ICARDA's strategy for biotechnology: objectives, organizational structure and areas of research

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RESUME - "La stratégie de l'ICARDA dans le domaine de la biotechnologie : objectifs, structure d'organisation et domaines de recherche". ICARDA (Centre International de Recherche Agronomique pour les Régions Sèches) a pour objectif majeur l'amélioration de l'agriculture dans les zones sèches du Proche-Orient et de l'Afrique du Nord. Ce progrès passe par les techniques classiques de l'amélioration des plantes mais aussi par les voies nouvelles des biotechnologies. La mise en oeuvre de biotechnologies est décidée en tenant compte de leur supériorité par rapport aux voies conventionnelles, de leur coût, de leurs impacts socio-économiques et des risques qu'elles comportent. Elles sont introduites dans les laboratoires d'ICARDA par des échanges de chercheurs et par des consultants. Ensuite, elles sont appliquées soit dans le cadre d'ICARDA, soit dans le cadre des Centres de Recherche Nationaux. A titre d'illustration diverses biotechnologies actuellement mises en oeuvre concernent les points suivants : immunodiagnostic, cultures de tissus, cultures d'anthères, cultures d'embryons, sondes oligonucléotidiques, RFLP, PCR, transfert de gènes...).

Mots-clés : Sécheresse - Amélioration des plantes - Biotechnologies - Résistance à la sécheresse - Immuno-diagnostic - Cultures in vitro - Sondes moléculaires - Transfert de gènes.

SUMMARY - The major objective of ICARDA (International Centre for Agricultural Research in Dry Areas) is agricultural improvement in the Middle East and North Africa dry areas. These advances are to be achieved by means of conventional plant breeding techniques, and new biotechnological methods. The applications of biotechnology are determined taking into account their superiority as compared with traditional techniques, their cost, their socio-economic impact and risks involved. Their introduction in ICARDA laboratories is achieved through scientists exchanges and through consultants. They are afterwards applied by ICARDA, or by National Research Centres. As an example, there are presently a number of biotechnologies involved in the following areas: immunodiagnostics, tissue culture, anther culture, embryo culture, oligonucleotide probes, RFLP, PCR, gene transfer...).

***Key-words:** Drought - Plant breeding - Biotechnologies - Drought resistance - Immunodiagnostics - In vitro cultures - Molecular probes - Gene transfer.*

Introduction

Improving farming in the dry rainfed areas of West Asia and North Africa (WANA) region is the main objective of the International Center for Agricultural Research in the Dry Areas (ICARDA). These areas experience hot dry summers and wet cold winters with highly unpredictable amounts and distribution of rainfall. ICARDA strives to improve the economic well-being of small farmers in these dry, harsh environment areas by cooperating with national scientists in breeding better crop varieties and using them in developing profitable and sustainable production systems. Emphasis is laid on achieving an assured economic return and preserving the natural resource base with minimal cost inputs.

Over the last ten years, ICARDA's research has contributed to the improvement of the lot of farmers in the dry areas around the Mediterranean sea through the development of improved cultivars of important food crops and production of appropriate production technology and by promoting their use by farmers. In so doing, all relevant classical techniques of plant breeding and crop improvement have been used.

The emphasis at ICARDA on collaborative research with and training of scientists in the National Agricultural Research Systems (NARSs) in the region has led to increasing self-reliance in national scientists in carrying out crop improvement research. They are, therefore, now anxious to apply new concepts and technologies in their crop improvement efforts and ICARDA has a role to play in identifying the relevant biotechnologies and ensuring their access to those NARSs that are ready to use them.

Objectives

ICARDA looks at the application of biotechnology in the improvement of ICARDA mandate crops with the aim of either realising those objectives that hitherto proved unattainable by the use of conventional research techniques or in improving the efficiency and economy of currently used techniques. Once identified, the appropriate biotechnologies have not only to be used at ICARDA but also rapidly transferred to NARSs. ICARDA's strategy for the use of biotechnology thus involves three steps.

The first step deals with the selection of the appropriate biotechnology. As the second step, the selected biotechnology is evaluated and adapted at ICARDA under the specific conditions of WANA. The third step includes the transfer of the appropriate and well-adapted biotechnology to the NARSs.

Step one

The appropriate technique is selected based on the following criteria:

1) *Expected advantages* of the new biotechnique in comparison with existing techniques.

2) Probability that the new technique will yield advantage.

3) Input necessary to adapt the technique to the specific conditions of WANA.

4) Running costs to NARSs if the new technique were transferred.

5) Infrastructure required by NARSs to adopt the new techniques.

6) Socio-economic impacts that will result from the new technique.

7) Risks, disadvantages and uncontrollable biohazards associated with the new technique.

To gain in-depth knowledge of these issues and to stay informed of the state of the art of biotechniques, scientists of ICARDA attend relevant international conferences and visit institutions and organizations leading in various techniques. In the workshops organized at ICARDA, scientists from the center, NARS and leading institutions involved in biotechnological research discuss the selection of the best technologies and prepare recommendations. The selected techniques are then presented at ICARDA's internal planning meetings where the initiation of any project is finally approved.

