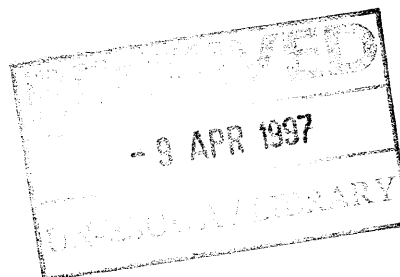


16 MAR 1989

E/ESCWA/ID/89/CONF.1/29

The First Arab Conference on  
Perspective of Modern Biotechnologies  
in the Arab Countries  
27-30 March 1989  
Amman - Jordan



PLANT PRODUCTION AND PROTECTION BIOTECHNOLOGIES IN THE  
NEAR EAST AND NORTH AFRICAN COUNTRIES

Ibrahim Y. Hamdan  
Food and Agriculture Organization

---

This paper has been produced without formal editing.

89-0334

ESCWA Documents converted to CDs.

CD # 5

Directory Name:

89-9433

CD5\ID\89\_CONF1.29

Done by: ProgressSoft Corp., P.O.Box: 802 Amman 11941, Jordan



**Plant Production and Protection Biotechnologies in the  
Near East and North African Countries**

**Ibrahim Y. Hamdan  
Biotechnology Department  
Food Resources Division  
Kuwait Institute for Scientific Research  
P. O. Box 24885  
13109 - Safat - Kuwait**

**February 1989**

## Contents

<b>Introduction</b> .....	<b>1</b>
<b>Potential Applications of Biotechnology to Plant Production and Plant Protection</b> .....	<b>2</b>
Plant Production Through Cell and Tissue Culture .....	3
Non-Recombinant DNA Approaches to Plant Production .....	4
Somaclonal Variation .....	4
Protoplast Fusion .....	5
Recombinant DNA Approaches to Plant Production and Protection .....	6
Transformation by (Ti) Plasmids .....	6
Transformation by Plant Viruses .....	8
<b>Plant Protection by Biological Pest Control</b> .....	<b>9</b>
<b>Basic Features Related to Biotechnology in Arab Countries</b> .....	<b>11</b>
Plant Biotechnologies in Selected Arab Countries .....	13
Saudi Arabia .....	13
Egypt .....	14
Morocco .....	16
Other Arab Countries .....	17
<b>Potentially Promising Plant Biotechnologies for the Arab Countries</b> .....	<b>19</b>
Natural Resources .....	19
Promising Plant Biotechnologies .....	20
Date Palm .....	21
Citrus .....	22
Olives, Almonds and Pistachios .....	22
Potatoes .....	22
Cereal Crops .....	23
Other Crops .....	23
<b>Constraints</b> .....	<b>24</b>
<b>FAO Experience in Regional Projects and Policy Towards Biotechnology</b> .....	<b>25</b>
Regional Projects .....	25
FAO Policy Towards the New Biotechnologies (with special reference to developing countries) .....	26
<b>Conclusions</b> .....	<b>27</b>
<b>References</b> .....	<b>29</b>

**PLANT PRODUCTION AND PROTECTION BIOTECHNOLOGIES IN THE NEAR EAST AND NORTH  
AFRICAN COUNTRIES**

**Ibrahim Y. Hamdan\***

**Biotechnology Department, Kuwait Institute for Scientific Research, Kuwait**

**Introduction**

Biotechnology is the application and integration of several disciplines, including microbiology, biochemistry, genetics and biochemical engineering, for the production of compounds that are beneficial to human welfare. Great advances were recently made in plant biotechnology, including the development of micropropagation systems for many plant species, the development of new plant varieties with highly desirable characteristics, the manipulation of the genetic material and its cloning into other organisms and the application of tissue culture technology to the micropropagation of plants. These represent major developments in technology that could lead to great potential applications in agriculture and the food industries.

Biotechnology has become established as the newest 'high technology' industry, but most of the expertise in this technology is limited to developed countries. Most biotechnological innovations and applications in these countries have been confined to priorities that are of minimal relevance to applications in developing countries. Furthermore, much of the expertise in developed countries is concentrated in the private sector that restricts developing countries' access to patented and proprietary technology.

Most Arab countries in the Near East and North Africa are in arid and semi-arid zones that are characterized by severe weather conditions, lack of fresh water and soil erosion. As a result, viable agriculture for the production of food of both plant and animal origin is strictly limited and below consumption levels. Coupled with a population growth rate that is expected to double in the next two decades, this indicates the magnitude of the food supply problem facing these countries.

The main challenges facing the Arab countries are related to food supply and conservation of resources. Through biotechnological approaches, different resources can be utilized by: increasing soil fertility through biological activities; increasing agricultural production through genetic manipulation of plants; and utilization of wastewater through biological treatment. Most present biotechnological activities in these countries are limited to conventional methods of plant breeding, industrial fermentation and soil microbiology

### **Potential Applications of Biotechnology to Plant Production and Plant Protection**

There is great interest in the potential applications of new biotechnology to plant production. The prospects were reviewed and discussed by several workers (Cocking et al., 1981; Barton and Brill, 1983; Brill, 1984). In the last five years, there have been important advances in plant molecular biology especially in plant viruses being used as vectors (Brisson et al., 1984), and in using a tumour-inducing, (Ti) vector to transfer genes to a monocotyledon (Hooykaas et al., 1984; Hernalsteens et al., 1984). Genes were successfully transferred between plants such as corn, weeds, potatoes, wheat, beans and tobacco via microinjection into pollen or protoplast fusion. Some other known genes that are likely candidates to transfer to plants for pest resistance

(Plunkett et al., 1982); and genes for resistance to the herbicides' glyphosphate (Comai et al., 1983); and genes for bacterial pesticidal proteins such as those produced by Bacillus thuringiensis (Klier et al., 1982).

### Plant Production Through Cell and Tissue Culture

Rapid development of plant cell and tissue culture techniques for plant propagation offer great potential for the future development of agriculture. A wide range of plant species have now been cultured and regenerated through plant cell, tissue or organ culture allowing many identical plants, including trees, to be grown. The plant species that can be propagated through tissue culture include asparagus, beets, cauliflower, cabbage, lettuce, spinach, alfalfa, millet, clover, potatoes, carrots, tomatoes, citrus, oil palms, date palms and many ornamental species (OTA, 1984). The majority of these plants are regenerated through shoot-tip culture. This approach is labour-intensive and costly, and can only be justified in the case of high-value speciality plants, e.g., oil palm, for which a micropropagation system has been developed. This system provides clones of desirable, uniform and consistent characteristics (Jones, 1983). For micropropagation of mass food crops to be successful, a major development in technology is required in the area of automated propagation and somatic embryogenesis (Fowler, 1986).

