oil annually (1992), but also in neighboring countries [CIRAD News, Oct. 6 (1993)]. In addition, the CIRAD has achieved a first by creating tropical

hybrid rice, in cooperation with a private company in Brazil and French Guyana. This work is based on the utilization of cytoplasmic male sterility in indica rice. At the end of 1992, 200 new hybrid lines were available for evaluation in 1993 [CIRAD, 1993].

The main public research and training institutions involved in biotechnologies are concentrated in Brazil's central-southern region (i.e., in the states of São Paulo and Rio de Janeiro). Others are found in the states of Minas Gerais, Paraná, and Rio Grande do Sul. In the state of São Paulo, there is strong emphasis on plant biotechnologies in relation to agricultural development and technologies concerning biomass transformation (production of bioenergy and recycling of sugar industry wastes and by-products). In the same state are the most advanced institutions in molecular biology, such as the Institute of Biomedical Sciences of the University of São Paulo and the Center for Molecular Biology and Genetic Engineering of the University of Campinas (UNICAMP).

The maize project involves the Institute of Biomedical Sciences, the University of Campinas Center for Molecular Biology and Genetic Engineering (Laboratory of Plant Molecular Biology), and the seed corporation Agrocere S.A. The joint project associating the CIRAD's Department of Annual Crops and Rhône-Poulenc Agrochimie in the production of maize hybrids in Brazil began to bear fruit in 1993 [CIRAD News 5:6 (July 1993)].

At the College of Agriculture Luiz de Queiroz, the University of São Paulo's faculty of agriculture, the O. J. Crocomo group first started using plant tissue culture in June 1971, and was one of the first teams to introduce these techniques in Brazil. In September 1988, the creation of the Center for Agricultural Biotechnology (CEBTEC) confirmed the quality of the group's research and development work:

1. Propagation of virus-free strawberry plants derived from meristem cultures of selected varieties, for commercialization by the private company Citoplant; papaya propagation; embryo culture for the propagation of the native palm species *Acrocomia aculeata* (macauba) from Minas Gerais, the oil of which could be used to produce soap, cosmetics, and fuel.
2. Shoot-tip micrografting of citrus species, with a view to producing super seedlings free of diseases and pathogens; this project, initiated in 1990, was funded by the company Citrovita, part of the industrial conglomerate Votorantim.
3. Interspecific hybridization in bean, to develop hybrids resistant to the common bean mosaic virus and tolerant of water stress. The project, funded by the European Union, was carried out in collaboration with the Faculty of Agronomy of Gembloux, Belgium.

The Center for Molecular Biology and Genetic Engineering of the UNICAMP, inaugurated in 1990, comprises four laboratories dedicated to plant molecular biology, microbiology, virology, and medical genetics. The Plant Molecular Biology Laboratory is working on the seed storage proteins of maize, sorghum, and an Asian grass, *Coix lacryma-jobi*; the genes coding for these proteins have been cloned to develop transfer vectors and to achieve their expression in seeds.

At the Virology Laboratory, in collaboration with a small Brazilian company specializing in pharmaceuticals and antiviral substances, with a pilot plant in Campinas, a mutant strain of baculovirus was developed for the biological control of the soybean caterpillar (*Anticarsia gemmatilis*) and the sugarcane borer (*Diatraea saccharalis*, Lepidopteran). Commercial production of this strain in live insects has been achieved, as well as its purification and formulation as an aqueous solution sprayed from airplanes or tractors in sugarcane and soybean fields. Production of a polyvalent virus, active against two or three insect pests (e.g., Lepidopteran defoliators), is expected. Collaboration exists between the Virology Laboratory

and similar research units in France (National Institute for Agricultural Research Plant Protection and Pest Control Units), United States (University of Florida, Gainesville), and Canada (York University, Toronto).

At the Federal University of Rio de Janeiro, the following work has been carried out at the Laboratory of Plant Physiology of the Institute of Biophysics:

1. Somatic embryogenesis of *Arracacia xanthorrhiza*, a native plant of Peru, the rhizome or root of which is used by Nestlé-Brazil in baby foods because it is rich in starch, carotene, and vitamin C; cell suspensions were grown to obtain large quantities of embryoids which could be coated for broader and easier use by farmers.
2. Meristem cultures of sweet potato cultivars to eliminate viruses and pathogens, followed by field trials around Rio de Janeiro to check the agronomic traits of the clones; yields were raised from 9 to 19 tonnes/ha; this simple procedure of "cleansing" the cultivars is, therefore, considered very efficient.

At the Institute of Biology, the Laboratory of Plant Genetic Engineering (Department of Genetics) initiated plant tissue culture in 1983 and plant genetic engineering in 1987. Genetic transformation is carried out on economically important species, such as rice, groundnut, potato, tomato, egg plant, and grapevine. Attempts have been made to introduce the genes coding for herbicide tolerance, insect resistance, or increase in seed nutritional value.

By the mid-1980s, it was realized that the Brazilian industry for the production of legume inoculants (soybeans) lacked an appropriate technology ensuring maximum efficiency of the inoculant used. The total production of packets (200 g) for 1990 was estimated at about 17 million (i.e., some 3500 tonnes) produced by seven inoculant manufacturers; 95% of these packets were inoculants for soybeans. In June 1990, the Brazilian Network of Laboratories for *Rhizobium* Strains Recommendation (certification) was established. It involved 11 research institutions and the seven private inoculant manufacturers. The objective was to improve the mediocre quality of the inoculants produced in Brazil, caused by companies giving priority to the low price of the inoculant at the expense of quality.

Sementes Agroceres, S. A., a large national private agrofood corporation, dedicating 4% of its turnover to research and development, had diversified its activities in vegetable seeds in 1968. It focused on the production of potato "seed" through in vitro tissue culture, and collaborated with Biótica S. A., an Argentinian private company headquartered in Buenos Aires, part of the Polichaco Group. The project was initiated in 1987. Sementes Agroceres S. A. used one of its subsidiaries to develop the basic production of potato seed. In Argentina, the field multiplication of potato seed sent by Agroceres was done by Biótica in collaboration with the National Institute for Agricultural Research. As a result of the project in 1991, the first 35,000 cases of certified potato seed were distributed on the Brazilian market by Agroceres. Production tests carried out in Paraná confirmed the higher yields from the biotechnology-derived potato seed: 38 tonnes/ha, compared with a national average of 13 tonnes/ha. Efforts toward increasing productivity and reducing production costs for field multiplication by Biótica led to promising results: a cost reduction from 90,000 dollars/ha in 1988 to 10,000 dollars/ha in 1992, the medium-term objective being 6000 dollars/ha [Castelhana Bruno and Silva Waack, 1992].

## 5. Argentina

The priority areas of the National Biotechnology Program were biochemical engineering; nitrogen fixation; plant tissue culture and plant molecular biology; production of vaccines; and production of diagnostic reagents.

In addition to research carried out at the University of Buenos Aires, the National University of La Plata, the National University of Rosario, and the National University of Cuyo are involved in biotechnology research on potatoes and other tuber or root crop species. At the University of La Plata, work focuses on potato, rice, and soybeans: gene mapping, selection of cultivars resistant to diseases, and environmental stresses [Bijman et al., 1990].

