UNDP/ICARDA/France

Workshop
on

Biotechnologies for the Improvement of Cereal and Legume Crops in West Asia and North Africa: Present Status and Future perspectives

April 7-10, 1991
Aleppo - Syria

UNDP/ICARDA/France
Contents

Program

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Biotechnologies for the Improvement of Cereal and Legume Crops in West Asia and North Africa: Present Status and Future Perspectives

April 7-10, 1991
Aleppo - Syria

Sunday, April 7, 1991

	Arrival
12.00 noon	Slide show
12.30	Lunch
13.30-14.30	Registration
14.30-15.30	Transportation to Hotel
15.30-17.30	Tour of Aleppo

Monday, April 8, 1991

09.00-09.30	Registration
09.30-09.45	Inaugural address **Dr. Nasrat Fadda**, Director General, ICARDA
09.45-10.00	Introduction to ICARDA, **Dr. Aart van Schoonhoven**, Deputy Director General-Research, ICARDA
10.00-10.15	Workshop overview, **K. Makkouk**, ICARDA
10.15-10.45	Opening address State of the art in agricultural biotechnology **Dr. Alain Deshayes**, INRA, Paris, France

10.45-11.00	**Coffee break**
	Session One
	GENERAL TOPICS
	Chairman **Dr. Alain Deshayes**
11.00-11.40	Lessons learned from biotechnology research and networking of rice. **Sir Ralph Riley**, Cambridge, United Kingdom
11.40-12.15	Status of biotechnology in the Arab countries: general review. **Dr. Ibrahim Y. Hamdan**, Kuwait Institute for Scientific Research, Kuwait
12.15-14.00	**Lunch**
	Session Two
	APPLICATIONS OF PLANT CELL AND TISSUE CULTURE FOR CROP IMPROVEMENT
	Chairman: **Dr. M.C. Saxena**
14.00-14.40	Haploid breeding for the improvement of ICARDA-mandated crops. **Dr. E. Picard**, CNRS-INRA - Universite Paris Sud, Gif sur Yvette, France
14.40-15.20	Wide-crossing in cereals and legumes. **Dr. George Fedak**, Ottawa Research Station, Agriculture Canada, Ottawa, Canada
15.20-15.40	**Coffee break**
	Session Three
	GENETIC TRANSFORMATION AND CROP IMPROVEMENT
	Chairman: **Sir Ralph Riley**
15.40-16.20	Potential of plant transformation in crop improvement. **Dr. Horst Lorz**, Institut fur Allgemeine Botanik, Universitat Hamburg, Germany

16.20-17.00	General discussion
17.00	Transportation to Aleppo
19.00-21.00	Reception

Tuesday, April 9, 1991

08.30	Transportation to Tel Hadya

Session Four

MOLECULAR TECHNOLOGIES FOR CROP IMPROVEMENT

Chairman: **Dr. Horst Lorz**

09.00-09.40	Recent advances in biotechnology-based diagnostics for the detection of plant pathogens. **Dr. Yves Bertheau**, France
09.40-10.20	Microbes and agriculture. **Dr. Guy Riba**, INRA, Station de Lutte Biologique, Guyancourt, France
10.20-10.40	**Coffee break**
10.40-11.20	Plant biotechnologies in developing countries: The plant breeder's perspective. **Sir Ralph Riley**, Cambridge, United Kingdom
11.20-12.00	General discussion
12.00-13.30	**Lunch**

Session Five

DIRECTIONS OF BIOTECHNOLOGY RESEARCH AT ICARDA

Chairman: **Dr. Aart van Schoonhoven**

13.30-13.50	Biotechnology for rhizobia: Present and future trends. **Drs. D. Beck** and **L. Materon**, ICARDA (Two separate presentations)
13.50-14.18	Development of biotechnology for cereal improvement at ICARDA. **Dr. P. Lashermes**, ICARDA
14.10-14.30	RFLP- fingerprinting in legume improvement. **Dr. F. Weigand**, Legumes Program, ICARDA
14.30-14.50	Production of ELISA kits for the detection of cereal and legume viruses. **Dr. K. Makkouk**, ICARDA
14.50-15.10	**Coffee break**

Session Six

BIOTECHNOLOGY RESEARCH IN COUNTRIES OF WEST ASIA AND NORTH AFRICA

Chairman: **Dr. Ibrahim Hamdan**

15.10-15.25	Present status and future prospects of biotechnology in Turkey. **Dr. Dogan Sakar**, Southeastern Anatolia Agricultural Institute, Diyarbakir, Turkey
15.25-15.40	Somaclonal variation for some agronomic characters in wheat (Triticum aestivum L.) **Akbar Shah Mohamed**, PARC, Pakistan
15.40-15.55	Nuclear x cytoplasm interactions controlling anther culture response in wheat. **Hasan Ekiz, B.D.** International Winter Cereals Research Center, Konya, Turkey
15.55-16.10	Gene mapping in lentil with random single seed descent derived lines. **Dr. M. Tahir**, Pakistan

16.10-16.25	Selection of Ascochyta resistance in chickpea using phytotoxic compounds. **Dr. Moncef Harrabi**, INAT, Tunisia
16.25-16.40	Inheritance of isozyme variation in Ascochyta blight resistant germplasm of chickpea (Cicer arietinum L.). **Mr. Ismail Kusmenoglu**, Ankara, Turkey
16.40	Transportation to Aleppo

Wednesday, April 10, 1991

08.30	Transportation to Tel Hadya
	Session 7
	BIOTECHNOLOGY RESEARCH IN COUNTRIES OF WEST ASIA AND NORTH AFRICA (contd.)
	Chairman: Dr. George Fedak
09.00-09.15	Tissue culture activities for the improvement of cereal and legume crops in Egypt. **Dr. Galal S. Youssef**, ARC, Egypt
09.15-09.30	Biotechnology in Jordan: Status and perspectives. **Dr. Hassan Abu Qaoud**, University of Jordan, Amman, Jordan
09.30-09.45	Heat shock protein and thermotolerance in wheat. **Mr. Said El-Madidi**, Morocco
09.45-10.00	Identification of inoculant strains of rhizobia in legume nodules. **Dr. Hassan Moawad**, NRC, Dokki, Cairo, Egypt
10.00-10.30	**Coffee break**
10.30-12.30	Working Groups (Simultaneous sessions)
	Working Group 1 Tissue culture and transformation techniques for crop improvement. Rapporteur: **Dr. P. Lashermes**

	Working Group 2 Molecular technologies for crop improvement Rapporteur: **Dr. F. Weigand**
	Working Group 3 Applications of biotechnology in agriculture microbiology. Rapporteur: **Dr. L. Materon**
12.30-14.00	**Lunch**
14.00-16.00	Round table discussion Moderator: **Dr. Alain Deshayes** Development of Biotechnology capacity in National Systems of the WANA region: facilities, training, networking and potential sources of funding
16.00-16.30	Closing remarks **Dr. Aart van Schoonhoven**

April 3, 1991
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Summaries

Workshop Overview

I would like to welcome every one here and to extend my thanks to the biotech steering committee and the biotech group at ICARDA who have helped develop, guide and implement this workshop. I also would like to thank all the speakers, and participants who accepted our invitation with a relatively short notice. Thanks also to the fact that the Gulf crisis is over, which led to the normalization of life including travel and permitted all of you to join us in this activity.