Plant cell and tissue culture techniques provide input to plant breeding efforts at two levels. The first is to provide the breeders with materials such as genetic and cytogenetic variants, haploids, wide cross hybrids and disease-free stocks. The second input is to provide the bridge between molecular genetic engineering techniques and plant breeding. Most of the schemes envisioned involve the transfer of genetic information and require that cells from the recipient plant be cultured; however, using improved genetic methods allows the direct manipulation of pollen or embryos, circumventing the necessity for regeneration (Lamphey and Moo-Young, 1988).

### Non-Recombinant DNA Approaches to Plant Production

The applications of biotechnology, via cell, organ and tissue culture techniques, to plant breeding in the long term are to create new plant varieties with increased vigour and yield, to incorporate value-added traits and to develop disease, herbicide and pesticide-resistant crops. Recent developments in plant breeding centered on using recombinant DNA to introduce new characteristics into plants; however, there are a number of non-recombinant DNA approaches that are useful and successful in developing new crops. The first approach is the exploitation of spontaneous or induced variation in cultured plant cells or tissues. The second is the use of protoplast fusion to mediate genetic exchange.

Somaclonal Variation. Somaclonal variation is a form of mutation breeding used to produce minor modifications in established varieties. The somoclonal variation technique takes advantage of naturally occurring genetic variations that appear in plants regenerated from somatic cells grown in tissue culture. This variation can pre-exist in the explant tissue, but it usually arises during the tissue culture procedure itself. The genetic changes that occur include nucleotide sequence, chromosome structure and chromosome number and phenotypic variability.

The variability in cell cultures of regenerated plants can be identified by morphological changes such as plant height, leaf shape, flower morphology and pigmentation. The genetic diversity of plants emerging from callus tissues was used to introduce some useful plant variants into established cultivars without using several crosses. Some examples of plants improved through somaclonal variations are carrots, celery, potatoes, sugar cane and tomatoes (Primrose, 1987).



**Protoplast Fusion.** Protoplast fusion is the process of fusing cells whose cell walls have been removed. Protoplast fusion enables the production of plant hybrids not possible by normal breeding methods and does not depend on normal sexual reproduction. Two basic techniques can be used for protoplast fusion. One is to use chemicals to act as fusogen such as polyethylene glycol, dextran and poly-L-ornithine. Alternatively, electrofusion can be used, whereby DNA is introduced into the cells by passing an electrical pulse through a solution containing DNA in which protoplasts are suspended. The fusion of one cell line with the cytoplasm of another cell line to form hybrids that can be used to transfer some traits into breeding lines is useful for cytoplasmic male sterility, disease resistance and herbicide resistance in some plants. The biggest disadvantage of this technique is that there is little control over what genetic information is retained or eliminated (Primrose, 1987). Some hybrids have been successfully fused with difficulties such as hybrids of wheat and rye (triticale) and hybrids of turnip and cabbage (raphanobrassica). In 1978, protoplasts of potato and tomato were fused resulting in a hybrid plant (pomato) that is not of immediate value, however, pomato is of great scientific interest and it may have agronomic advantages in future such as producing tubers as large as those of the potato and larger fruits containing alkaloids inherited from the parents (tomatine and solanine). Potato, being a species that is difficult to improve genetically, unlike tomato, could be improved to be disease-resistant by developing, in a few generations a donor tomato disease-resistance variety that could be transferred to a potato variety in a single operation by protoplast fusion, whereas it would take about 20 years with conventional plant breeding methods (Sasson, 1984).

### Recombinant DNA Approaches to Plant Production and Protection

The advantages to plants of using genetic engineering over non-recombinant approaches are that genes can be introduced into a plant that do not exist in any member of the same plant family. Second, gene manipulation could be used to make substantial alterations to key cellular proteins. Cloning vectors are required for genetic engineering of plants that are based on those derived from the tumor-inducing (Ti) plasmid of the bacterial strain Agrobacterium tumefaciens and on those based on plant viruses. Plant protoplasts can also be induced to take up naked DNA in a way that is similar to the transformation of animal cells. To genetically engineer a plant for commercial applications, certain requirements must be satisfied: (1) introduction of the genes of interest to all plant cells; (2) stable maintenance of the new genetic information; (3) transmission of the new gene to subsequent generations; and (4) expression of the cloned genes in the right cells at the right time (Primrose, 1987).

Transformation by (Ti) Plasmids. Different types of the tumor-inducing (Ti) plasmids that are responsible for transformation of the pathogenic properties of Agrobacterium spp. contain one or more large plasmids. Because of the natural ability of the Ti plasmids to insert DNA into plant cells, they have become the subject of extensive studies for their use in genetic engineering of plants (Depicker et al., 1983). Plants could be made genetically resistant to herbicides, pesticides, frost and insecticides. Plants or mutant organisms that express novel forms of natural resistance are the source of genetic determining resistance. Many single-structured genes occurring in nature have been transferred and expressed in plants (Comai and Stalker, 1986). Plants that have been engineered by introducing coding regions of single genes under promoters functional in plants are moving from laboratory to field trials.

Genetic variability among sexually compatible plants has already been exploited by plant breeders for obtaining high yielding crops. However, unlike plant breeding, the sexual compatibility barrier may be crossed by using protoplast fusion and recombinant DNA technology. Genes were isolated, modified in vitro and mutagenized then inserted to plant genomes where they were expressed. An important step in this direction was made by using Ti-plasmid of Agrobacterium tumefaciens as a vector. The Ti plasmid inserts a fragment of its DNA called T-DNA into the host plant DNA. The integration of T-DNA is directed by specific sequences of about 25 base pairs located at both ends of the T-region on the Ti-plasmid. DNA sequences of more than 50 kilobases inserted within the T-region are integrated into the plant genome. The plasmid-derived T-DNA codes for enzymes involved in the biosynthesis of opines, which are unusual amino acids not found in the normal tissues, inhibit plant differentiation (Kudrika, 1986). Since mobilization functions of small wide host range plasmids can substitute the Ti plasmid's 25 base pairs direct repeat T-DNA borders, Agrobacterium host range may be expandable (Buchanon-Wollaston et al., 1987).