Tecnoplant S. A., a leading in vitro micropropagation private company in Argentina and Latin America, has been working since 1985 on the selection and massive propagation of vegetable and fruit species. The company sells banana, kiwi, apple, cherry, pear, vine, strawberry, raspberry, and blackcurrant plantlets in pots, ready for planting in the field, or already with roots for immediate use by the customer. It also "cleans" varieties delivered by customers, carrying out under contract the propagation of Colombian and Costa Rican banana varieties, asparagus, strawberry, and other berries. At the request of large-scale producers and planters, the company has developed new lines for massive propagation.

Tecnoplant S. A. also provides advisory services for micropropagation, multiplication, management, harvest, and postharvest technologies; marketing; and such. In 1992, Tecnoplant S. A. became the Division of Plant Biotechnology of Bio Sidus S.A., Argentina's second largest pharmaceutical company and leading national laboratory (e.g., producing and marketing human recombinant alfa-2, interferon, and human recombinant erythropoietin).

## 6. Chile

Most plant biotechnology research has concentrated on cash crop species: citrus, jojoba, wine and table grapes, kiwi fruit (University of Chile); kiwi (Companía Chile de Tabaco); sweet cucumber (*Solanum muricatum*), citrus, carnation (Universidad Católica de Valparaíso); blackberry, papaya, *Eucalyptus*, ferns, and other ornamentals (German Gallardo Comp.); bulbs and blackberry (Tecvív Ltd); garlic and potatoes (Universidad Austral); papaya, potatoes (Universidad Pontificia de Chile); potatoes (University of Talca); blackberry, asparagus, and various fruits (University of Concepción) [Bijman et al., 1990].

At the Universidad Pontificia de Chile, the research program includes improvement and conservation of virus-free potato germplasm, mass production of healthy elite individuals, large-scale propagation of varieties resistant to stress and pathogens, and vegetative and sexual multiplication of little-known fruit species and Andean endangered species. Joint programs with other universities on genetic engineering are being developed [Bijman et al., 1990]. For more detailed information, see Sasson [1993].

## 7. The Caribbean

According to Ali (1992), with the exception of Cuba, the Caribbean is, in terms of production, still overwhelmingly in the first phase of biotechnologies in the following areas: plant selection and improvement, baking, baker's yeast manufacture, brewing, alcohol production, mushroom cultivation, methane generation, and anaerobic and aerobic waste treatment.

At the Scientific Research Council, Kingston, Jamaica, the use of leafy cuttings, miniset technology, and tissue culture proved to be superior to yam propagation methods. Yam was threatened by a disease discovered in the 1960s known as internal brown spot. Research on controlling the disease commenced at the University of the West Indies and was subsequently pursued at the Caribbean Agricultural Research and Development Institute (CARDI). During the 1980s, CARDI established a plant tissue culture laboratory in Barbados, with a view to developing a virus-tested yam multiplication project. In the same laboratory, more than 32 varieties of cassava, imported between 1984 and 1988 from the International Center of Tropical Agriculture, are conserved. After the cassava bacterial blight disease reached Barbados in

1987, breeding work was extended to the improvement of disease resistance in this crop species [Pistorius et al., 1990].

In Trinidad and Tobago, at the University of the West Indies, St Augustine, biotechnological research is being carried out on sugarcane to improve yield and resistance to disease and pests, with a view to counteracting a plunge in international sugar prices through lower production costs and increasing productivity. Food crops such as plantain are also the subject of priority research, with emphasis on plant tissue culture, development of bioinsecticides and mycorrhization [Pistorius et al., 1990].

In Cuba, at the National Center for Scientific Research, haploid sugarcane lines have been generated, with some giving higher yields than their parent lines under natural conditions; efforts have also been made to isolate cell lines resistant to salt, aluminium toxicity, and microbial toxins. Microbial biotechnologies include the large-scale cultivation of microorganisms for the decomposition of lignocellulosic biomass and the production of high-value compounds, such as amino acids, citric acid, and enzymes. Furthermore, yeast grown on molasses, used as fodder for livestock, has been partially substituted for imported soybeans [Pistorius et al., 1990].

Transgenic potato plants resistant to the most current viruses were obtained at the Center for Genetic Engineering and Biotechnology, and field trials were carried out in 1992. The Cuban scientists were of the opinion that genetic engineering was the fastest approach to "cleansing" and improving potato planting material, together with the development of effective diagnostic kits for the identification of the various viruses. The increasing production of virus-free potato planting material has enabled Cuba to save half of the foreign currency spent on importing virus-free potato tubers (seed). In addition to potato plantlets produced in vitro, potato micro-tubers are also produced in vitro and can be used as planting material.

At the Biotechnology Institute Las Vilas, Santa Clara, clonal multiplication of banana following in vitro micropropagation from axillary buds has produced about 5 million plantlets annually, with less than 1% genetic variation. This was performed in unsophisticated facilities at 25°–30°C; the yield from tissue culture-derived plantlets was 30% higher than that from banana trees propagated by conventional vegetative means. A Porto Rican sugarcane variety grown for 40 years in Cuba has also been massively propagated.

## 8. *Cassava Biotechnology Network*

Many constraints concerning cassava breeding and improvement, which remained difficult to resolve through conventional research, are now amenable to biotechnologies within an international collaborative program in the form of a network involving both developing and developed country organizations.

In 1994, cassava (*Manihot esculenta*) was the primary carbohydrate food for more than 500 million persons throughout the world. In 1990, an estimated 150 million tonnes were produced, 22% of this in Latin America. The starchy roots of cassava produce more calories per unit of land than any other crop except sugarcane. Cassava leaves provide vitamins and proteins when eaten as a vegetable, a common practice in sub-Saharan Africa.

Tolerant of drought and low soil fertility, cassava is grown primarily in marginal agroclimatic zones and by small farmers. Cassava can be processed into different forms for a wide range of uses, much of this processing being done locally, providing jobs and income in rural areas. Cassava production, processing, and marketing are often women's activities.

Of the 98 species of *Manihot* designated in the most recent monograph of the genus, 17 are native of North America. The main center of species diversity is in Brazil, with a secondary center located in Mexico [Bertram and Schaal, 1993].

In September 1988, the Cassava Biotechnology Network (CBN) was formed. The CBN was supported by the Special Programme Biotechnology and Development Cooperation, Directorate General for International Cooperation, Netherlands. Coordination was provided by the International Center of Tropical Agriculture (CIAT, Cali, Colombia) and, as a voluntary service to the research community, by members of the CBN Steering Committee.