At ICARDA we look to "biotechnology" as a discipline which encompasses an array of techniques providing new abilities to manipulate germplasm. We are interested in intentional integration of biotechnology with conventional crop improvement programs, as opposed to this integration occurring on its own, or not occurring at all. Such integration will increase opportunities to maximize efficiency of using available funding, personnel and facilities.

Two or three years ago, ICARDA started vigorously to approach sources for funding biotech research, and succeeded to have support from Japan and France. More recently ICARDA was awarded a grant from UNDP/France to improve biotech capabilities at ICARDA and extend it to the National programs of the region. One of the first outcomes of this grant is this consultancy workshop.

The main two objectives we had in mind when we thought of this workshop are:

* To provide a forum to discuss the latest applications of biotechnology for legume and cereal improvement and use this forum to define areas of collaborative research.
* To develop a network of research scientists working on the new technologies in West Asia and North Africa on cereal and legume crops and encourage creating a linkage among them through collaborative research.

During the three days deliberations we would like to make use of this gathered expertise to identify research objectives that completely integrate cellular and molecular technologies with conventional crop improvement. The technologies which we have identified have been grouped into three sessions including:

- Applications of plant cell and tissue culture for crop improvement.
- Genetic transformation and crop improvement
- Molecular technologies for crop improvement.

Those sessions will be proceeded by an introductory session to discuss
- Status of biotechnology in the Arab Countries
- Lessons learned from the biotechnology research and networking of rice.

We selected rice, because quite a bit of effort has been dedicated to this crop and a number of donor agencies, International Institutes and advanced laboratories were all involved in this activity.
Sir Ralph Riley kindly agreed, with a short notice, to present to you this case.

The program also includes a session to present to the group the on-going research in biotech at ICARDA followed by a session to learn some of the biotech research which is in progress at selected countries of the WANA region.

In the morning of the third day participants will be divided into working groups based on their interests.

Working Group 1. Tissue culture and transforation techniques for crop improvement.
Working Group 2. Molecular technologies for crop improvement.
Working Group 3. Applications of biotechnology in agriculture microbiology

There are sheets at the registration desk for the three working groups. Please sign-up for the working group that you would like to join.

The working groups are intended to encourage discussions per defined area of research in order to identify, explore and develop new linkages for collaborative research and also to define priorities and constraints. This is the participants opportunity to enhance the meeting's value by presenting unique perspectives not able to be included in the formal program.

The workshop program is ended with a round table discussion on "development of biotechnology capacity in National Systems of the WANA region: facilities, training, net-working and potential sources of funding. We look to this round table discussion which is the last session of this workshop as a very special opportunity that distills your discussions and summarizes your insights and recommendations. Originally we asked Dr. Max Rives to moderate this round table discussion, but Dr. Rives had apologized recently because he was unable to travel. Dr. Alain Deshayes kindly accepted to replace Dr. Rives as the moderator for this round table discussion.

We did not intend a proceedings for this workshop. However, inorder to document the highlights of what will be presented, I would like to ask the help of all those who will give a presentation to hand on to me or to the registration desk a one page summary of their presentation. This would help us to produce a concise report about the workshop which we will distribute later to all participants and other concerned individuals or institutions.

K. Makkouk
Biotechnology Coordinator

STATE OF THE ART IN AGRICULTURAL BIOTECHNOLOGY.

Alain Deshayes, INRA, Paris, FRANCE.

Biotechnologies are concerned with the use of potentialities of tissues and cells in artificial conditions and the use of molecular techniques to have a better understanding of genome structure and eventually to modify the genome. Therefore Biotechnologies are not a "Science" but only a set of additional techniques that can be applied in practice.

Three categories of technologies can be considered: (i) in vitro culture, (ii) diagnostics and molecular markers, and (iii) gene cloning and genetic engineering.

Some in vitro techniques have been developed several decades ago. Micropropagation for example was found to be a very efficient and cheap tool to multiply clones and hybrids of agronomic interest. Others, like cultures of immature embryo and embryo rescue, allow to have several generations per year and to obtain inter-specific hybrids which could not develop naturally. Production of somatic embryos is used in some species, like trees, to multiply a genotype. The haploid technique is very useful for breeders to analyze genetic variability coming out from crosses between two parents and to obtain homozygous lines more quickly than by selfing. Protoplasts are very convenient biological material for some physiological and genetic studies. Protoplosts can be mutagenized, fused and engineered by genetic engineering.

The second set of techniques are those used for the diagnosis of plant pathogens. Diagnostics have mostly been based on polyclonol antibodies with high specificity and reliability. Their availability will accelerate the process of breeding for resistance to many pathogens. The recently developed PCR technique offer also a very powerful tool for pathogen detection.

One of the major problems which faces breeders is to find markers closely linked to characters of agronomic importance. The use of RFLPs has allowed genetic maps with hundreds of markers to be created. Correlation of these markers with traits of interest will make selection and breeding procedures easier, more precise and therefore more effective. Traits of interest can be monogenic but also multigenic. RFLP techniques allows us to localize on the chromosome quantitative trait loci (QTL). Several QTL have already been mapped in tomato for pH, weight and sugar content. Furthermore, technology is advancing very quickly and it would be easier in the near future to use it routinely.

The use of recombinant DNA techniques which emerged almost twenty years ago offer powerful possibilities to isolate genes and to analyze their molecular structure. Because of genetic engineering techniques, gene regulation can be studied in a new and efficient way. Genetic engineering is also a very elegant way to by-pass sexual barriers. Theoretically, any gene can be expressed in any organism provided the coding sequence is put under the control of adequate regulating sequences. Genetic engineering offers new possibilities for plant breeding by introducing genes of agronomic interest into the plant genome.

Transgenic plants with genes confering resistance to herbicides and to pests, such as insects, viruses or fungi, have already been field tested with success. Chimeric genes have also been constructed to confer male sterility or to modify product quality like protein or oil content. Many other projects of interest are under investigation in many laboratories.

Progress is still required to make all crops accessible to those technologies. ICARDA's mandated crops such as Wheat, barley, lentil and chickpea are at present either not easily, or not accessible at all to in vitro culture or DNA techniques. But with more work one can be confident that progress will be made in the near future.

It is frequently said that biotechnologies can solve all problems of our societies for the benefit of human health, for agriculture and for agro industry. People who are not doing biotechnologies do not feel involved in the game. Biotechnologies are indeed a big challenge for the coming few decades, but they are nothing if the biological material is not well defined and well handled. They are only tools to reach goals in different and new ways or to accelerate the breeding process. It is of major importance that physiologists, geneticists, breeders and pathologists be associated with the biotechnology work, otherwise some disappointment could occur with time.

RICE BIOTECHNOLOGY: PROGRESS AND PROSPECTS. Gary. H. Toenniessen, Rockefeller Foundation, New York, U.S.A. and Ralph Riley, Cambridge, UNITED KINGDOM

Considerable progress has been made in the development of cellular biology and molecular genetic techniques that can be applied to the genetic improvement of rice. Tissue culture techniques such as anther culture, embryo rescue, and use of somaclonal variants have contributed to the release of new rice varieties. These technologies are of proven benefit to rice breeding and through research are becoming applicable to a broader range of rice cultivars and breeding objectives. Most molecular genetic techniques are still at an early stage of development but progress has been more rapid with rice than any other cereal. Species specific probes and genetic maps of rice have been produced and increasingly are being applied in breeding. Further development of these tools into a map-based system for cloning rice genes is underway. Regeneration of fertile plants from protoplasts has been achieved for both japonica and indica rice. This is the basis of rice genetic transformation systems that now exist in several laboratories. Transgenic rice plants containing alien marker genes are scheduled to be field tested this year. Several experiments are underway to introduce potentially useful cloned genes into rice. Numerous other research projects are aimed at identifying, constructing and cloning a wide variety of genes from plants, microbes and animals which might

install useful traits if introduced into the rice genome. This paper reviews the progress to date and describes several promising research projects.