A bacterial gene that is resistant to methotrexate was successfully inserted into T-DNA, and cells into which this gene was introduced became resistant to methotrexate. The regulatory nucleotide sequence of the bacterial gene could be expressed in plant cells. However, the expression of other genes inserted into Ti plasmids was not obtained systematically and the nature of gene expression mechanisms in a given cell environment still has to be determined. On the other hand, as the tumor-inducing function of T-DNA was not affected when gene coding for opine synthesis was removed, these genes could be replaced by others, such as nitrogen fixation genes (nif), to have integration into the host-cell genome (Sasson, 1984).

The microbial genes transferred into plant cells via the Ti plasmid of Agrobacterium spp. and genes include those encoding Kanamycin resistance and resistance to herbicides. Target species in which success was achieved include Petunia, tobacco, tomato, cotton, poplar, alfalfa, soybean and corn. Although the Ti plasmid of Agrobacterium spp. was thought to be incapable of transferring genes to monocot plants, some success was obtained with asparagus (Hernalsteens et al., 1984; Demain, 1988). With the manipulation of the Ti-plasmids, foreign genes can be inserted and expressed into plant cells from which normal plants can be regenerated. Coding sequences of these foreign genes are put under control of the promoters that are functional in plants. These techniques can be used to transfer genes with desirable characteristics, from which normal plants can be regenerated, thus paving the way for more advances in plant biotechnology.

Transformation by Plant Viruses. Plant viruses have good potential as cloning vectors and provide a natural example of genetic engineering because viral infection of a cell results in the addition of new genetic material that is expressed in the host. In both microbial and animal cells, viruses have been used in vector development, so it is expected that plant viruses could be considered candidates for plant gene vectors. Additional genetic material incorporated into the genome of a plant virus might be replicated and expressed in the plant cell with the other viral genes (Gardner, 1983). There are a number of criteria that need to be satisfied to use the plant virus as a vector: (1) the virus must be able to spread from cell to cell via the plasmodesmata; (2) the modified viral genome should not elicit disease symptoms to the infected plants; (3) the virus should have a broad host range; and (4) it is preferable that the virus vector has a DNA genome such as the Caulimovirus group. This group of

viruses contain a circular double stranded DNA genome. A typical member is cauliflower mosaic virus (CaMV), which is widely distributed throughout the temperate regions of the world and is responsible for a number of economically important diseases of cultivated crops (Primrose, 1987). This virus multiplies in the nucleus, but its DNA is not integrated into the plant DNA. It results in a systemic infection of the plant. One disadvantage of its use as a vector is the fact that only small pieces of DNA, less than the size of an average gene, can be stably integrated into the viral genome (Nestor, 1983).

### Plant Protection by Biological Pest Control

Several strains of bacteria, fungi, protozoa and viruses are effective in controlling certain types of harmful insects. Appropriate strains must have no toxicity to man and plants and, at the same time, be effective against the intended target pest. Such strains might be useful to control insects if there is feasible technology for the systematic continuous mass production of these bioinsecticides. For the production of bioinsecticides, several methods can be applied: submerged fermentation for producing Bacillus thuringiensis and Bacillus moritai, and surface fermentation for the fungus Bacillus bassiana; the in vivo approach is used exclusively for producing insect viruses and protozoa. Before using any of the bioinsecticides, safety measures should be applied to test for their acute toxicity, irritation, teratogenicity, carcinogenicity, invertebrate specificity and phytotoxicity.

Extensive tests in the USA demonstrated that at least four entomopathogenic viruses (Heliothis, Orgyia, Lymantria, Autographa); two bacteria (B. popilliae, B. thuringiensis); two fungi (B. bassiana, H. thompsonii); and three protozoa (Nosema locustae, N. algerae, Mattesia trogodermae) are not toxic or pathogenic to man, other

animals, or plants. Sufficient testing was also conducted to demonstrate the safety of a fungal preparation (Boverin, B. bassiana) produced in the Soviet Union and a virus (Matsukemin, Dendrolimus CPV) and a bacterium (Rabirusu B. moritai) produced in Japan (Ignoffo and Anderson, 1979).

In Japan, agriculturally useful antibiotics have been widely used to protect plants against diseases and pests. Some examples are: (1) Cycloheximide (from Streptomyces griseus) as wettable powder against onion downy mildew and shoot blight of Japanese larch; (2) Kasugamycin (from Streptomyces kasugaensis) as dust against rice blast; (3) Polyoxins (produced by Streptomyces cacaoi var. asoensis) as dust, wettable powder and emulsion against rice sheath blight and fungal diseases of fruits and vegetables; (4) Validamycin A (from Streptomyces griseus) as wettable powder or liquid against bacterial diseases of fruits and vegetables; (5) Oxytetracycline (from Streptomyces viridifaciens) as wettable powder against citrus canker and peach bacterial leaf spots; and (6) Tetranactin as emulsion against carmine mite of fruits and tea. The primary site of action of these antibiotics relates to chitin synthesis of cell wall, cation leakage from mitochondria, biosynthesis of inositol and protein from DNA synthesis. There are limitations in the use of antibiotics in agriculture and difficulties in analysis because they are used in small amounts and there is a likelihood of development of plant pathogens resistant to antibiotics. Therefore, the use of chemicals and antibiotics in alternate years has been recommended to overcome such a possibility (Subnarao, 1985).

The concept of herbicides of microbial origin stems from the effort of plant pathologists to use exotic or endemic pathogens to kill weeds, e.g., for the first case, the use of Puccinia chondrillina from southern Europe to control skeleton weed (Chondrilla jancea) in Australia; and for the second, the use of Cercospora rodmanii to

control water hyacinth (Eichhornia crassipes) that chokes waterways in the USA (Subharao, 1985).

### **Basic Features Related to Biotechnology in Arab Countries**

The information available on biotechnological activities in the Arab countries is limited. Most of the information available is either old or incomplete and does not include all Arab countries. A limited survey was conducted by the Arab League Educational, Cultural and Scientific Organization (Al-Tamimi, 1987) in which only 13 institutions from seven Arab countries responded. The salient features of biotechnological activities, according to this survey, were as follows:

- ◉ Most biotechnological activities are limited and confined to classical microbiology, and plant breeding courses in universities and only few specialized biotechnology research centers have established tissue culture facilities.
- ◉ Most universities and research centers have a small culture collection unit for their own use, with no regional coordination.
- ◉ All manpower involved in the disciplines related to biotechnology at these institutions obtained their higher degrees from outside the Arab world, and few are specialized in biotechnology and genetic engineering; however, there are many local undergraduates in related fields such as microbiology and biochemistry. The trained technical staff involved in biotechnology are limited, and there is a need for manpower development planning.
- ◉ The space and equipment allocated for biotechnology are not satisfactory, and the budget is not adequate. As a result, advanced research work and expansion in biotechnology and genetic engineering is limited. Few institutions have allocated budgets for biotechnological activities.