In 1994, the cassava biotechnology research projects under way were as follows:

1. Numerous projects on tissue culture and micropropagation in Barbados, Cameroon, Cuba, China, Indonesia, Nigeria, Panama, Peru, Samoa, Venezuela, Zaire, and others; CIAT and IITA (International Institute of Tropical Agriculture, Ibadan, Nigeria)
2. Nine projects on cassava regeneration in China, France, Netherlands, United Kingdom, United States, Zimbabwe; CIAT and IITA
3. Seven projects on transformation of cassava in Brazil, Canada, United Kingdom, and United States; CIAT and IITA
4. Six projects on molecular mapping, markers and fingerprinting in France, United Kingdom, and United States; CIAT and IITA
5. Three on virus resistance in The Netherlands, United States, and Zimbabwe
6. Seven on cyanogenesis in Denmark, The Netherlands, Thailand, and United States; CIAT and IITA
7. Two on photosynthesis in Australia and the United States
8. Two on cryopreservation in France; CIAT
9. Numerous projects on cassava processing in Argentina, Brazil, Colombia, Congo, France, Ghana, India, Nigeria, South Africa, Tanzania, United Kingdom; CIAT

The cassava genetic-mapping project, or *Manihot* Genome Project, funded by the Rockefeller Foundation, has been carried out by Angel, Tohme, and Bonierbale at the CIAT. The project is expected to contribute to construction of a saturated genetic linkage map of cassava useful in various genetic studies and cultivar improvement.

A 1991 meeting, supported by the Rockefeller Foundation and organized by the FAO, explored the possibility of applying advanced genetic techniques to improve the storage life of cassava. The project envisaged called for three 5-year phases. Phase 2 would culminate in region-specific field tests of transgenic cultivars; and phase 3 in the diffusion of transformed, locally preferred cultivars with longer storage life in the target environments [*CBN Newslett. I(1):15 (1993)*].

The route of regeneration of cassava plantlets from tissue culture used by almost all groups was somatic embryogenesis from cassava leaf lobes. The International Laboratory for Tropical Agriculture (ILTAB)/Scripps group achieved a breakthrough in improving the efficiency of somatic embryogenesis and plant recovery in cassava (Mathews et al., 1993).

In 1994, several groups were involved in cassava transformation in Canada, United States, The Netherlands, United Kingdom, Colombia, and Brazil. Cassava calli were easily transformed using *Agrobacterium*; somatic embryos were transformed by *Agrobacterium* and by particle gun, and chimeric embryos expressing the *gus* gene were produced, as well as chimeric plantlets [Fauquet, 1993].

## C. Arab States

### 1. Egypt

Research in plant biotechnologies is being carried out at the Cell Research Section of the Agricultural Research Center, Giza, Cairo. In 1990, the Ministry of Agriculture and Land Reclama-

tion decided to create a National Agricultural Genetic Engineering Laboratory (NAGEL), comprising the following major units: nitrogen fixation, germplasm development and storage, molecular biology and cytogenetics, fermentation and food processing, and a computer center. The NAGEL's main research objective is to transfer agronomically desirable traits, such as tolerance to salt and drought, and pest resistance between species or genera, using embryo rescue and tissue culture techniques. Callus initiation and plant regeneration are being studied in wheat, barley, maize, sorghum, rice, alfalfa, and broad bean (*Vicia faba*). Current research also includes protoplast fusion for interspecific or intergeneric crosses that are impossible to achieve through conventional breeding methods.

At the NAGEL, Eweida et al. [1992] were able, for the first time, to regenerate three commercial potato cultivars (*Solanum tuberosum*) from leaf and tuber disks, with a chimeric gene encoding the coat protein of potato X virus. The Egyptian researchers then carried out the trials on the transgenic potato plants to test their resistance to the X virus when challenged with it [in *Proceedings of the 1992 Miami Bio/Technology Winter Symposium, Advances in Gene Technology: Feeding the World in the 21st Century*, vol. 1, Miami, 20–24 January 1992, p. 58].

A 4-year date palm production improvement program was initiated in 1988. It included the selection of 1000 pollinators in Aswan, New Valley, and Assiut sites, the selective pollination of female trees, and the continuous selection of natural offshoots that could be used in tissue culture experiments. Imported tissue culture-derived date palm plantlets were also tested in greenhouses and nurseries on several sites. The planting of tissue culture-derived date palm saplings was carried out in 1991 and 1992.

Interest in the application of rhizobial inoculants in Egypt had arisen in the 1930s, and the first inoculum production was achieved in 1939 in the Ministry of Agriculture, and the National Research Center had been involved in biological nitrogen fixation research and development since the early 1960s.

The Faba Bean Nile Valley Project in Sudan and Egypt, conducted in association with the International Center for Agricultural Research in Dry Areas and funded by the International Fund for Agricultural Development, contributed to the understanding of the rhizobia needed for efficient crop production. Exchange of strains and evaluation of cultures and rhizobium inoculants of faba bean were achieved.

At Ain-Shams University Faculty of Agriculture, a Microbial Resources Center (MIRCEN) was created in 1978 as part of the world MIRCEN network set up by UNESCO, the United Nations Environment Programme and the International Cell Research Organization. The Soil Biotechnology Unit, Department of Microbiology, Faculty of Agriculture, Cairo University, Giza, Cairo, was established in late 1990 to carry out research on nitrogen fixation and biofertility of desert soils. Priority was given to investigations on "integrated nitrogen fixation by symbiotic and associative systems," such as intercropping of grain–legume species (inoculated with the specific rhizobia) and grasses (inoculated with associative nitrogen fixers), and mixed canopy of fodder species, including nitrogen-fixing trees (*Leucaena* and *Sesbania*) and grasses (rye grass, sorghum, barley, and Sudan grass). The unit maintains a collection of rhizospheric microorganisms and plant growth-promoting rhizobacteria, as well as of *Frankia* strains for the inoculation of *Casuarina* spp.

A research project on nitrogen fixation in agricultural and natural ecosystems was carried out for 7 years, in collaboration with the Department of Agricultural Microbiology, Institute of Atomic Science in Agriculture, Wageningen, The Netherlands. Another research project, part of the Egyptian Major Cereals Improvement Project, was carried out in collaboration with the Agricultural Research Center, Giza, Cairo, and New Mexico University, on nitrogen fixation in cereals and soybeans as affected by nonsymbiotic and symbiotic microbial strains and mycorrhizal inoculants. Inoculation of wheat and maize seeds with efficient strains



of *Azotobacter* and *Azospirillum*, respectively, in the presence of organic amendment (2.0 tonnes/feddan; 1 feddan = 0.44 ha), could save half the normal field amount of nitrogen fertilizer, while at the same time obtaining higher yields of both cereals; inoculation of soybean varieties (Clark and Caland) with a mixture of *Bradyrhizobium japonicum* strains, in the presence of organic amendment (2.0 tonnes/feddan), 15 kg nitrogen units/feddan, and rock phosphate (33.0 kg/feddan), resulted in high grain yields [N. Magdoub, 1990, personal communication].

Large-scale production of vesicular-arbuscular mycorrhizae (VAM) spores was carried out within the framework of a collaborative project between the Unit of Biofertilizers, the Agricultural Research Center, and Dundee University, United Kingdom. VAM inoculation has been effective in reducing the preemergence damping off and severity of root-rot disease in soybeans. The most recent project in biofertilization used *Crotalaria* sp. and *Sesbania rostrata* as green manures [Y. Z. Ishac, 1990, personal communication].