STATUS OF BIOTECHNOLOGY IN THE ARAB COUNTRIES: GENERAL REVIEW. Ibrahim Y. Hamdan, Kuwait Institute for Scientific Research, KUWAIT.

Most Arab countries are located in arid and Semi-arid zones that are characterized by severe weather conditions, lack of fresh water and soil erosion. As a result conventional agriculture for production of food and feed is strictly limited and below consumption levels.

Recent advances in biotechnology offer good prospects to Arab countries. These prospects include innovative biotechnological techniques for (a) the production of food and feed by bioconversion systems; (b) biological treatment and utilization of wastes, (c) biological nitrogen fixation for soil fertility, (d) pollution control for oil spills and (e) tissue culture for improvement and micropropagation of plants.

The available information from Arab countries indicates that biotechnology applications are limited to traditional and classical methods for agriculture production. Some countries have established tissue culture laboratories for the propagation of some crops, particulary date palms, whereas others are still preparing to establish a plant biotechnology infrastructure. Crop species that play an important economic role in the Arab countries and expected improvement from the application of modern biotechnological techniques is likely, include: date palm, cereal and legume crops, potatoes, citrus, olives, almonds and pistachios.

Several constraints face the development of biotechnological applications in most Arab countries, similar to those experienced in other developing countries. These constraints mainly relate to priorities and strategies, man power availability and economic infrastructure and development. The nature of biotechnology as a multidisciplinary adds to these constraints, since it requires integrating several disciplines of life sciences which necessitates a special training program. Inspite of these constraints, the research and development initiated so far represents an important starting point, and will have a long-term impact on the commercial development of agriculture.

DOUBLED HAPLOID METHOD FOR BREEDING ICARDA MANDATED CROPS. E. Picard, Laboratoire Biotechnologie du Blé-Université Paris sud - Ferme du Moulon 91190, Gif sur Yvette, FRANCE.

ICARDA mandated crops are barley, wheat (T. aestivum and T. durum), lentil, Kabuli chickpea, faba bean and other forage crops. Apart from barley and wheat, the available amount of published work on the doubled haploid (DH) method for the other crops is very rare. Therefore, the communication is mainly concerned with cereals. In this crop, the principal objective of ICARDA researchers is to ensure a sustainable increase of productivity in the harsh, stressful and variable environment of the rainfed agricultural system of the WANA region (Annual Rep. ICARDA 1989). Thus we have to introduce DH methodology within the framework of the plant breeding program of ICARDA and develop, the principal impacts of this new system of selling. In fact, the breeding process is a complex one and additional problems came from the stresses which are present in the region such as drought, heat and salinity. The main steps in a breeding program are: 1) Evaluations and introduction of Genetic variability 2) Combination of lines chosen in relation with the objectives; single crosses or more complex populations can be developed
3) Extraction of recombinant lines by selling and 4) Testing the derived lines to select the best ones for the users.

A haploid plant is a plant developed from male or female gametic cells or from abnormalities of zygotic fusions. When chromosomes are doubled by colchicine treatment for example and fertility restored, a completely homozygous line is obtained. Therefore the first advantage of the DH method compared to other plant breeding methods is to save time in producing pure lines. But in comparison with the pedigree method or pedigree derived methods the biotechnologist has to bring other arguments defending the DH method to make it attractive for plant breeding and plant genetic analysis. Those arguments are:

- Absence of heterozygosity, and, subsequently, no dominance effect which permits avoiding errors due to heterotic effects.
- Useful recessive genes are expressed as well as dominant genes in a DH line.
- Plant breeder has a better, overall judgement with DH lines especially with barley and other cereals.
- DH methodology allows an easier study of genetic effects due to additive and epistatic effects.
- As there is no segregation during the generations, DH method offers a better security in the evaluation of environmental effects and genotype X environment effect.

Conservation of original allelic combination originating from meiotic recombination is another interesting consequence of DH method. A lot of work has been done all around the world to develop DH lines (technique) and compare

the DH method with other conventional methods. The conclusion is clear: DH method has the same probability of giving rise to valuable lines as the other methods.

But additional advantages should be helpful when DH lines are produced through in vitro process (anther, ovary, ovule, isolated microspore cultures). During such process, gametoclonal and in vitro selection could occur and modify the expression of genes or provoke genomic modifications (amplifications, deletions, cryptic rearrangements) which bear on agronomic traits such as heading date, quality and yield. In vitro selection can be more organized around precise objectives by applying during in vitro culture special stress: thermic shocks, salinity, osmotic stress, etc. First example have been published for salinity tolerance of barley after in vitro selection during anther culture. A collaborative project exists between ICARDA , Paris Sud University and INRA-ENSAM Montpelier on in vitro selection for thermic tolerance through anther culture of wheat.

Finally, isolated microspores dividing efficiently are very suitable tools for genetic transformation and cell manipulation .

WIDE CROSSES IN CEREALS. George Fedak, Plant Research Center, Agriculture Canada, Ottawa, CANADA.

Procedures for integration of alien chromatic , carrying desired genes into crop plant chromosomes involve the following steps

1. Crossing with the use of growth regulators eg. 2.4D injected into stems of maternal parent followed by application of Ga3 to pollinated plants
2. Use of embryo rescue, ovule or anther culture to overcome lack of endosperm development in intergenic hybrid seeds.
3. Genome analysis on hybrid by studying meiotic chromosome pairing; amount of chromosome pairing will determine if induction of recombination will be required at later stages.
4. Chromosome doubling of intergeneric hybrids to produce amphiploid by using colchicine treatments or callus cultures.
5. Backcrossing onto hybrids or amphiploid for several cycles to produce addition and finally substitution lines for all donor chromosomes. Verify identity or homology of addition lines by means of chromosome handling by molecular methods.
6. Screening the series of addition/substitution lines for traits in quarantine.
7. Induce recombination in critical addition/substitution lines by moves callus culture, mutants of meiotic pairing genes, or crosses with species that overcome miotic pairing control genes.in species such as wheat.
8. Screen recombination progeny for desirable traits derived from donor parent,

check for cytological stability of derived lines and identify site of alien chromatic integration by means of in situ hybridization, or southernblotting using species-specific probes.

Wide crossing is meant to complement traditional plant breeding methods by introgressing traits that are not available in previous gene pools of the respective crop species. Some of these traits are resistance to diseases such as BYDV, Helminthosporium & Fusarium. Tolerance to abiotic stresses is available in alien species and although difficult to manipulate some potential traits for their transfer.

POTENTIAL OF PLANT TRANSFORMATION IN CROPS IMPROVEMENT.

Horst Lörz, University of Hamburg, GERMANY.

Transformation of crop plants is seen as an additional tool in plant breeding to overcome barriers of incompatibility and to do plant breeding with single, defined genes. Short summaries are given on different aspects of barley cell and protoplast culture, transformation and initial experiments to manipulate isolated gametes of maize.