- o Most research activities are confined to the country itself, and few are carried out with regional or international cooperation.
  - o Most institutions offer training courses, workshops and conferences in collaboration with international organizations.
  - o Interactions between institutions dealing with biotechnology and private industry are lacking.
  - o Most institutions included in the survey indicated that they had no role in formulating national policy and few countries have national plans for biotechnology and genetic engineering development.
  - o Priorities of research listed as:
    - Tissue culture for plant propagation.
    - Diagnosis for diseases in humans, plants and animals.
    - Improvement of agriculture and food industries using recombinant DNA technology.
  - o The recommendations of the institutions on the development of national and Arab policies for biotechnology development were as follows:
    - a. Formulation of a national committee on biotechnological development.
    - b. Development of a national plan for biotechnology and genetic engineering, taking into consideration the multidisciplinary aspect of the field.
    - c. Development of a mechanism for follow up and implementation.
    - d. Increase of financial support for biotechnological research and development.
    - e. Enhancement of regional and international cooperation.
-



- f. Formulation of national policies connected with integrated Arab collaboration.

### Plant Biotechnologies In Selected Arab Countries

Saudi Arabia. The main biotechnological activities in Saudi Arabia related to plant production are conducted at the Date Palm Research Center (DPRC) at King Faisal University in Hofuf. The center has conducted extensive and advanced research activities in date palm propagation and improvement via biotechnological techniques such as cell and tissue culture. Date palm was found to be capable of plant initiation from several tissues and organs and offered good potential for producing a large number of genetically uniform plants. Recently, the first date palm plant reached production stage and confirmed the genotype of the plant and obtained a true-to-type plant from a well-known variety. The center is considered one of the leading research centers in date palm micropropagation in the Arab world and the only one in Saudi Arabia (A. Al-Ghamdi, 1989, personal communication). Several reports dealing with date palm tissue culture appeared in the last five years and were published in national and international conferences and symposia proceedings (Abu El-Nil, 1986a,b,c; Abu El-Nil et al., 1986; Al-Ghamdi, 1987). Other tissue culture laboratories for date propagation are established at King Saud University in Riyadh. Another laboratory is under construction at the Ministry of Agriculture and Water.

In December 1987, a national seminar on genetic engineering and biotechnology was held in Riyadh, and was sponsored by King Abdul Aziz City for Science and Technology (KACST) and the United Nations Industrial Development Organization (UNIDO) in collaboration with King Saud University (KSU). The seminar recommended setting up of a national Biotechnology Advisory Group (BAG) with the participation of government

agencies, industry and universities, as well as the Arab Gulf University in Bahrain. The main task of the BAG would be to initiate and coordinate a biotechnology program, develop a biotechnology information base for Saudi Arabia and organize training programs/workshops on specific aspects of biotechnology while promoting interaction between various disciplines and the industry (UNIDO, 1987).

Egypt. Most biotechnological applications for plant production in Egypt utilize conventional plant-breeding techniques, and research activities are being carried out at research centers and at most universities. Most advanced plant biotechnologies are conducted by Egyptian scientists in collaborative work in the USA and Europe during short visits. Few research activities utilizing advanced plant biotechnologies are carried out inside Egypt due to the lack of equipment and the continuous supply of chemicals. Research activities in plant biotechnology are carried out at the cell research section of the Agriculture Research Center (ARC) in Giza, which is in the process of becoming the Biotechnology Center in the Ministry of Agriculture. The main objective of the Cell Research Section in the center is to transfer agronomically desirable traits such as tolerance to salt, drought and pests between species or genera by utilizing the embryo rescue and tissue culture techniques. Some progress has been achieved towards this objective, in spite of the limitation of equipment and continuous supply of chemicals. Callus initiation and regeneration was started in commercial varieties of wheat, barley, maize, sorghum, rice, alfalfa and vicia faba. Micropropagation was initiated in alfalfa and vicia faba to produce mutants that are difficult to obtain somatically or sexually. Future research plans at the Center include the use of protoplast fusion for interspecific or intergenetic crosses that will not be possible to achieve by conventional breeding methods. Other research plans include work on drought and

salinity resistance for wheat, rice and alfalfa using callus, cell suspension and protoplast culture (A. Al-Naggar, 1989, personal communication).

Research work at the Department of Horticulture at Zagazig University include using the tissue culture techniques for the micropropagation of ornamentals such as carnations and gladiolus (A. R. Awad, 1989, personal communication). The other research work conducted at the Botany Department at Zagazig University is in the field of cytogenetics in wheat, in which some important traits and protein fractions in wheat varieties were determined by electrophoresis techniques. The results obtained will be useful for breeding purposes and for molecular biological studies (A. A. Mahmoud, 1989, personal communication). Future work planned at the Horticulture Department is to propagate woody trees by tissue cultures, and develop salt and drought-tolerant and virus free plants. On the other hand, future projects planned by the Botany Department are the induction of haploid plants in wheat and barley from culturing the anthers' pollen grain. Mixing these haploids produced from divergent varieties by hybridization may lead to a new variety with good economic traits (hybrid vigour).

Research activities at the Horticulture Department at Assiut University are utilizing tissue culture techniques for hardening vegetable crops and selecting salt and/or drought tolerance in the callus culture in garlic followed by regeneration of the plantlets (Fahmy et al., 1984; N. M. Kandeel, personal communication, 1989). Research activities at the Department of Genetics at Assiut University are mainly concerned with genetic systems governing the variability in a number of plant attributes that contribute to the success of the plant under dry conditions. Selection for drought resistance in barley and wheat was conducted for developing new cultivars for moisture-deficient infertile sandy soils in Egypt (Omara, 1988; M. K. Omara, 1989, personal communication).

The Egyptian Academy of Science and Technology, signed an agreement with UNDP and UNIDO for establishing an affiliated laboratory to the International Center for Genetic Engineering and Biotechnology (ICGEB) in Trieste. At the National Research Center, a Division of Genetic Engineering and Biotechnology was established with six departments (Molecular Biology, Microbial Genetics, Human Genetics, Cell Biology, Microbial Biotechnology and Plant Tissue Culture). The Agriculture Research Center is formulating the plan of action on applications of genetic engineering for the development of agriculture (Hamdan and ElNawawy, 1988).