## 2. Saudi Arabia

The Date Palm Research Center is considered one of the leading research centers in date palm micropropagation in the Arab States. In vitro-derived date palms have reached the production stage, confirming that the in vitro plantlets are genetically similar to the mother variety [Abu El-Nil, 1986a,b; Abu El-Nil et al., 1986; Al-Ghamdi, 1987]. Other tissue culture laboratories for date palm propagation were set up at King Saud University in Riyadh and at the Ministry of Agriculture and Water.

The results obtained in Saudi Arabia, as well as in Morocco, Algeria, and Tunisia, have shown that in vitro micropropagation can be very successful. However, future research is needed to develop date palm clones resistant to the bayoud disease caused by *Fusarium oxysporum* f. sp. *albedinis* (*Fusarium* wilt). In the date-producing Arab States, the application of tissue culture techniques to improve date palm varieties, coupled with processing, packaging, and marketing efforts, will lead to major changes in the export capacity of high-quality dates. For more detailed informations, see Sasson [1993].

## 3. Morocco

In 1981, there was only one tissue culture laboratory; a decade later it coexisted with ten research laboratories and five commercial laboratories; cooperation has been established between the public and private sectors. Of the three Maghreb countries, plant biotechnology transfer to the commercial sector is the most advanced in Morocco.

Research and development groups working on the following crop species have been identified: cereals, citrus, date palm, banana, strawberry, potato, and the argan tree. Close collaboration has been established between the different services of the Ministry of Agriculture involved in tissue culture techniques and also with the growers' associations. Such a close relation is paying off; an increasing number of farmers are requesting more tissue culture-derived plants of potato, citrus, strawberry, banana, and date palm [M. Aaouine, 1990, personal communication].

Under the National Institute for Agricultural Research (INRA), Morocco's main agricultural research institution, an important research program was carried out on the date palm, at the Institute's Regional Center in Marrakech. This program was initiated to control the bayoud disease, caused by *F. oxysporum* f.sp. *albedinis*; since its appearance at the end of the 19th century, the disease has wiped out two-thirds of Morocco's palm groves. Disease control is of vital importance, because the date palm provides a living for millions of people and is an effective means against desertification.

Date palm clones resistant to, or tolerant of, the pathogenic fungus were isolated by the INRA scientists as part of a wide-ranging breeding program. Some of these clones were producing good quality dates. An in vitro micropropagation project was designed with a view to producing healthy plantlets on a large scale through meristem and axillary bud cultures; somatic embryogenesis was also obtained. The micropropagation techniques have been transferred to the industrial production units of a private company, also responsible for distributing the plantlets to farmers.

A 4-year collaborative project has been carried out by the three Maghreb countries, with the assistance of the European Economic Community and a French laboratory, with a view to enhancing the in vitro propagation of date palm and developing clones resistant to the bayoud disease. A collaborative project on date palm, also aimed at controlling bayoud disease, was implemented by the Agronomic and Veterinary Institute Hassan II (Rabat) tissue culture laboratory, in Agadir and Purdue University, West Lafayette, Indiana.

Virus-free citrus plantlets were commercially produced, using shoot-tip grafting in vitro. Shoot multiplication and rooting of new rootstocks was obtained, with a view to producing virus-free trees on selected resistant rootstocks with improved yield and a higher quality of fruit. This was achieved by the Société de Développement Agricole, the objective of which was to produce about 200,000 virus-free scions annually to meet national needs for grafting and replanting.

Virus-free potato tubers were produced using multiplication of nodal cuttings and microtuber production, with a view to establishing a national program for certified tubers, eliminating tuber imports, and improving yield and quality of local potato varieties. This was achieved by the partly state-owned Société de Gestion des Terres Agricoles. In 1990, according to the FAO's estimates, Morocco exported 59,000 tonnes of potatoes.

Virus-free strawberries are being produced on a large scale, with a view to ending imports, producing new cultivars rapidly, and improving yield and fruit quality. Similarly, commercial propagation of pineapple using tissue culture techniques has been achieved and aimed to meet local demand for planting material. Virus-free plants have been obtained for sweet potato and grapes, whereas commercial propagation has been achieved for banana, olive, pistachio, prune, joboba, and verbena. Some relatively salt-tolerant banana cultivars have been propagated through tissue culture, and drought-tolerant tomato cultivars have been regenerated in vitro. The private corporation Domaines Royaux de Meknès own an industrial tissue culture unit, which markets more than 50,000 plantlets of date palm, 250,000 plantlets of banana, and 400,000 plantlets of verbena annually [M. Aaouine, 1990, personal communication].

A project using biotechnologies to improve cereal and food legume varieties is under way under the aegis of the National Institute for Agricultural Research, in cooperation with the International Center for Agricultural Research in the Dry Areas. The project, carried out in Settât, near Casablanca, aims to contribute to a wider program concerning genetic improvement of wheat (soft and durum), barley, triticale, lentils, chickpeas, and field beans. Androgenesis and plant regeneration from callus has been achieved with cereals (wheat and barley), with a view to selecting stress-tolerant genotypes and improving the yield and nutritional value of cultivars.

The argan tree (*Argania spinosa*) is an endemic wild tree growing in southwestern Morocco over an area of about 828,000 ha in semiarid and arid zones. Because it is well adapted to very dry and low-fertility soils in regions where the annual rainfall is less than 200 mm, this tree plays a prominent role not only in the overall economy of the region (Souss), but also in the protection of the environment. The tree belongs to the Sapotaceae family, the representatives of which are found mainly in the tropics, some of them having an important economic or

industrial role (e.g., the sheabutter tree or karite, *Butyrospermum parkii*, and the gutta percha tree). The argan tree is the farthest northern representative of this family [Nouaim et al., 1991].

The seed is used to produce an edible oil, but argan oil production represents only 1.6% of total annual edible oil consumption in Morocco. Oil extraction is rather rudimentary and not complete: about 100 kg of fruits and 8–10 h of work are needed to produce 1–1.2 L of oil. Its low concentration in linoleic acid and high content of linolenic acid, confer a high nutritive value on argan oil, considered as hypocholesterolemic. The properties of argan oil for skin protection against ageing led Galenic Laboratories of manufacture a series of products under the label "Argane," and the company Colgate Palmolive produced a soap containing 1% of argan oil called "Antinea." These high-added-value products could foster interest for the argan oil and argan tree [Nouaim et al., 1991].

The argan tree is above all considered a fruit tree and secondarily a fodder and a forest tree. Well-maintained plots, even in arid zones, could produce up to 800 kg of fruits per hectare (i.e., at least 250 kg of dry pulp, 200 kg of broken shells, 20–25 kg of oil cake, and more than 8 L of edible oil, which is sold at a high price) [Nouaim et al., 1991].

The acreage of the argan forests has been decreasing; population and livestock growth, the spreading of intensive crops, as well as the infringement of regulations in protected areas, are the main causes of deforestation. It has been estimated that at the current deforestation rate, the argan tree will almost entirely disappear in the year 2051 [Nouaim et al., 1991].