Embryogenic cell suspension cultures of barley (Hordeum vulgare L. cvs. Igri, Gimpel, Princess and Baroness) were established from anther-derived embryogenic callus. Suspension cultures of the cultivars Igri and Gimpel were regenerable. The most successful cultivar was Igri from which a number of independent cell lines producing plantlets were established. Plants could be transferred to soil; up to now 50% of more than 200 regenerated plants were morphologically normal and fertile. The relative frequency of sterile plants increased as the cell suspension got older. Suspensions older than one year produced embryogenic callus but only albino plantlets could be regenerated (Jahne, Lazzeri, Jager-Gussen and Lörz, 1991, Theor. Appl. Gent. in press).

Fertile plants were regenerated from barley (Hordeum vulgare L. cv. Igri) protoplasts isolated from regenerable suspension cultures. These suspensions were initialed from anther-derived embryogenic callus. Plants were routinely regenerated from these suspension cultures, which maintained their regenerative capacity for several months. It was first possible to isolate protoplasts from suspension after three months of culture and after four months protoplasts maintained the regenerative capacity of the donor cells and formed embryogenic callus. Plants were regenerated from protoplast-derived calli, although the proportion of albino plantlets was high. Viable regenerants were transferred to soil and fertile plants were recovered. (Jahne, Lazzeri and Lörz 1991, Plant Cell Reports, in press).

Protoplasts isolated from a barley cell suspension (cv Dissa) were transformed

with plasmid DNA containing the neomycinphotransferase II (NPT) and ß-glucuronidase (GUS) genes, using polyethylene-glycol (PEG) to induce DNA uptake. Transformed microcalli were selected in media containing G418 sulphate. NPT activity was detected in all antibiotic-resistant cell lines, but not all NPT-positive cell lines have GUS activity. Southern analysis confirmed the presence of sequences homologous to the NPT and GUS genes in DNA of G418-resistant callus. (Lazzeri, Brettschneider, Luhrs and Lörz, 1991. Theor. Appl. Genet. in press).

Electrofusion-mediated in vitro fertilization of maize using single sperm and egg cells was performed. Sperm cells were released from pollen grains after rupture of the latter by osmotic shock in fusion medium (0.55 M mannitol). Egg cells were isolated by enzyme treatment (pectinase, pectolyase, hemicellulose, and cellulase) followed by mechanical isolation. The conditions generally used for the electrical fusion of protoplasts of somatic cells were also applied to the protoplasts of gametic cells of maize. Electrofusion was performed with single pairs of gametes under microscopic observation. The mean fusion frequency was 79%. Isolated egg cells of maize showed protoplasmic streaming during 22 days of culture, but they did not divide. However, after fusion of the sperm with the egg cells, these fused cells did develop, with a mean division frequency of 83%, and grew to multicellular structures. Egg cells and fusion products were cultivated with a maize feeder-cell system (Kranz, Bautor and Lörz 1991, Sex. Plant Reprod., in press).

BIOTECHNOLOGY APPLIED TO DIAGNOSIS OF PLANT PATHOGENS.

Yves Bertheau, INRA, INA P-C, 16 rue Claude Bernard, 75231 Paris Cedex 05, FRANCE.

Diagnosis of plant pathogens still has a restricted market but it is growing. Several techniques are available and in current use and most of the commercial and non commercial detection tools are based on ELISA. Although several improvements, such as the DIB ELISA technique, are under study, the serological methods appear to be limited by their detection level and their dependance on environmental conditions (masking, gene expression...). Thus more accurate and rapid techniques are actively looked for, particularly to detect latent infections.

The use of nucleic acids for the detection of phytopathogens is one of the ways studied. Nucleic acids staining of infected plants was a first approach (e.g. for Mollicutes) but it appeared to be limited by its low detection level, its duration as well as the need of well trained people. Nuclear probes (specific or non-specific for the target organism) and nucleic acid hybridization improved greatly the detection level when radioactively labelled. However the use of radioactive material is highly handicapped in terms of legal policy, security, wastes and

duration of the labelling. The use of cold (non radioactive) probes solves most of these problems but reduced the detection level by 20 to 100 fold.

Description in 1985, then in 1988, of PCR (Polymerdse chain Reaction) could enhance the use of the stable cold probes. This DNA amplification is yet widely used for cloning, direct sequencing and detection. In 1989, INRA started a National program (Co-ordinator: Y. Bertheau) to evaluate the ability of PCR in detecting RNA viruses, mollicutes, eubacteria and fungi using several commercial enzymes and thermal cyclers. The PCR technique and the results obtained during these 2 years were presented to the technical institutes and organization of producers during a recent meeting (Paris, February 1991). A special issue of a French journal will also summarize this meeting. Moreover practical training courses will be held in Paris.

PCR appears to be a rapid (less than 5 hours without any optimization) and valuable technique for detection of pathogenic organisms, study of competition between introduced organisms or in risk assessment. Associated to its very low detection level, PCR is valuable for polymorphism studies of the amplified fragment (s) by RFLP, DGGE or TGGE. However several problems have to be solved (reliability of cyclers, quantification, preparation of the samples which differ from one plant to another, carry-over contamination). The availability of techniques such as RAPD and genomic substraction will increase the interest in PCR. Commercial kits are being developed, the price of which depends on several factors (patents, reduction of reactional volumes, prices of the cyclers and enzymes...).

USES OF MICRO-ORGANISMS IN PLANT PROTECTION. Guy Riba, Lutte Biologique, INRA, 78285 Gyancourt, FRANCE.

Five strategies have been defined in microbial control of plant enemies:

1. Respect of naturally occurring organisms must be maximized in all agricultural conditions.
2. A fungus, Puccinia chondrillina has been introduced in Australia in order to control the weed, Chondrilla juncea growing in pasture. Baculovirus of nuclear polyhedrosis of Oryctes rhinoceros has been introduced in Pacific island around 1975 and since then reduced the population of this palm pest. The last example developed in 1989, is the use of specific strain of Beluveria which reduced considerably, the population of Hoplochelus marginalis, a white grub of sugar cane and Geranium cultures in the Reunion island.
3. Nomuracea rileyi is an entomopathogenic hyphomycete able to induce naturally occurring epizootics within Anticarsia gemmatalis population evolving in soybean fields in south Brazil and south of US. Such anticipation of epizootics can be induced by an early inoculation of few propagules in the

region where natural epizootics occur too late.

4. Innundative treatment are recommended when no other strategy can be developed. This is the traditional use of biopesticides which are really diversified microorganisms able to control weeds, diseases and pests.
5. Finally, biotechnological tools are efficient enough to permit the transfer of useful genes from such micro-organisms to others. Objectives are, host spectrum (genes fusion in Bacillus thuringiensis), enhancement of virulence, gene expression, recombination of genes by protoplast fusion (entomopathogenic fungus), modification of host-defence mechanisms.

Risks assessment of microbial control has to be estimated since cases of induced resistance are known, and genes transfer may occur.

In the near future, uses of micro-organisms for plant production and plant protection will be more diversified and has to be compatible and integrated with other agricultural practices. This emphasizes the extreme necessity to organize exhaustive surveys everywhere and specifically in arid and semi arid areas.

BIOTECHNOLOGIES IN DEVELOPING COUNTRIES: THE PLANT BREEDER'S PERSPECTIVE. Ralph Riley, Cambridge, UNITED KINGDOM.