Morocco. The main activities that are of concern to the Maghreb countries, in general, and Morocco, in particular, are concerned with the micropropagation of the date palm tree taking into consideration the threat posed by the Bayoud disease caused by Fusarium oxysporium f. sp. albidenis. This problem could be solved by the selection of genetically resistant varieties with good quality fruits. Due to soil contamination, the in vitro culture was the only viable alternative to control this disease (Benbadis, 1988).

A proposed collaborative project on date palm grafting for controlling the Bayoud disease is planned by the Tissue Culture Laboratory in Agadir, Morocco, and Purdue University, USA, (M. Aaounine, 1989, personal communication). Other main activities of this lab. in the field of tissue culture technology are: clonal mass propagation, recovery of pathogen-free plants, variety improvement using haploid plants, somatic embryogenesis, callus and cell suspension cultures for selecting stress-tolerant genotypes.

The research work of interest conducted at the Hassan II Institute of Agronomy and Veterinary is on selection of drought tolerance in barley, H. vulgare, using

- conventional and tissue culture techniques such as: crosses between different genotypes, screening in vitro for drought tolerance, creation of haploids by crossing cultivars with H. bulbosum and haploid embryo rescue (B. Ouafae, 1989, personal communication).

The research activities at the Faculty of Sciences, Mohammed V University in Rabat are mainly concerned with cereal and vegetable crop improvement through plant biotechnology techniques. Wheat plants through pollen, anther cultures and somatic embryogenesis are produced. These activities are carried out in collaboration with French laboratories. One of the projects is financed by the Organization of Francophone Universities (AUPELF), the other project on wheat biotechnology is partially financed by the International Center for Agriculture Research in Dry Areas (ICARDA) (H. Chlyah, 1989, personal communication).

Collaboration between the three Maghreb countries (Morocco, Algiers and Tunis) resulted in formulation of a work group concerned with propagation of the date palm tree by in vitro techniques. Such action indicates the seriousness of the Maghreb countries' involvement in biotechnological disciplines (Benbadis, 1988).

**Other Arab Countries.** Information on research activities in plant biotechnologies in other Arab countries are either not available or do not exist. The existing research activities in most of these countries are mainly based on conventional plant breeding methods and classical agricultural practices.

In Kuwait, early agricultural research activities were pioneered by the General Authority for Agricultural Affairs and Fish Resources (AAFRA), particularly in the field of protected agriculture and hydroponics. Plans are underway for the establishment of a tissue culture laboratory at the Kuwait Institute for Scientific Research (KISR). A

national plan for biotechnology and genetic engineering is being prepared in collaboration with UNIDO. The plan includes a program for plant biotechnology to develop drought and salt-resistant plants through genetic engineering techniques and micropropagation for ornamental plants, legumes and date palm trees via tissue culture and other advanced biotechnological techniques (UNIDO, 1987).

In Jordan, the Higher Council of Science and Technology, is making plans for the establishment of a national council for biotechnology that will be responsible for all research and development in this field. For this purpose, two committees were formed, one for food and agriculture and the second for medical and pharmaceutical aspects (A. Al-Tamimi, 1988, personal communication). The research activities for plant production conducted in Jordan are mainly related to conventional plant breeding such as collection and evaluation of cereal genetic resources with an emphasis on collecting germplasm of barley and durum wheat for possible use in breeding for environmental stress tolerance (Jaradat et al., 1987). A collaborative research program on wild emmer, Triticum dicoccoides, wheat evaluation is planned with its main objective to assess genetic variability for morphological and agronomic traits in the wild emmer wheat collection in Jordan and assess the range of tolerance of the collection to drought stress (A. A. Jaradat, 1989, personal communication).

Tissue culture laboratories for date palm propagation were established in Iraq and Tunis. No information is available on the research accomplishments of these laboratories or the activities related to plant biotechnologies in the remaining Arab countries.

## Potentially Promising Plant Biotechnologies for the Arab Countries

### Natural Resources

The Arab countries are characterized by diversified environments, that include different plant varieties adapted to harsh environmental conditions. The plant cover is sparse and patchy, and consists mainly of shrubs and grass; the vegetation is dominated by species that have a discontinuous and suppressed leaf canopy as a mechanism to control transpiration. Ephemeral plants germinate, grow and complete their life cycles rapidly after the rains and remain as seed throughout the long intervening dry periods; perennial grass sprouts from rhizomes or bulbs during rains, and perennial shrubs and trees like Acacia species that have woody stems and leathery leaves are physiologically adapted to harsh environments. On the other hand, Mediterranean-type vegetation occurs along the coasts of Morocco, Algeria, Tunisia, Lebanon, Syria and the northern part of the Libyan coast. Excluding the hyper-arid Sahara zone, forests cover 16% of Morocco, 12.5% of Algeria, 9.5% of Tunisia and only 0.4% of Jordan. Land use in the Arab world includes mainly rangelands (270 million ha constituting 19% of the global area of the region), and arable land occupying 3.64% of the total area and used for rain-fed agriculture (40 million ha) and irrigated agriculture (10 million ha) (Younes, 1984).

In the Arab countries, the average number of date palms between 1971-76 was estimated at 62,000,000 trees, which represent 5% of the total arable land and of which Iraq accounted for 20.8% of the world total acreage followed by Morocco (14%), followed by Saudi Arabia, Algeria, Egypt, Libya, North and South Yemen, Tunis, Oman and Sudan (Mohamed, 1983). In 1982, the production of dates in the Near Eastern and

North African countries accounted for about 73% of the total world production (1.9 million t). Iraq and Saudi Arabia were leading, followed by Egypt, Algeria, North and South Yemen and Sudan (Sawaya, 1986).

Large quantities of citrus and olive trees are grown in large areas in most Arab countries located on the Mediterranean in North Africa and the Near East. These countries also produce various kinds of vegetables and cereals. In addition, large varieties of plants and shrubs that are adapted to desert conditions are found in the arid regions of these countries; however, there is a lack of information on their identification and taxonomy. Also, little work has been done on the flora in the region; although the flora of some countries have been documented, most of the work is not updated (Younes, 1984).