Such gloomy prospects led the Moroccan Forestry Service to undertake reforestation programs as well as projects to control sand formation. In the early 1990s, about 3000 ha were reforested and some 4700 ha of sand dunes were fixed. The main regeneration process of the argan tree is by offshoots; the second regeneration process is by seeds. At the French National Institute for Agricultural Research (INRA), in Dijon, an in vitro predevelopment unit has been working on in vitro culture of the argan tree since 1989. French scientists noticed that the plantlets obtained from in vitro cultures are true to the mother plant and that, in particular, the characteristics of the taproot are preserved [Nouaim et al., 1991]. At the French INRA and the Faculty of Sciences of Agadir, Morocco, scientists observed that the argan tree roots contain endomycorrhizae, which play an important role in the transfer of water and phosphorus, both of which are limiting factors in the arid soils underlying the argan forests; furthermore, as the roots of the tree have no absorbing hairs, the fungal mycelium can replace them and even explore a large volume of soil. Both the taproot and lateral roots contain endomycorrhizae. It was thus suggested to inoculate seedlings and tissue culture-derived plantlets with endomycorrhizae such as *Glomus mosseae* or *Gigaspora margarita*, so that the young saplings could benefit from the physiological advantages offered by these symbiotic fungi [Nouaim et al., 1991].

To sum up, the value and importance of plant biotechnologies have been recognized in Morocco; growers are increasingly requesting pathogen-free plantlets of banana, date palm, strawberry, and potato. Furthermore, owing to Morocco's lower labor costs and the absence of taxes on farmers' income, foreign corporations are investing in this area. In the coming years, plant biotechnologies will be applied to a wider range of crop and forestry species, and private sector involvement will increase.

At the Faculty of Sciences, Mohammed V University, Rabat, research has been carried out since 1980 on nitrogen fixation by grain legumes. Research in the early 1990s concentrated on the effect of several limiting factors on nitrogen fixation: soil acidity, water stress, and iron deficiency. Furthermore, the research program has been extended to subterranean clover and to actinorhizian trees (*Casuarina* sp.). The main objective of this research work is both to help raise grain legume yields, while decreasing inputs of nitrogen fertilizer, and to contribute to reforestation schemes with nitrogen-fixing tree species.

At the Agronomic and Veterinary Institute Hassan II, the research program of the Soil Microbiology Laboratory includes the following themes:

1. Ecology of *Rhizobium* spp. in different Moroccan soils
2. Characterization of *Rhizobium* spp. natural populations
3. Competition and survival of *Rhizobium* strains introduced into Moroccan soils
4. Determination of inoculation need for the main grain and fodder legumes cultivated in Morocco, and establishment of a map showing the regions where legume inoculation is or is not needed
5. Salt tolerance of the alfalfa-*Rhizobium* symbiosis in the Tafilalet oases
6. Introduction, in collaboration with the Forestry Department, of new nitrogen-fixing tree legumes, such as *Leucaena leucocephala*

Extension and development activities have resulted in on-farm demonstration inoculation trials on soybeans, chickpea, and berseem (*Trifolium alexandrinum*) in various regions of Morocco; training field technicians in legume inoculation; and organizing extension days with farmers to familiarize them with legume inoculation techniques.

#### 4. Tunisia

The creation in 1983 of the National Center for Biotechnology, in Sfax, was the result of a national plan for biotechnology research, oriented mainly toward agriculture, the agrofood industry, and health. A national commission for biotechnology coordinates the research carried out at the Sfax center, the Pasteur Institute (for health aspects), the Faculty of Sciences, the National Institute for Scientific and Technological Research, the National Institute for Agricultural Research of Tunisia (INRAT), and the National Institute for Agriculture of Tunisia (INAT) [Daaloul et al., 1990].

At the INRAT, *in vitro* shoot-tip micrografting is being used in citrus to produce virus-free plants; meristem and bud cultures have been applied to peach, almond, pistachio, and jojoba; and a laboratory has been created for date palm *in vitro* micropropagation and multiplication, with FAO assistance.

At the INAT, *in vitro* tissue culture has been applied to potato, artichoke, strawberry, and ornamentals; all *in vitro* culture stages have been mastered, including the rooting of plantlets; collaboration has been undertaken with an agricultural development institution to scale up the process to the commercial stage. Crop breeding, especially toward stress- and disease-resistance in barley and wheat, also rely on plant biotechnologies [Daaloul et al., 1990].

Biological nitrogen fixation research on grain legume species was initiated in 1980 at the Laboratory of Agronomy of the INAT, on native rhizobia that induce broad bean nodulation. Later on, similar work was carried out on pea, chickpea, groundnut, and common bean. Inoculation trials showed positive effects on broad bean, field bean, pea, and chickpea. These effects might be due to both the *Rhizobium* and the phosphate added to the peat carrying the inoculum.

Introduction of alfalfa, which could contribute to the intensification of cereal cultivation over 300,000 ha of fallow fields, was carried out in cooperation with Australia. Such an innovation seemed to be successful in zones receiving more than 150 mm of annual rainfall, but sufficient seed supply was needed. Preliminary inoculation trials, carried out with the assistance of the International Center for Agricultural Research in Dry Areas and the International Development Research Center (Ottawa), showed a positive effect of the inoculum on alfalfa and on *Medicago hispida*.

*Acacia cyanophylla* has been used in Tunisia since the 1930s by foresters, because it was a good pioneer plant species which, after 12–18 years, could be replaced on the rehabilitated

soils by higher-value species or citrus. It was also used as a fodder tree on very large areas: about 47% of fodder units produced annually in this country originated from *A. cyanophylla*. Nitrogen fixation research on *Acacia cyanophylla* was initiated in 1983 and a culture collection of native *Rhizobium* isolates was constituted. The majority of these strains belong to the *Bradyrhizobium* genus. The overall purpose of the research work is to isolate and multiply highly efficient *Rhizobium* strains, as well as endomycorrhizae that could improve *A. cyanophylla* phosphorus nutrition and to clone the most effective plants using tissue culture. The most efficient symbiotic associations thus identified could be used, first in field trials, then in plantations.

#### D. Sub-Saharan Africa

In 1994, in vitro micropropagation was being conducted for the following cash crop or plantation species: oil palm (Ivory Coast, Nigeria); date palm (Algeria, Egypt, Morocco, Tunisia); cocoa (Nigeria); coffee (Kenya, Zimbabwe); tea, camphor tree, and pyrethrum (Kenya); sugarcane (Kenya, Zimbabwe); olive tree (Egypt, Morocco); strawberries (Egypt, Morocco, Tunisia); pineapple (Zimbabwe); tobacco (Zimbabwe), forest tree species (Egypt, Kenya, Senegal, Tunisia); and ornamentals (Kenya, Mauritius).

For food crop species, the following are being micropropagated: banana (Burundi, Cameroon, Egypt, Gabon, Ghana, Morocco, Nigeria, Senegal, Tunisia); plantain (Cameroon, Gabon, Ghana, Nigeria); tuber plants (Algeria, Burundi, Egypt, Kenya, Mauritius, Morocco, Tunisia, Zimbabwe); cassava (Burundi, Cameroon, Congo, Gabon, Nigeria, Zimbabwe); taro (Cameroon, Ivory Coast, Gabon, Ghana); yams (Cameroon, Ivory Coast, Gabon, Ghana, Nigeria); sweet potato (Burundi, Kenya, Zimbabwe); rice (Senegal); soybeans, maize, and groundnut (Zimbabwe).