The Green Revolution in wheat and rice saved many parts of the developing world from famine in 1960s and 1970s. To a considerable extent it was due to the magnificent application of knowledge that had been generated from the 1930s onwards in such disciplines as genetics and breeding, pathology, physiology and agronomy. We must learn the lesson from this and continue to build new scientific knowledge and to understand how it can be applied in agriculture. Developing countries must have capability in research, and access to research findings, in biotechnology. This will provide the best insurance against food deficits in the future. Therefore expanded research investment is necessary immediately. When possible research should be done in developing countries but in some instances the quickest benefits from biotechnology may be obtained when the research solutions are sought from advanced laboratories. In addition, the scientific community has a special responsibility to ensure that in bringing the benefits of biotechnology to developing countries care is taken to assess whether there are associated environmental risks.

Agricultural improvement is already resulting from the use of varieties into which foreign genes have been introduced. Better varieties have been obtained from anther and microspore culture. Protoplast fusion has made possible hybrid oilseed rape varieties with considerable hybrid vigour in seed production. Biochemical markers have already increased the precision of conventional breeding. RFLPs will soon provide even greater precision. The benefits of

biotechnology are already tangible and there are more to come if the developing countries, the donors and the scientific community are prepared to make the necessary commitments.

BIOTECHNOLOGY FOR RHIZOBIA: PRESENT AND FUTURE TRENDS.

D. Beck, ICARDA, P.O. Box 5466, Aleppo, SYRIA.

The overall objective of rhizobia work at ICARDA is to increase N2 fixation and thereby reduce fertilizer needs for cereals in the farming systems of the region. One area of major potential for application of biotechnology tools to this objective is in Rhizobium ecology.

In ecological studies, the aim is to introduce a superior rhizobial strain into an existing, less effective indigenous soil population. The introduced strain must compete successfully for nodulation sites on the legume root, and subsequently colonize the soil so that repeated inoculation is not necessary. It is possible to select for competitiveness and survival ability in conjunction with superior N2 fixation ability, but introduced strains must be identifiable against a diverse background of native populations.

Currently, immunodiagnostics utilizing polyclonal antisera for identification of chickpea and lentil rhizobia by ELISA and FA methods are used for ecological studies at ICARDA with reasonable success. Cross reactions with native rhizobia are however often a problem, and more discriminating techniques are needed for detailed investigations. The possibility of disseminating ELISA kits for use by regional scientists in Rhizobium ecology studies is being explored.

In cooperation with the Microbial Ecology Dept. of the Univ. Lyon, methodologies for rhizobia identification, including RFLP utilizing non-radioactive probes and enzyme polymorphism, are being tailored for use in in vivo studies. In conjunction with the methodologies development, a large collaborative effort is going into classification of chickpea rhizobia, involving INRA (France), Univ. Lyon (Fr.), Univ. Ghent (Belgium) and ICARDA. Using DNA/DNA hybridization, DNA/RNA hybridization, and host compatibility studies two distinct groups of chickpea rhizobia have emerged. These findings will clearly place chickpea rhizobia in the overall Rhizobia classification scheme and will change the approaches to study the chickpea symbiosis.

The other major area of work utilizing biotechnology tools is in genetic transformation of rhizobia. A cooperative project with U.S. universities to introduce the delta toxin gene from Bacillus thuringiensis var. tenebrionis (toxic to Coleoptera spp.) into rhizobia has made good progress. The objective is to control the obligate nodule feeding larva of the Sitona beetle, by engineering rhizobia to produce the toxin within the nodule. This is accomplished by conjugating a recombinant plasmid containing the toxin gene (produced in E. coli)

downstream from the nod-H promoter in lentil rhizobia strains. A major complementary effort on selecting competitive lentil strains which will be engineered to produce the toxin is being initiated with Dr. H. Moawad of NRC, Egypt.

Techniques being developed in advanced labs in the U.S. and Europe for engineering rhizobial specificity, competitiveness and energy efficiency will be explored at ICARDA as they become available.

PRESENT AND FUTURE TRENDS OF BIOTECHNOLOGY FOR RHIZOBIA. Luis Materon, Microbiologist, PFLP, ICARDA, P.O. Box 4566, Aleppo, SYRIA.

Areas of activities conducted at this program were briefly described. They included description of projects involving the legume (annual medics, clovers and forage legumes), Rhizobium and the environment. Emphasis on these projects centers on solving ecological factors affecting symbiosis and rhizobia. Technology transfer and training were also components of this general activities. Approximately 80% of his time is invested in the above projects. The remainder of the time is invested on biotechnology projects.

Research conducted as a joint project with Dr. Bertrand Early and Robert Selander of Pennsylvania State University (USA) was published as Genetic Structure of Natural Populations of the Nitrogen-Fixing Bacterium Rhizobium meliloti (Applied and Environmental Microbiology 56:187-194).

Some highlights of this research were: The genetic structure of 232 isolates of R. meliloti was examined by analysis of electrophoretically demonstrable allelic variation of 14 metabolic, presumably chromosomal, enzyme genes. 115 isolates were collected in West Asia and belong to the ICARDA collection. Fifty distinctive multilocus genotype (electrophoretic types) were identified, and cluster analysis revealed two primary phylogenetic divisions separated at a genetic distance of 0.83. The divisions represent two primary distinct evolutionary species. One of the divisions appeared to be adapted to annual medic species of the Mediterranean basin. With the development of molecular methods for determining chromosomal genotypes in bacteria is now possible to estimate phylogenetic relationships.

Future activities in the PFLP Microbiology Unit will concentrate in plasmid manipulation of Rhizobium meliloti. Emphasis will be given to plasmids containing traits that improve the behavior of the strains once applied on the seed and also when the rhizobia remain in the soil in the absence of the plant. Some of these traits are competitiveness and persistence.

A joint activity with the INRA-Montpelier group on amplification of the dot gene of R. meliloti for malate transport. The unit will conduct the symbiotic work on these improved strains. other activities will concentrate on continuing the work

on genetic structure of rhizobia with an emphasis on other species of pasture and forage rhizobia.

Finally, The possibility to commence work on sheep rumen microbiology was mentioned. Enhancing feedstuff utilization by molecular techniques on bacteria to alleviate problems associated with current feed practices in the ICARDA region may warrant investigation.

BIOTECHNOLOGY RESEARCH ACTIVITIES FOR CEREAL IMPROVEMENT AT ICARDA. Philippe Lashermes, ICARDA, P.O. Box 5466, Aleppo, SYRIA.

The project seeks to develop and adapt methodologies that will accelerate and improve the efficiency of cereal breeding. Activities are focused on the production of doubled haploid (DH) lines, and on the development and assessment of DNA molecular marker techniques for barley breeding.

Two different methods of haploid plant production have been developed. These are anther culture and interspecific crosses followed by embryo rescue (pollination with pollen from Hordeum bulbosum or maize). Both have become integrated in the breeding programs of bread wheat and barley.

Research was initiated in 1990 with the objective to identify genes or combination of genes associated with adaptation to low rainfall environments. In preliminary work, an estimation of genetic distance based on RFLP and PCR markers, between reference barley genotypes has been done. On the basis of divergence at the molecular level and agro-morphological data, the cross Tadmor//ER/Apm has been selected for further effort. A mapping program has been initiated using a population of 150 F_5 inbred lines produced through SSD from the cross Tadmor//ER/Apm. Genetic analysis and manipulation of physiological and morphological characters such as growth habit, leaf color, growth vigor, leaf water potential, c-13 discrimination, etc. may be considerably simplified. Information on the number of loci involved, mode of action, and interaction effects will be generated.