#### **Promising Plant Biotechnologies**

According to Sasson, the application of biotechnological techniques to plants has several advantages: production can be continuous without limitation by season or climate; the quality of the products are reliable and free from pathogens; and the recent developments in plant biotechnologies can contribute to crop improvement and protection of threatened species. The application time of these biotechnological technologies is classified into three categories: (1) short-term applications (three years)--in vitro vegetative propagation, pathogen-free plant production and germplasm storage and exchange; (2) medium-term application (3-8 years)--somaclonal and gametoclonal variation, embryo rescue, in vitro fertilization, anther cultures and haploid plant production; and (3) long-term applications (8-15 years)--somatic hybridization, wide-cross hybridization, mutant cell lines, chromosome transfer and gene transfer (Sasson, 1988).



Selected crop species playing an important role in the economy of the Arab countries will be used as examples to gain insight as to what might be expected from the application of current plant biotechnologies in future.

Date Palm. Date palm is considered one of the most important commercial crops in the Arab world (FAO, 1984). Although the date palm is long-lived, it produces relatively few offshoots suitable for transplanting in its lifetime. Vegetative micropropagation by tissue culture is therefore the most promising technique for multiplying the species and producing high quality, high yielding and resistant individuals to fungal diseases. Plantlets obtained from date palm tissue cultures by researchers in Morocco, Algeria and Tunisia prove that date palm cloning is feasible (Sasson, 1988). Also, researchers in the Date Palm Research Center in Saudi Arabia confirmed the genotype of a date palm and obtained a true-to-type plant of a well-known variety (A. Al-Ghamdi, 1989, personal communication). On the other hand, future research is still needed to cope with uncertainty with respect to early flowering and uniform plants. Ammar et al. (1987) reported that it is 7-8 years before a date palm bears fruit, a time period that would make date palm tissue culture plantlets a questionable proposition.

It is also important to create date palm clones resistant to Bayoud disease caused by Fusarium oxysporum f. sp. albedinis. This disease is threatening the 4.5 million date palm trees in Morocco (Sasson, 1988). In the date-producing Arab countries, that are first among the world's date producers, high quality dates are still imported although domestic production now exceeds consumption. In these countries, the application of tissue culture technology for high quality date varieties coupled with improved processing, packaging and marketing efforts will offer these countries an

excellent opportunity to become a world leader in the international trade of dates and date products.

Citrus. The Mediterranean Arab countries in North Africa and the Near East are well-placed geographically to act as a supply center to Europe of high quality citrus products. The citrus trees in these countries are suffering from virus attacks such as Psorosis, Tristeza and Exocortis. Somaclonal variation and tissue culture methods could play an important role in producing virus-free plants using meristem culture and the use of virus indexing to detect early virus attacks (Mardi, 1985).

Olives, Almonds and Pistachios. These plants are an important crop in some Arab countries; however, the propagation of high quality varieties is a slow and limited process. Therefore, using tissue culture techniques should be considered to improve these crops' quality and propagation.

Potatoes. Arab countries import large quantities of expensive potato tubers that account for 30-60% of pre-harvest costs. This compares with 5% for corn and 10% for wheat. Planting true potato seed as opposed to tubers could be more than half the pre-harvest cost. In addition, true potato seeds are virtually free of disease, and it takes only five ounces of coated seed to plant one acre compared with one ton for tubers, causing enormous savings in transport and storage. Using meristem tip culture to eliminate viruses is now an established procedure for potatoes, and micropropagation is now used extensively to multiply the virus-tested stock for further multiplication in insect-proof greenhouses and then in the field in which the insect population can be controlled to slow down the disease reinfection rate. Virus-indexed tissue cultured plants can be outplanted to the field (Wattimena et al., 1983) so that in

Arab countries, where the disease reinfection rate is high, importing expensive potato tubers could be eliminated. However, the cost of the micropropagated tubers from insect-proof greenhouses is still too expensive to be cost-effective; therefore, more development on automation is needed to cut costs since labour accounts for much of the cost (Aynsley, 1987).

Cereal Crops. Developing cereal crop varieties that can give good yields under severe climatic conditions in the Arab world is of primary concern. Recent work in Europe on the genetic manipulation of cereals has shown it possible to engineer these species (including wheat and barley) and so introduce desirable traits such as drought and disease resistance. Studies in a number of institutions have shown it possible to regenerate wheat and barley plants from cell cultures (Fowler, 1987); although in its infancy, this may provide a longer term and more rapid method of assessing new cereal varieties and getting them to the field.

Other Crops. Other important crops in the Arab world include legumes; on which the effect of plant biotechnology could be more immediate than for cereal crops. Research on the development and tissue culture of legumes has been low compared with other species. Other developments with different crops in the Arab world that should be given consideration for exploitation by tissue culture and other related techniques to meet rising demand are: peas, beans, tomatoes, onions, garlic, strawberries, cotton and ornamental plants. Automated techniques of plantlet production and fluid planting could make considerable input into commercial viability and cost/benefit ratios in the long term in these areas. Detailed consideration needs to be given to ways of harvesting this technology in the Arab world together with assessing potentials for developing other crops.

### Constraints

There are several constraints facing the development of biotechnological applications in most Arab countries that are similar to those experienced by other developing countries. These constraints are mainly related to setting priorities and strategies, manpower availability and economic infrastructure and development. The nature of biotechnology as a multidisciplinary field adds to these constraints because it requires integrating several disciplines of life sciences, which necessitates a special training program. These constraints include the following:

- o Absence of research and development priorities, strategic plans and policies in the area of biotechnology.
- o Lack of appreciation by decision makers of the importance of biotechnological applications to plant production and protection.
- o Inadequate funds allocated for research and development, in general, and biotechnology, in particular.
- o Low priority for long-term research and development plans for biotechnology.
- o Lack of clear national plans and regional cooperation plans for developing biotechnology.
- o Lack of undergraduate courses in biotechnology at most universities.
- o Brain drain of competent experienced scientists to the West due to employment problems related to economic infrastructure and development.
- o Shortage of competent experienced local technical staff in the field of biotechnology.
- o High turn-over rate of expatriate scientists resulting in disruption of research progress.

- Bureaucratic purchase and customs procedures disrupt the regular and continuous supply of perishable high-grade chemicals and reagents.
- Frequent breakdown in power supply causing disruption in laboratory fermenters and growth chambers that are operated continuously.
- Lack of adequate maintenance system for equipment and repair.
- Inadequate library facilities, making it difficult to keep up with latest developments.
- General lack of infrastructure for scientific research.