Many African staple crops (e.g., cassava, sweet potato, yam, and plantain) are vegetatively propagated and so far have received little research funding (orphan crops). Meristem culture, shoot-tip and node-cutting cultures are routinely used to eliminate viruses from improved cassava clones and to micropropagate them. The virus-free improved clones have been distributed by the International Institute of Tropical Agriculture (IITA) to national agricultural research institutions for evaluation and testing. The in vitro reduced growth storage method was applied to conserving clonal germplasm: a total of more than 2000 accessions are being maintained at the IITA.

Embryo culture techniques for the germination of mature and immature cassava embryos are being developed. In IITA scientist has reported that production of plantlets from callus produced from cassava leaf has become possible using several cassava varieties resistant to the Africa cassava mosaic virus.

##### 1. Senegal

On 20 March 1992, the Research Unit on In vitro Culture (URCI, Unité de Recherche en Culture In Vitro) was inaugurated in Dakar, as a joint laboratory between the Senegalese Institute for Agricultural Research (ISRA) and the French Scientific Research Institute for Development in Cooperation (ORSTOM). The unit, located at the ORSTOM/ISRA Center in Dakar, aimed to make available a biotechnological tool for improving forest species through in vitro tissue culture. Set up with funding from the French Ministry for Cooperation, the laboratory's first objective was to create a seed pool of selected clones for producing improved seeds for reforestation. In a second stage, the laboratory will apply biotechnologies to improving other plant species (e.g., horticultural and large-scale cultivation crop species). It is expected to

play a regional role through welcoming foreign researchers seeking to become better acquainted with plant tissue and cell culture.

## 2. *Ivory Coast*

The Ministry of Scientific Research has taken steps to facilitate the integration of plant biotechnologies into the national agricultural research program. In 1991, a committee consisting of scientists and representatives of the main agricultural companies was set up to investigate a plan for plant biotechnology development in the Ivory Coast [Mulongoy et al., 1993].

Plant biotechnology research at the National University in Abidjan includes micropropagation of virus-free yam and plantain. Other research interests include yam improvement through the production of haploids, invertase activity in relation to yam quality during storage, and isoenzyme characterization of yam and eggplant to document the diversity of these genera [Mulongoy et al., 1993]. A project of close collaboration between the Research Institute of Plant Biology of the University of Montreal, and the National University, Abidjan, with funding from the International Development Research Center (Ottawa), aims to transfer plant tissue culture techniques for *in vitro* micropropagation and clonal multiplication of yams. The research project has led to the production of yam microtubers *in vitro*; it has been followed by laboratory and field work in the Ivory Coast, oriented toward substituting the traditional vegetative propagation methods with a technique more suited to the large-scale distribution of superior yam clones.

The first laboratory for oil palm *in vitro* culture was set up in the early 1980s at La Mé by the French Institute of Research on Oil and Oilseeds, now part of the CIRAD's Department of Perennial Crops. Ivory Coast was the second-largest producer of palm oil in Africa and, therefore, was interested in improving multiplication techniques for oil palm. In the 1970s, 50,000 plantlets of oil palm, resulting from 1000 crosses were produced. Plantlets of elite varieties were distributed to farmers and to Palmindustrie, a governmental company. At La Mé, the oil palm germplasm was stored *in vitro* at low temperature or in liquid nitrogen [Mulongoy et al., 1993].

In 1993, palm oil represented more than 20% of the world production of vegetable oil and ranked second behind soybean oil. A larger share of world production depends on increasing productivity through the utilization of higher-yielding oil palm varieties or clones tolerant to the principal diseases. In West Africa, yield was no higher than 20–23 tonnes of fresh fruit bunches per hectare, even when there was a sufficient water supply, because solar radiation was insufficient in the face of the important cloud blanket. The same oil palm varieties yielded 30 tonnes/ha or even more in Southeast Asia, where solar radiation was higher than 30% (CIRAD, 1993).

In the Ivory Coast, a diagnosis of the status of the 130,000 ha of oil palm groves has been carried out by the CIRAD at the request of Palmindustrie. The study shows that industrial exploitations replanted with plant material bred over the 1970s and 1980s yielded about 25% more than the first generation at the same age. It has been demonstrated that the best clones of oil palm (derived from tissue culture) yield 25–30% more than the average hybrid progeny from which they originated. More than 2500 ha have been planted worldwide with oil palms derived from somatic embryos. Some flowering abnormalities are explained by the variations in the amounts of endogenous plant hormones. The identification of molecular markers would allow the early elimination of calli likely to give rise to abnormal clones. On the other hand, reducing the production cost of plantlets is considered a priority objective: research is being carried out on developing embryogenic suspensions in a liquid culture medium; a process of

this kind has the advantage of offering substantial savings in labor and laboratory space (CIRAD, 1993).

As West Africa is the natural center of origin of the oil palm, there is a large genetic collection of both wild and highly developed breeding lines, as well as a long tradition of oil palm research. Much of the data required for selection of parents for production of progenies suitable for cloning are already in the archives. The African countries are in a strong position to introduce new genetic variation, including disease resistance, and to compete with Southeast Asian material derived from the relatively narrow base of the Deli Duras. On the other hand, there is a need to acquire the DNA technology for clonal monitoring and for oil palm genetics to aid the breeding program [Jones, 1992].

In the breeding of high-yielding cocoa varieties, hybrids of selected clones have been identified in the Ivory Coast by the CIRAD in collaboration with IDEFOR (Forestry Institute); capable of producing up to 3 tonnes of marketable cocoa per hectare, they are widely used. Productivity improvement is also sought through the creation of varieties resistant to diseases (*Phytophthora* spp.) and pests. The genomic sequence of the swollen shoot virus has been identified in collaboration with the French National Institute for Agricultural Research, paving the way to new diagnostic methods [CIRAD, 1993].

Research work is being conducted at CIRAD's center in Montpellier on the genetic diversity of cocoa, using DNA markers in about 300 clones. Restriction fragment length polymorphism (RFPL) and random-amplified polymorphic DNA (RAPD) markers are used to establish a map of the cocoa genome. In the medium term, genes for resistance to diseases and for organoleptic qualities of cocoa are expected to be identified. In collaboration with Francereco (Nestlé group), the conditions for obtaining somatic embryos from petals have been determined in nine genotypes. In 1992–1993, plants derived from somatic embryos were being grown in pots in greenhouses [CIRAD, 1993]. For more detailed information, see Sasson [1993].

### 3. Nigeria

Of the 24 government research institutes in 1994, about 10 were involved in some kind of biotechnology research, largely related to agriculture and food processing. The National Center for Genetic Resources and Biotechnology, created in 1986 on the Moor Plantation, Ibadan, is coordinating research activities carried out in 9 satellite laboratories.