The present development of RFLP technology does not allow the efficient handling of the large number of plants. Attempts are being made in collaboration with Montana State University to assess the Polymerase Chain Reaction (PCR) as an alternative strategy. The PCR is a fast and simple non-radioactive technique, but requires an understanding of the molecular basis of variation between genotypes at specific loci. Once an informative RFLP locus is identified, it may be converted to a PCR based marker.

RFLP-FINGERPRINTING IN LEGUME IMPROVEMENT. Franz Weigand, Legume Program, ICARDA.

Molecular marker techniques have gained widespread applications in plant genetics and breeding. Isozymes and RFLP are valuable tools for linkage analysis and to set up genetic maps for crop plants. More recently the technique of DNA-fingerprinting has also been introduced for the analysis and characterization of plant and fungi genomes. This technique makes use of the existence of hypervariable DNA sequences in nuclear genomes and uses appropriate "fingerprinting" probes to detect them. DNA-fingerprinting of chickpea using oligonucleotide probes has shown that depending on the probe, species, variety, or individual, specific patterns can be obtained. The banding patterns are somatically stable and inherited in a codominant Mendelian manner. DNA-fingerprinting thus has many possible applications in legume germplasm evaluation and improvement. In germplasm evaluation it can be used for identification of duplicates in the collection, for precise variety identification, characterization of genetic variability within varieties, landraces and wild species and precise determination of the degree of homozygosity.

In chickpea improvement, the technique is used to improve the backcross breeding strategy to develop Ascochyta rabiei resistant varieties as the DNA fingerprinting allow to quantify the similarity between backcross progeny and recurrent parent, which reduces the number of backcross generations required. Another objective is the identification of loci in chickpea, linked to Ascochyta resistance. This involves phenotypical screening of backcross progenies for Ascochyta resistance under controlled conditions and the identification of bands from fingerprint patterns tightly linked to resistance traits.

Pathotyping of A. rabiei is another important area for the application of DNA fingerprinting at ICARDA as different races of different aggressiveness exist in the Mediterranean region, which need to be adequately characterized. The development of a "Fingerprint passport" will allow the identification of races and their geographical distribution, identification of new races and facilitate the selection of suitable chickpea varieties for different production areas.

In cooperation with the University of Frankfurt, a non-radioactive digoxigenin labeled oligonucleotide DNA fingerprinting was developed, which is used at ICARDA and also practiced in the biotechnology training course, as this can be used by national programs.

DIAGNOSTICS AVAILABLE AT ICARDA FOR THE DETECTION OF CEREAL AND LEGUME VIRUSES IN SEEDS, LEAVES AND APHID VECTORS. K.M. Makkouk, Virology Laboratory, ICARDA, P.O. Box 5466, Aleppo, SYRIA.

Availability of methods for the detection of plant viruses which are sensitive, rapid, simple to use and economical has a wide application in agriculture production. Reagents for the detection of barley yellow dwarf virus (PAV isolate), broad bean mottle virus, broad bean wilt virus, and broad bean stain virus have been developed and are available in the form of Kits. Two serological tests are being used: (i) enzyme-linked immunosorbent essay (ELISA) and (ii) Virobacterial agglutination (VBA). Sensitivity of the ELISA test could be increased by using an enzyme amplification mixture. ELISA and VBA kits are available for distribution to laboratories of national programs in West Asia and North Africa. Each kit has enough reagents to test 2000 samples. The kits permit the detection of the above viruses in seeds, leaves and aphid vectors. Details on relative advantage and limitations of the tests used will be presented.

PRESENT STATUS AND FUTURE PROSPECTS OF BIOTECHNOLOGY IN TURKEY. Dogan Sakar, TURKEY.

Biotechnology in Turkey started with the tissue culture technique during 1977. Since then, several tissue culture laboratories were established at Universities and at the Department of Agriculture. The objectives of these laboratories were to use micropropagation for the production of virus-free plants. Later on, research has been conducted on anther culture methodology and somaclonal variation. Most of the research was on horticultural crops.

Food legumes and cereals are important components of Turkey's farming system and very good progress have been made toward the development of disease resistant and high yielding cultivars with good seed quality characters. However, to speed up the breeding cycle and to transfer desirable genes (stress tolerance) from wild relatives to cultivated species requires basic research on food legume biotechnology.

Well defined objectives, training and support of dedicated young scientists and very close collaboration with other national and international programs are the prerequisite to achieve these goals.

SOMACLONAL VARIATION FOR SOME AGRONOMIC CHARACTERS IN WHEAT. Akbar Shah Mohmand, Pakistan Agro. Res. Council, Islamabad, PAKISTAN.

Efficient tissue culture and regeneration methods were established by using mature embryos as explant source. Genotypic differences were evident in the variants of three wheat genotypes tested. Variants were regenerated from three wheat cultivars, Glennson, Pavor and PAK-16171 and were evaluated for some agronomic characters. Calli were initiated from germinating embryos on linsmaier and skoog (LS) medium plus 2 mg/ml 2,4-dichlorophenoxyacetic acid (2,4-D), 2% sucrose and 1% agar. Plants were obtained on LS medium containing 0.5 mg/ml benzyladenine (BA) and 0.1 mg/ml indoleacetic acid (IAA). Significant variation was observed in the ll characters recorded, between the variants and parent and among the variants. They differed in spike length, number of grains per spike and 100-grain weight. The most remarkable differences were observed in the flag leaf slize (i.e. length, width, and area) which was three to four times greater in variants 71B88R, 71C88R1 and two to three times greater in variants PA88R1 and PB88R1 than the parent lines. Such variation will be of tremendous importance for utilization in future breeding programs.

NUCLEAR/CYTOPLASM GENETIC INTERACTIONS CONTROLLING ANTHER CULTURE RESPONSE IN WHEAT (Triticum aestivum L.). Hasan Ekiz Bahri Dagdas, IWCRC, P.O. Box 325, Konya, TURKEY.

The anther culture responses of alloplasmic lines of three spring wheat (Triticum Aestivum L.) cultivars, were compared to the nuclear donor cultivars. In each series, the observed effects of alien cytoplasms were significant for callus number per 100 anthers plated, plant regeneration and green plant proportion. In addition the study of alloplasmic lines with the common alien cytoplasms showed that cytoplasmic nuclear genetic interactions were significant. The number of calli per 100 anthers ranged from 32 to 122, 4 to 198, and 2 to 41 while green plant proportion ranged from 26 to 63, 9 to 65 and 12 to 75 % for the Selkirk, Siete cerros 66 and Penjamo 62 series, respectively. Cytoplasms from Triticum dicoccum v. Pseudomacrotherum, T. aegilopoides, T. persicum fuliginosum, T. dicoccum khapli and T. turanicum notabile interacted favorably with T. aestivum nuclear genotypes and mostly were superior to the normal T. aestivum cytoplasm.

SELECTION FOR RESISTANCE TO ASCOCHYTA RABIEI USING TOXIC METABOLITES. M. Harrabi, H. Halile, R. Strauge and S. Riazuddin, INAT, TUNISIA.

Results indicated clearly that Ascochyta rabiei produced toxic biological products. Four products were purified and characterized, one of these compounds, cytochalisin, was characterized totally from an isolate from Pakistan. Since these metabolites have fungistatic effect, it would be possible to use them as a screening tool to select for resistance at early stages of cultivar development, since tissue culture techniques for chickpea have been well established. Resistant protoplast could be regenerated to give resistant plants provided that the resistance at the protoplast level is maintained at the required plant level.