### **FAO Experience in Regional Projects and Policy Towards Biotechnology**

#### **Regional Projects**

There are currently 40 FAO regional projects with research components: 16 research and development projects in Africa, nine in Asia and the Pacific and 15 regional research projects in the Near East (FAO, 1988). The projects in the Near East include the following:

- Development and strengthening of agricultural research in the Arab countries (under formulation).
- Near East applied research for land and water use.
- Palm and dates center.
- Near East regional animal production and health.
- Near East regional cooperative research and development network on small ruminants.

**FAO Policy Towards the New Biotechnologies (with special reference to developing countries)**

Biotechnology in food and agriculture presents both opportunities and challenges. FAO is therefore formulating a comprehensive policy for its assistance on biotechnology. The following policy recommendations by the Food and Agriculture Organization are based on its statutory missions and recognition of the characteristics of biotechnology as an emerging technology (B. Muller-Haye, 1989, personal communication; Buttel, 1988):

- o Carry out a realistic assessment of the potential and limitations of this technology, which is essentially a tool or a means and not a goal in itself. Moreover, the short-term benefits of biotechnology must not be overstated. Many proponents of biotechnology have exaggerated the speed with which major improvements in animal and plant agriculture will be achieved.
- o Promote multiple applications of biotechnology since there should be synergy between agricultural and non-agricultural biotechnological research and development in national biotechnologies. The latter are now contributing to a wide range of activities.
- o Advise on research priorities directed towards those technologies that are of most benefit to developing countries. The emphasis should be on capital intensive approaches, traditional food crops and the problems of marginal zones.
- o Assist member countries to substantially upgrade the skills of their scientific communities and to acquire scientific equipment, a necessary condition for member countries to increase their capacity to utilize the new biotechnologies.
- o Develop and promote strategies to enable the best endowed countries to make the most of their research capacity and to enable the least endowed to expand their capacity to make use of research done elsewhere.

- Develop strategies to maximize the degree to which private sector research results can be made available to developing country-oriented biotechnological researchers.
- Monitor and assess the socio-economic impacts of new biotechnologies applied to both developed and developing worlds.
- Assess the impact on existing markets of new agricultural or food products developed through or based on new biotechnological techniques and evaluate the implications for the nutritional adequacy, safety and consumer acceptance of food supplies.
- Develop guidelines for member countries to avoid major environmental costs arising from the application of new technologies applied to agriculture.

### Conclusions

The various biotechnological techniques briefly reviewed in this paper need to be assessed and prioritized for their potential application in the near East and North African countries. The highlights of the current status of plant biotechnology in these countries indicate that biotechnological applications in most countries are still limited to traditional and classical plant-breeding and production methods. A few countries have established tissue culture laboratories for the propagation of some crops, particularly date palm trees. Other countries are still in the process of preparing for the establishment of a plant biotechnology infrastructure. In spite of the constraints facing most Arab countries, the research activities initiated so far represent an important starting point for the establishment of the infrastructure for biotechnology, and will have a long-term impact on the commercial development of agriculture.

The integration of the natural resources and expertise between Arab countries and the adoption of a long-term strategic plan utilizing recent developments in plant

biotechnology through regional and international collaboration will help to increase commercial development of agriculture. Due consideration should be given to establish a strong foundation for research and development in the area of biotechnology in the long term, with access to the appropriate skills and expertise based in developed countries, through interactive links at both academic and industrial levels. These plans and policies should aim at adapting local know-how and national research capabilities to local biotechnological development problems. They should also take into consideration the development of local competent trained personnel to be able to participate in future commercial biotechnological ventures in the region.

---

\* This paper was prepared by Ibrahim Y. Hamdan for the Research and Technology Development Division, Food and Agriculture Organization of the United Nations, Rome.



## References

- Abu-El Nil, M. M. 1986a. Refinery methods for date palm micropropagation. Proceedings, 2nd Symposium on Date Palm, Al-Hasa, Saudi Arabia.
- Abu-El Nil, M. M. 1986b. The effluent of amino acid nitrogen on growth of date palm cultures. Proceedings, 2nd Symposium on Date Palm, Al-Hasa, Saudi Arabia.
- Abu-El Nil, M. M. 1986c. Effect of different auxins on cultures induction and growth of date palm in vitro: a structure and future study. Proceedings, 2nd Symposium on Date Palm, Al-Hasa, Saudi Arabia.
- Abu-El Nil, M. M.; A. S. Al-Ghamdi and A. Turjoman. 1986. Role of tissue culture in propagation and genetic free improvement of date palm: Perspective review. Proceedings, 2nd Symposium on Date Palm, Al-Hasa, Saudi Arabia.
- Al-Ghamdi, A. S. 1987. Cell and tissue culture technique as a means for vegetative propagation and genetic improvement of date palm (Phoenix dactylifera L.). Proceedings, National Seminar on Genetic Engineering and Biotechnology, Riyadh, Saudi Arabia, Article No. 5.
- Al-Tamimi, A. 1987. Arabic Strategic Plans for Biotechnology. Tunis: ALECSO, Arab League, pp. 13-17.
- Ammar, S., A. Benbadis and B. K. Tripathi. 1987. Floral induction in date palm seedlings. (Phoenix dactylifera Van Deglet Nour). Cultural in vitro 65(1):137-142.
- Aynsley, A. J. 1987. The role of biotechnology and genetic engineering in Saudi Arabian development. Proceedings, National Seminar on Genetic Engineering and Biotechnology, Riyadh, Saudi Arabia, Article No. 6.

- Barton, K. A. and W. Brill. 1983. Prospects in plant genetic engineering. Science 219:671-676.
- Benbadis, A. 1988. Present status of plant biotechnologies in Magreb country. In 8th International Biot. Symposium. Edited by G. Durand, L. Bobichon and J. Florent. 11:1354-1361.
- Brill, E. J. 1984. Safety concerns and genetic engineering in agriculture. Science. 227:381-384.
- Brisson, N.; J. Paszkowski; J. R. Penswick; B. Cronenborn; I. Potrykus; and T. Hohn. 1984. Expression of bacterial gene in plants by using a viral vector. Nature 310:511-514.
- Buchanon-Wollaston, V., J. Passiatore and E. Cannon. 1987. Expanding Agrobacterium host range. Nature 328:172.
- Buttel, F. H. 1988. Policy towards the new biotechnologies and developing world: Strategies and prospects. FAO working paper, Food and Agriculture Organization of the United Nations, Rome.
- Cocking, E. C.; M. R. Davey; D. Pental; and J. B. Power. 1981. Aspects of plant genetic manipulation. Nature 293:265-270.
- Comai, L., L. C. Sen and D. M. Stalker. 1983. An altered *aroA* gene product confers resistance to the herbicide glyphosphate. Science 221:370-371.
- Comai, L., and D. M. Stalker. 1986. Mechanisms of herbicide resistance and their manipulation by molecular biology. Plant Molecular and Cell Biology 3:456.
- Demain, A. L. 1988. Genetics and Applied Microbiology: The Future is Now. Recent Advances in Biotechnology and Applied Biology. VII GIAM Proceedings. Edited by S. T. Chang, K. Chan and N. Woo. Hong Kong: Chinese University Press, pp. 3-10.