Crop species studied extensively (including at the International Institute of Tropical Agriculture, IITA, Ibadan) are cassava, yam, groundnut, cowpea, sorghum, maize, rice, cocoa, and rubber. Since 1977, the IITA has been working on hybrid maize and, by 1983, Nigerian research institutes had followed suit, resulting in on-farm testing in 1984. That same year, the first private company, Agricultural Seeds Co., became involved in producing hybrid maize seed. Furthermore, Pioneer Hi-Bred Seed Nigeria initiated production of hybrid maize in 1990 and, in 1992, bought up the Agricultural Seeds Co. In early 1992, hybrid maize covered an estimated 100,000 ha of the country's 2 million ha planted with maize [in the *IITA Annual Report, 1991*].

For about four decades, Nigerian researchers had been evaluating rice germplasm in investigations aimed at developing resistance to blast disease and brown leaf spot disease, both prevalent in Nigeria. New, resistant varieties were developed and distributed to farmers in the early 1990s, both in Nigeria and across sub-Saharan Africa. The development of high-salinity tolerant rice might change some 2 million ha of Nigerian coastal marshlands into paddy fields [Ogunseitan, 1991].

With use of combinations of tissue culture and other techniques, the research goal was to create as large a collection of soybeans as possible that were sufficiently promiscuous

(nodulating freely with natural *Rhizobium* present in Nigerian soils), photoperiod-insensitive, nonshattering, higher-yielding, with good seed longevity [Ogunseitan, 1991].

Nigeria is the leading yam producer in sub-Saharan Africa. In the face of spiraling population growth, intensive cropping systems are needed. It would be necessary to lower production costs devoted essentially to planting material, billowing, weed control, and stick setting (especially in forest areas), not to mention those linked to harvesting tasks, to ensure the competitiveness of yam versus other crop species. Research work should also play a vital role in selecting plants with a different stem morphology, tuber traits, growth periods, and multiplication rates. Postharvesting losses are heavy. Their mitigation could significantly reduce the need to extend the cultivated acreage and the resulting drop in soil fertility. At the Laboratory of Plant Tissue Culture and Biotechnology, Department of Botany, University of Nigeria, Nsukka, Enugu State, plantlets were regenerated from explants of a few edible yam species derived from stem nodes (with or without leaf), internodes, and meristems. Furthermore, methods for the culture of zygotic embryos have been devised to recuperate embryos from plants with a high abortion rate.

Cocoa is still the leading agricultural export of Nigeria, the sixth-largest cocoa producer. The Cocoa Research Institute of Nigeria (CRIN) was set up in 1953 on the Moor Plantation, Ibadan. Although cocoa was introduced into Nigeria about a century ago, active research began only about 30 years ago. With the advent of biotechnologies, in vitro propagation and germplasm conservation benefited from their application. During the third Nigerian breeding program, somatic embryos were obtained by Esan in 1975 from cultured cotyledon and hypocotyl tissues of very young cocoa embryos. Attention focused mainly on micropropagation and in vitro germplasm conservation of cocoa at the CRIN. For more detailed information, see Sasson [1993].

#### 4. Cameroon

At the Jay P. Johnson Biotechnology Laboratory of the Institute for Agricultural Research, Buea, Cameroon, culture media have been developed for the regeneration of yam plantlets (*Dioscorea rotundata*) from stem nodes, as well as for the induction of multiple shoots and rooting of microcuttings derived from these shoots. For macabo (*Xanthosoma sagittifolium*), a method for inducing secondary shoots on the initial bud has been devised, followed by the rooting of the resulting shoots and production of plantlets.

The project on the genetic improvement of plantains and other bananas aims to improve banana production and to increase exports. In 1993, production increased from 30,000 to 100,000 tonnes and laboratories were built for phytopathology and plantlet production at an affordable price for planters. The project's board of directors brought together representatives of the Institute for Agricultural Research and the CIRAD's Department of Fruit and Horticultural crops (CIRAD-FLHOR). Funding amounted to about 2.7 billion CFA over 5 years.

The Regional Center for Banana and Plantain, an original Euro-African regional cooperation structure, was set up in 1989 on the joint initiative of the Government of Cameroon and the CIRAD. It was then extended to include the Central African Republic, Congo, and the Institute for Agricultural and Livestock Research, jointly run by Burundi, Rwanda, and Zaire. On 7 July 1993, the directorate of the CRBP was inaugurated in Njombe, Cameroon [CIRAD News, October 1993, 6]. For more detailed information, see Sasson [1993].

#### 5. Kenya

The Kenya Agricultural Research Institute (KARI) was been set up in 1902 and since the early 1950s, 15 stations for agricultural research and extension have been established throughout the



country under the aegis of the government. Early research work was initiated on the breeding and selection of cereal species, particularly wheat and maize, followed by work on coffee and tea. Most of the applications of plant biotechnologies are found in the research carried out on these crop species.

Most research on potatoes is being carried out at KARI's National Plant Quarantine Station, Muguga, which screens and multiplies the material supplied by the International Potato Center. Further large-scale propagation through tissue culture is undertaken by the KARI's National Potato Research Station located in Tigoni. New varieties are disseminated by the Agricultural Research Corporation. It is estimated by the International Labor Organization that the adoption of plant biotechnologies for the propagation and cultivation of potatoes might increase labor productivity by 24%, while doubling the contribution of potato production to national income [Olembo, 1991; Pistorius, 1991].

At the Crop Department of the University of Nairobi, research is being carried out on meristem culture and somatic embryogenesis of several crop species, with a view to breeding pest-resistant plants. Viruses have been identified in the same department by the enzyme-linked immunosorbent assay (ELISA) technique, increasingly used in research stations and in quarantine and germplasm conservation units. The International Potato Center has introduced the technique into the region to detect potato viruses. In addition to the work carried out on citrus and strawberries, research has also been under way on cassava, sugarcane, and coffee. Maize tissue culture is also performed at Kenyatta University, near Nairobi [Clark and Juma, 1991].

Coffee is the greatest export-earning commodity in Kenya, ahead of tourism and tea. Coffee breeding in Kenya began in 1971, stimulated by losses resulting from the coffee rust and coffee berry disease. At the Ruinu Coffee Research Foundation, a small team of researchers developed tissue culture methods for micropropagation purposes. Somatic embryogenesis has been obtained on secondary calli derived from leaf explants. Regeneration of coffee plantlets from tissue cultures is still difficult to achieve on a large scale and with a high success rate [Olembo, 1991; Pistorius, 1991]. In 1986, the Coffee Research Institute released a new coffee variety, Ruinu-11, with a short stature, derived from Catimor. The current breeding program is directed toward resistance. There is also interest in developing RFLP and isoenzyme markers to assist coffee breeding. New coffee-breeding programs are being conducted on two segregated populations: one from a cross between Kenyan Catimor and Colombian Catimor, and another resulting from a cross of robusta tetraploids with arabica.

Tea cloning also plays an important role in the cultivation of this extending cash crop: in 1991, about 10% of the large-scale tea plantations were planted with clonal material obtained through tissue culture. Small farms, representing 67% of total acreage, are all planted with clonal material [Olembo, 1991; Pistorius, 1991]. For more detailed information, see Sasson [1993].