Our data show that both protoplast and electrolyte loss bioassay have good sensitivity to the toxin. Electrolyte leakage might be used as the basis of a quantitative bioassay provided conditions are carefully standardized. Since good correlations were found between toxin-induced leakage and fungal reaction at the adult stage, we propose that this bioassay be used as a routine screening for early selection for resistance to A. rabiei.

INHERITANCE OF ISOZYME VARIATION IN ASCOCHYTA BLIGHT RESISTANCE CHICKPEA LINES. Ismail Kusmenoglu, Field Crops Central Research Institute. P.O. Box 226 Ulus, Ankara, TURKEY.

The objectives of this study were to study inheritance of isozyme polymorphism in Ascochyta blight resistant lines of chickpea and determine the relationship between Ascochyta blight resistance and isozymes. Forty one germplasm lines were scored for 28 enzyme systems using horizental starch gel electrophoresis. In addition eight F2 populations were scored for allozymes of four isozymes. Variation for four isozymes within three enzyme systems were found in the germplasm. Inheritance of genes encoding for one previously described (6 pgd-2) and three new isozymes Est 1,2., Est-5, and Gal-1) of chickpea indicated polymorphisms were present for each of these isozymes. Gene duplication in the region of Est 4, 5 was observed.

These isozymes showed monogenic inheritance. The investigation of possible linkage between Gal-1 and the other three isozymes loci indicated independence. No relationship between Ascochyta blight resistance and three isozymes (Est 1,2, Gal-1, and 6 pgd-2) was observed. The F2 population segregating for Est-5 have not been screened yet for Ascochyta blight resistance.

RESEARCH ACTIVITIES ON CEREALS AND LEGUMES IN EGYPT. G.S. Youssef, ARC, Giza, EGYPT.

Our main objectives is the production of interspecific and intergeneric crosses for transferring agronomically desirable characteristics i.e. insect (aphids) resistance, high protein content and salt tolerance from related or distantly related species or genera. Therefore, biotechnological tools are becoming of increasing importance in our program such as:

1. **Embryo rescue**
   Young embryos 10-12 days after fertilization were excised and immediately sterilized and grown on MS medium, containing 0.2 mg/L 2,4D. Most embryos have germinated forming shoots and roots. Plantlets were transferred on free hormone media.
2. **Callus initiation and regeneration**
   Callus initiation has been accomplished in most cereal species such as wheat, barley, sorghum rice in addition to vicia faba. Mature embryos were excised (after sterilizing the seeds) and grown on MS media containing 2 mg/l 2,4D+0.5 mg/l kinitin. For regeneration, the callus was transferred after 3 passages on hormone-free MS media (for wheat and barley or sorghum) or MS+IAA and 6BA for other legumes or clovers. Micropropagation has been worked out in some crops such as wheat, sorghum, vicia faba, clover, tobacco with the purpose of propagating some mutants which are otherwise difficult to reproduce by sexual or asexual means. Small pieces (1/2 cm) of leaves, stems, flowers, roots, after sterilization, were grown on MS media supplemented with 6BA and/or IAA.
3. **Anther culture.**
   Using anther culture was initiated in our laboratory to producing homozygous lines as well as to facilitate protoplast fusions. Spikelets at uninuclear stage were picked carefully and grown on N6 or potato media.
4. **Protoplast isolation and culture.**
   protoplasts of some wheat varieties were isolated in our lab with moderate density using cellulase R10, cellulase RC or YC. Work is being continued to isolate protoplasts in high density using different kinds of media. Protoplast fusion between wheat and other grasses is also planned.

With the above techniques the following activities are in progress:i) Screening wheat callus and the regenated plants for salt tolerance, ii) The production of herbicide resistance callus in wheat, iii) protoplast isolation, culture and fusion in wheat and some wild relatives, iv) Producing haploids from anther culture in wheat and barley.

BIOTECHNOLOGY IN JORDAN : STATUS AND PERSPECTIVES. Hassan Abu-Qaoud, University of Jordan, Amman, JORDAN.

The use of new techniques for agricultural development in Jordan is highly desirable. Plant biotechnology involves the application of scientific methods and techniques to regenerate plants in vitro. Plant biotechnology research includes the use of tissue culture procedures (micropropagation, cell selection, embryo rescue, haploid techniques and protoplast technology) and the use of crop transformation through genetic engineering. The need of biosafety regulations, Intellectual property and training, networking and funds are necessary to establish industrial biotechnology in Jordan.

Research priorities include : 1) Improving legumes through manipulation of Rhizobium symbiosis, 2) Establishing mycorhizal association, 3) Manipulation of biological control, 4) Micropropagation, 5) Plant breeding and protoplast-techniques. There are two cooperative projects between Jordan, Canada and France that are being established. The two projects aim at building tissue culture facilities to micropropagate several important crops as well as to establish a foundation for further biotechnology applications. The shortage in trained manpower and Scientists is considered as a major limiting factor. In addition, the inadequate funds is another important limiting factor for applying plant biotechnology in Jordan.

HEAT SHOCK PROTEINS AND THERMOTOLERANCE. Said EL Madidi and Michel Zivy, Station de Genetique vegetale, INRA-CNRS-UPS, Gif sur Yvette, FRANCE.

In all organisms, exposure to high temperatures has a large influence on protein synthesis. The synthesis of most proteins stops or decreases, and the synthesis of heat shock proteins (HSP) is activated. Several papers have shown that HSP play a role in thermotolerance, but the exact function of most of them is still unknown. It seems that at least for one of them the function could be to protect the confusion of other proteins during heat shock. It has been observed that HSP are synthesized in the field by cotton and soybean, especially when plants were not irrigated. If HSP are responsible for thermotolerance, then the variability of these proteins could be responsible for a variation of thermotolerance between genotypes at the cellular level.

It must be kept in mind that we deal only with thermotolerance at the cellular level although differences of tolerance to high temperatures between genotypes can also be due to other phenotypic traits. Two questions need to be answered (i) is there a genetic variability at the cellular level of HSP synthesis? and (ii) is there genetic variability at the cellular level for thermotolerance?. The variability

between genotypes could be of different types: they could synthesize different HSP or synthesize the same HSP in different quantities. These quantities could be influenced by temperatures and plant organs. It is time consuming to compare a large number of genotypes in different conditions and on different organs. So we had first to study HSP synthesis in different conditions to know if a large amount of information is lost when only one organ is studied in only one experimental condition.

HSP synthesis was studied by two dimentional electrophoresis. The advantage of this technique is that it allows us to study the regulation of HSP synthesis as well as their genetic variation. In different genotypes tested, whether hexaploid or tetraploid wheat, we observed that HSP synthesis is progressive in function of temperature. There is no threshold effect, and the differences between genotypes were the same whatever the temperature tested. No differences between 35 and 40°C and the synthesis of HSP decreases, whereas at 45°C there is no HSP synthesis at all.

HSP variability was about 30% which is relatively high, as compared to other proteins, and was not affected by the organ studied(leaves or roots) and the species (tetraploid or dexaploid wheat). The thermotolerance of different genotypes was tested by the evaluation of capacity of recovery after heat treatments. This test allows us to quantify the thermotolerance of the genotypes.

We started to compare genotypes that behave differently under the same conditions. This study is in progress. Two dimensional gels are scanned and compared using the Kepler software, to compare quantitatively HSP synthesis in the different genotypes. In a preliminary experiment, it appears that HSP are less abundantly synthesized by chinese spring, a heat susceptible genotype than in Katia, a heat tolerant genotype.