- Depicker, A., M. Van Montagu and J. Schell. 1983. Plant cell transformation by Agrobacterium plasmids. In Genetic Engineering of Plants: An Agriculture Perspective. Edited by Alexander Hollaender. New York: Plenum Press, pp:143-176.
- Fahmy, F. G., R. F. Abdo and N. M. Kandeel. 1984. Production of potato virus-free plants by means of callus method. Assuit Journal of Agric. Sci. 15:263-274.
- FAO. 1984. FAO Production Yearbook. Food and Agriculture Organization of the United Nations, Rome.
- FAO, 1988. Collaboration agricultural research networks for the GCC countries. Presented at the Agricultural Research Network for the GCC Countries Symposium. Kuwait, Oct. 17-19.
- Fowler, M. W. 1986. Biotechnological applications of plant tissue cultures. In Perspective in Biotechnology and Applied Microbiology. Edited by D. Alani and M. Moo-Young. London: Elsevier Applied Science Publishers, pp. 295-315.
- Fowler, M. W. 1987. A strategic assessment of plant cell and tissue biotechnology potentials in Saudi Arabia. Proceedings, National Seminar on Genetic Engineering and Biotechnology, Riyadh, Saudi Arabia, Article No. 4.
- Gardner, R. C. 1983. Plant viral vectors:CaMV as an experienced tool. In Genetic Engineering of Plants: An Agriculture Perspective. Edited by Alexander Hollaender. New York: Plenum Press, pp. 121-142.
- Hamdan, I. Y. and A. El-Nawawy. 1988. Current status of biotechnology in Asian Arab countries. In Proceedings of the 8th International Biotechnology Symposium. Edited by G. Durand, L. Bobichon and J. Florent. Paris: French Microbiology Society, pp. 1362-1376.
- Hernalsteens, J. P.; L. Thia-Toong; J. Schell; and M. Van Montagn. 1984. An Agrobacterium - transformed cell culture from the monocot Asparagus officinalis. EMBO Journal 3:3039-3941.

Hooykaas, Van Slogteren, G. M. S., P. J. J. Hooykaas and R. A. Schilperoort. 1984.

Expression of Ti plasmid genes in monocotyledon plants infected with Agrobacterium tumefaciens. Nature 311:763-764.

Ignoffo, C. M., and R. F. Anderson. 1979. Bioinsecticides. In Microbial Technology.

Edited by H. J. Peppler and D. Perlman. New York: Academic Press. Inc., pp. 1-28.

Jaradat, A. A., S. Jana and L. N. Pietrzak. 1987. Collection and evaluation of cereal genetic resources of Turkey and Jordan. RACHIS 6(1):12-14.

Jones, L. H. 1983. The oil palm and its clonal propagation by tissue culture. Biologist 30:181-188.

Klier, A.; F. Fargette; J. Ribier; and G. Rapaport. 1982. Cloning and expression of the crystal protein genes from Bacillus thuringiensis strain Berliner 1715. EMBO Journal 1:791-799.

Kudrika, D. J. 1986. Interactions of Agrobacterium tumefaciens with soyabean (glycinema) leave explants in tissue culture. Canadian Journal Genetics Cytol. 28:808.

Lamprey, J., and M. Moo-Young. 1988. Biotechnology: principles and options for developing countries. In Microbial Technology in the Developing World. Edited by E. J. Dasilva, Y. R. Dommergues, E. J. Nyns and C. Ratledge. Oxford: Oxford University Press, pp. 335-375.

Mardi, M. O. 1985. Plant tissue culture and its potential use. Arab Journal of Science 7:40-45 (In Arabic).

Mohammad, A. R. 1983. The economics of date palms in the Arab world. Proceedings, First Symposium on the Date Palm. Al-Hassa, Saudi Arabia, pp. 716-724.

Nester, E. W. 1983. Vectors. In Genetic Engineering of Plants: An Agriculture Perspective. Edited by Alexander Hollaender. New York: Plenum Press, pp. 119.

- Omara, M. K. 1988. A genetic analysis of earliness in wheat and the response to the selection for flowering time under favourable clay soil and moisture stress conditions in sandy soils. Assiut Journal of Agric. Sci. 19(5):15-48.
- OTA. 1984. Commercial Biotechnology: An International Analysis. Washington, D.C.: Office of Technology Assessment.
- Plunkett, G.; D. Seneear; F. Zuroske; and C. A. Ryan. 1982. Proteinase inhibitors I & II of wounded tomato plants: Purification and properties. Arch. Biochem. Biophys. 213:463-472.
- Primrose, S. B. 1987. Modern Biotechnology. London: Blackwell Scientific Publications, 176 p.
- Sasson, A. 1984. Biotechnologies: Challenges and promises. UNESCO, Sextant Series 2, Paris, 315 p.
- Sasson, A. 1988. Biotechnologies and development. Technical Center for Agricultural and Rural Cooperation (CTA), UNESCO, Paris, 361 p.
- Sawaya, W. 1986. Dates of Saudi Arabia. Riyadh: Safar Press, 200 p.
- Subharao, N. S. 1985. The role of biotechnology in agriculture and forestry. In Biotechnology and Bioprocess Engineering. Edited by T. K. Ghose, New Delhi: United India Press, pp. 511-514.
- UNIDO, 1987. Genetic engineering and biotechnology monitor. UNIDO Publication Issue 20, pp. 1-2, Rome.
- Wattimena, G., B. McCown and G. Weis. 1983. Competitive field performance of potatoes from microculture. Am. Potato J. 60:27-33.
- Younes, T. 1984. The state of biology in the Arab states. Biology International 7:7-8.