## 6. *Zambia*

Zambia's use of legume inoculants has been increasing rapidly and the demand has challenged the facilities' ability to keep pace. Established in 1984, the Mt. Makulu Research Station Inoculum Production Facility has grown rapidly and promoted the application of biofertilizers over inorganic nitrogen use. An estimated 8000 ha of soybeans were inoculated during the first year, followed by annual increases of up to 40,000 ha of soybeans being inoculated with the appropriate *Bradyrhizobium* strains. The average production increase in legume inoculants on a peat-base carrier for several species has been 10–15% since 1987. A total of 75,000 packets,

each 250 g (18.75 tonnes), were supplied during the 1991–1992 production season, of which 95% were for soybeans.

## 7. *Zimbabwe*

In Zimbabwe, over the 1980–1990 period, agriculture had contributed an average 15% to the gross domestic product, and 55% of the active population are working in agriculture, which represented 42% of foreign currency earnings [Fritscher, 1992].

Zimbabwe, by far the most important forestry and agricultural producer in the Southern African Development Coordination Conference (SADCC) region, applies a hydroponic method of raising seedlings of forest species on a large scale. The so-called speedling technology consists of germinating and raising seedlings in polystyrene cells, under tightly controlled regimens of temperature, irrigation, and fertilization. After having been developed in the United States, speedling nurseries first appeared in South Africa during the 1970s, when several commercial vegetable producers adopted and invested in this technology. By the 1980s, very large nurseries were operating, servicing not only the agricultural and horticultural sectors, but also the South African forestry industry, the largest in Africa [Tiffin and Osotimehin, 1992].

Although the speedling technology was not developed in Zimbabwe, its success was based on local experimentation and know-how, local materials, and entrepreneurial self-reliance. In 1989, Delta Corporation approached Brookfield Seedlings which, along with Tabex (a major Zimbabwean tobacco and horticultural conglomerate) and other business investors, took over its tissue culture laboratory and established it on-site at Brookfield. In 1991, the laboratory started to function commercially and aimed to develop disease-free cuttings of coffee, strawberries, bananas, citrus, sweet potato, and other economically important crop species. The laboratory also led to a new venture in the production of mushroom spores for domestic consumption, instead of importing them from Europe [Tiffin and Osotimehin, 1992]. For more detailed information, see Sasson [1993].

## 8. *Republic of South Africa*

In late 1992, the Foundation for Research Development (FRD)—a government-sponsored body responsible for developing South Africa's human resources in science, engineering, and technology—compiled information on current biotechnological activities in the country, carried out by academic and research institutions, as well as industrial companies. A survey was conducted in which 50 companies involved in biotechnologies were asked to state their top priority for development:

1. Agricultural and plant biotechnologies (20%)
2. Production of food and protein substitutes (18%)
3. Medical biotechnologies and diagnostics (12%)
4. Pharmaceutical and therapeutic substances (8%)
5. Veterinary and animal biotechnologies (8%)
6. Environmental biotechnologies (8%)
7. Molecular biology (4%) [FRD, 1992].

Research groups in universities and "technikons" work mainly in the new biotechnologies, the majority being interested in medical biotechnologies (49%) and plant biotechnologies (45%) [FRD, 1992]. There seems to be general recognition that agricultural biotechnologies should have high priority to improve food production and that certain areas of health and the environment should also be considered priority areas.

Under the Agricultural Research Council, the Vegetable and Ornamental Plant Institute (VOPI), Pretoria, has maintained over 1000 plant types of over 80 species with acknowledged economic and horticultural value. In addition to being a reliable source of disease-free potatoes, sweet potatoes, garlic, and flower bulb species, the VOPI has a semicommercial tissue culture facility.

The Agricultural Research Council has cooperation links with the International Potato Center (CIP, Lima, Peru), the African Potato Association in Tunisia, and the Gene Bank in Lusaka, Zambia. It has been involved in research activities in plant breeding (Kenya) and sweet potato (Malawi).

At the Stellenbosch Institute for Fruit Technology, the Division of Plant Biotechnology and Pathology produced the first genetically engineered crop species, the strawberry cultivar Seleka, with herbicide resistance. Research has concentrated essentially on improving resistance to bacterial and fungal diseases, leaf roll virus, plum poxvirus, ripening inhibitors, cultivar typing, plant transformation, and plant tissue culture. Enhanced resistance has been incorporated into plants against bacterial canker, fire blight, and spots of stone fruits caused by *Pseudomonas* sp. and *Xanthomonas campestris* var. *pruni*; apple blisters caused by *Pseudomonas syringae*; and blossom blights of Forella pears. Diagnostic kits were developed to detect bacterial and viral diseases in passion fruits. To these achievements could be added the regeneration of apricot, apple, and pear plants. Research is also being conducted on the interaction of apple and plum plantlets and vesicular-arbuscular mycorrhizae.

Transgenic Royal and Gala apples, virus-resistant apricots, herbicide-tolerant strawberry cultivars (Seleka, Trobella, and Tioga) have been developed since this line of research was initiated in 1988. Cooperation exists with the Bayer Corporation, and the South African Committee for Genetic Experimentation is overseeing the release of genetically engineered plants. The Institute provides on-farm services and advice.

The South African Sugar Association Experiment Station (SASEX), which has links with 35 countries (including Malawi, Swaziland, and Zimbabwe), has developed new sugarcane varieties, using both conventional breeding and biotechnologies. Current research focuses on resistance to the sugarcane stem borer, *Eldana saccharina*. The SASEX's excellent tissue culture facilities lend themselves to the training courses it runs for both local scientists and those from neighboring countries. In addition, SASEX has hosted regional courses for farmers and specialists from Fiji, Sri Lanka, Thailand, Brazil, and other countries, as well as for farmers from South Africa, Malawi, and Swaziland.

At the University of Cape Town, life science and biotechnological research is well established and recognized worldwide. The following projects are being carried out:

1. Development of isotope tools for assessing drought tolerance in cotton- and tobacco-breeding programs
2. Gene targeting within the Poaceae family to improve resistance in cereal crop species to drought, disease, and herbicides
3. Genetic engineering to control the maize streak virus
4. Development of a biopesticide against the sugarcane stem borer, *Eldana saccharina*
5. Production of flavors and fragrances from indigenous plant species

#### 9. *Mauritius*

At the University of Mauritius several grain legume species have been selected for pulse production locally to cut down on imports: *Vigna mungo*, *Phaseolus vulgaris*, *Lablab purpureus*, *Glycine max*, *Lens culinaris*, and *Cicer arietinum*. Research work is being carried

out on the selection of effective and persistent strains of *Rhizobium* for these legume species to be grown under various soil conditions and fertilizer [Manrakhan, 1990].

After screening selected species of economic importance to Mauritius, their suitability for successfully cloning through tissue culture has been studied. In 1985, a Food and Agricultural Research Council (FARC) was set up with a view to linking research with commercial exploitation. It set up a cell and tissue culture laboratory, initially for *Anthurium* and horticultural crops in which Mauritius has earned a solid export reputation, and for several vegetables and other ornamentals, mainly for export purposes [Manrakhan, 1990].

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