IDENTIFICATION OF INOCULANT STRAINS OF RHIZOBIA IN LEGUME NODULES.

H. Moawad, National Research Centre, Dokki, Cairo, Egypt.

Methods available for identification of rhizobia are discussed. Fluorescent antibody (FA) technique was employed to identify inoculant strains of rhizobia in nodules of lentil and soybean grown in five field sites. The technique was very useful to quantify the response to inoculation in terms of nodule occupancy by introduced strains and the competitive relationships between inoculant and native strains. Positive inoculation response was achieved with lentil only in one site, whereas in the other two sites indigenous rhizobia outcompeted the inoculant strains. Favorable response however was obtained using soybean inoculants, with strain 110 being more competitive.

Recommendations

Recommendations

The participants were divided into three working groups depending on the research subject of their interest and discussed priorities, constraints, actions need to be taken, the roll of International Centers with emphasis on ICARDA, networking and other issues of common interest. The recommendations made are summarized below:

Working Group 1
Tissue culture and transformation techniques

A) Prioritization of problems:

1. The WANA region is characterized by a high stress environment. Abiotic stresses such as drought, cold, heat as well as biotic stresses (insect, disease, virus) are particularly relevant for the use of biotechnology. However, it was pointed out that physiological characters such as drought and heat tolerance are complex traits and require a long term strategy to resolve.
2. Two areas of biotechnology were identified:
   i. Tissue culture techniques such as anther culture, micropropagation and embryo rescue (wide crossing) which can be largely developed in the national programs.

   ii. Protoplast and genetic transformation are areas which require collaboration with advanced institution, central facility on a regional basis, and focused objectives.

B) Identification of constraints

1. Lack of infrastructure and adequate facility appeared as a major constraint for biotechnology development in the region. However, large differences between the countries of the region were mentioned.

2. Human resources is also an important bottleneck. Training is strongly required at the technicians level as well as scientists level. Organization of short term courses at ICARDA and long term courses in advanced institutions were proposed. It was recommended to associate training with facilities improvement whenever is possible.

3. For some countries access to information is an obstacle.

C) Proposals for action

Regional collaboration was proposed but no consensus was obtained. More discussion, in a smaller group is required.

D) Role of ICARDA

Several roles for ICARDA have been proposed:

1. To focus on research aspects particularly relevant to the region.
2. To provide a central training program
3. identification of problem/project
4. coordination of regional activity
5. support in approaching funding institutions
6. Test ground of new technologies.

E) Bio-safety aspect

A major role could be played by ICARDA in defining regulations required for the region. It has been proposed to use the guidelines developed and largely discussed in Europe and north American as a basis.

Working Group 2
New Molecular Marker Techniques for Improvement of ICARDA Mandated Crops

A) Techniques

Advantages and disadvantages of available techniques were discussed.

1. Isozymes
   fast, cheap, simple, easier to be established. Useful for e.g. taxonomic studies, but less promising for the development of trait specific markers.

2. RFLP
   more complex, more expensive, need for extensive staff training. High potential for development of molecular markers for agronomically important traits. DNA-fingerprinting has applications in detecting genetic variability in crops as well as pathogens.

3. PCR
   very recent technique with high potential to be used on routine basis in breeding programs, once markers have been developed for agronomically important traits.

B) Technique selection

Selection of proper techniques depend on specific objectives and will be different.

Selection of the technique should be based on:

1. Expected advantage over existing techniques
2. Probability of success
3. Cost of evaluation and regional adaptation

4. Infrastructure cost to NARS
5. Recurrent cost to NARS
6. Socio-economic impact
7. Environmental impact

C) Role of ICARDA

ICARDA mainly to act as a link between advanced institutes and NARS ICARDA to collect information on new techniques, their potential and risks, and to share this information with NARS through workshops, training courses and visits ICARDA has to evaluate and test the suitability of new techniques for application and integration in crop improvement programs. Selected techniques will be transferred to NARS through practical training which will improve the level of technical skill and knowledge for integration of new techniques into NARS breeding programs

ICARDA should put more research emphasis in crops where less research is done worldwide and less emphasis to crops where already much work is carried out by advanced institutions.

Working Groups 3
Applications of Biotechnology in Agricultural Microbiology

A) The following areas of microbiology were regarded by the working group as priorities to be assisted by the current and novel techniques of biotechnology:

1. Enhancement of nitrogen fixation by legumes grown in the WANA region. New techniques such as non-radioactive cold probes may be utilized to develop ecological maps of these microorganisms.

2. Characterization of the most important legume-rhizobial systems providing food and feed in the region. This topic was considered important in order to further develop the inoculant industry. Genetically improved rhizobia after being previously tested through plant passage can be then commercially produced. It was emphasized that the ecology of these microorganisms must be studied first before their release into the environment.

3. Expand studies in another important biofertilizer: the vesicular arbuscular mycorrhizae. These fungi are very important in extracting phosphate from soils containing low concentration of phosphorus. This will alleviate problems when plants are in need of this element.

4. Concerning other microorganisms such as plant pathogens it was discussed that diagnostics is an important area that need to be considered. Polyclonal and monoclonal antibody probes for plant bacteria, fungi and

viruses that attack crops should be developed. Sensitive and reliable tests are critical for the movement of germplasm to assure that seed is certifiably disease-free from key pathogens. It was concluded that techniques should be suitable for the needs of each country.

5. Initiate investigations in the area of insect pathogens such as Bacillus thuringiensis (Bt) and other microorganisms. It is important to develop a germplasm collection of these microbes for exchange and analysis. Aphids attacking important crops in WANA region may be controlled by microorganisms.

6. Treatment of waste water by efficient microorganisms for a better recycling isan area that need to be studied. Bioconversion of animal and plant products to produce compost, gas, etc. It was also discussed that countries of the area should have some knowledge on microorganisms able to break down oil spills.

B) The level of expertise on these areas is low. It can be improved if experts and technicians of other areas of research are trained to handle molecular techniques. Ir was emphasized that the researchers should always work with ecologists. Time frame to develop expertise depends on human resources and projects. In summary both junior and senior scientist should have an opportunity to upgrade their knowledge in the new techniques of biotechnology.

It was considered of importance that the researchers should be linked with other groups of scientists to develop a continuous exchange of information.

It was proposed that a Biotechnology Resources Center (such as MIRCEN) run by an agency of the United Nations be created to serve this region of the world. The Center will assist scientist in biotechnology matters and will be able to freely distribute genetically engineered microorganisms, molecular primers, probes, antibodies, hybridomas, plasmids, enzymes and the like. The Center will also keep a data base and will coordinate and disseminate information through electronic or normal mail.

C) ICARDA will assist scientists of the WANA region in matters relating to biotechnology regardless the crop under study. It was discussed that molecular tools have a lot in common and scientist working in this area should keep in touch with ICARDA. Assistance can be given in the form of training, cooperative projects and information. It would be ideal to have a system of electronic exchange of information through computer linkage among scientists of the WANA region.
ICARDA will also assist informing scientists where to obtain funding and

inform on opportunities to travel to scientific meetings and biotechnology courses elsewhere.

D) The issue of biosafety was considered of extreme importance. It is recommended that national programs should have a National Committee to formulate regulations on the release of genetically engineered microorganisms. Guildlines from other countries can be adopted and modified according to the needs of the respective country. It was suggested that international guidelines such as those of the United Nations be also consulted. The National Committee should include scientists from universities, research centers and the industry.

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Biotechnology Workshop
April 7-10, 1991

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April 7-10, 1991
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