Step two

The second step involves establishing the new techniques in the laboratories at ICARDA. If necessary, short-term consultants are contracted or ICARDA's scientists are sent abroad to bring back expertise in the new technique for establishment and use at ICARDA. In this context the students registered for a Ph.D. degree in a leading University under a joint supervision program are also assigned the task of establishing a new technique as they move back and forth between the University and ICARDA.

The process of establishing a new technique at ICARDA is closely watched to ensure that it is compatible with local environmental conditions and suitable for the genetic material specific to the region. If necessary, modifications are made in the technique. In order to develop usable techniques that still need a lot of basic research, the projects are outcontracted to some leading institutes worldwide. Special funding for this work is arranged because ICARDA considers itself responsible for encouraging this type of research with application possibilities for ICARDA mandate crops.

Step three

The third and final step involves the application of new techniques practically. The new technique is used by the crop commodity improvement programs and plant genetic resources unit of ICARDA, and the NARS who are the most important stakeholders in ICARDA. The transfer of knowledge of the new technique is accompanied by seminars and workshops in which the potential of new technology is practically demonstrated. If a NARS decides to adopt a new technique, ICARDA offers training. This is mainly in the form of advanced training for scientists and is based upon the expectation that these scientists will spread the new technique rapidly within their own country.

An additional important component of ICARDA's strategy of technology transfer is the creation and promotion of regional networks. The objective of these networks is to promote new technologies by free exchange of information and material between NARSs, and in special cases to share costs for expensive techniques.

Organization of Biotechnology Research at ICARDA

ICARDA has established a Biotechnology Research Unit to function as a link between advanced research institutions, ICARDA commodity programs (germplasm enhancement, germplasm conservation, farm resource management, etc.), and national programs, for the applications of biotechnology. Scientists dedicated to biotechnology, although they may be members of commodity programs, share common laboratory facilities and research objectives. The biotechnology laboratories are located next to those for pathology, virology, microbiology and entomology to ensure optimum use of facilities. The integration of biotechnology activities through a matrix type approach should promote collaboration and complementarity between biotechnologists and enhance interaction with other scientists to promote application of appropriate biotechnologies into conventional programs.

The biotechnology laboratory, although relatively modest, is well equipped to carry out research on tissue culture as well as molecular biology. The team includes four scientists providing a minimal critical scientific mass.

Research areas

Although immunodiagnostic activities have been conducted at ICARDA since the early eighties, biotechnology activities at ICARDA began in earnest in 1987 with research on cereal haploid plant production and tissue culture work in chickpea and lentil. The program has grown steadily and diversified. Current research covers all ICARDA mandated crops (winter cereals and cool season food legumes) and a wide variety of techniques. Because of the complexity, cost and rapid pace of biotechnology research, ICARDA does not endeavour to become a leader in basic research. However, special attention is given to the establishment and maintenance of close contacts with advanced institutions engaged in biotechnology research, to enable ICARDA to be ready to apply proven techniques most appropriate to the Center's objectives. Collaboration exists with a large number of biotechnology institutes in the industrialized countries such as Japan (Tropical Agriculture Research Center), Federal Republic of Germany (Max Plank Institute at Cologne and Munich, the Universities of Frankfurt and Hohenheim), France (University of Paris-south, and INRA), Italy (University of Naples) The Netherlands (IPO) and USA (Washington State University, Oregon State University, Montana State University).

Haploid plant production

Haploid plant production followed by chromosome doubling offers the possibility of developing completely homozygous lines from heterozygous parents in a single regeneration. Regarding crop improvement, the production of doubled haploid lines provides a rapid technique for the incorporation of new characters into agronomically superior germplasm, and higher selection efficiency. The objective is to establish efficient methods of haploid plant production in barley, bread wheat and durum wheat, and support conventional breeding efforts. Two different methods have been developed: anther culture and interspecific crosses.

The principle of in vitro anther culture is to divert the normal development of the male gametophyte by an abnormal pathway, to a sporophytic pathway resulting in callus or embryo formation. Although this technique is considered potentially the most efficient, since a plant may be produced from every microspore within the anther, the culture responsiveness varies considerably among and within cereal species. A routine procedure has been established in bread wheat allowing a large production of haploid plants. In contrast, success has been limited in durum wheat and further research is necessary. In barley, high production of haploid plants requires growth of the donor plants in a cool environment which, in the context of ICARDA, can be only obtained with expensive facilities.

Interspecific crosses followed by in vitro culture of immature embryos can lead to the production of haploid plants through elimination of "wild" chromosomes during embryo development. The bulbosum technique (pollination with pollen from *Hordeum bulbosum)* has been well established in barley. In wheat, a novel technique involving pollination with maize pollen and subsequent application of an exogenous hormone into bread wheat florets has been developed.

Both anther culture and interspecific cross techniques have become integrated into cereal breeding programs and doubled haploid lines have already been tested under field conditions.

Molecular marker techniques

DNA FINGER PRINTING:

A nonradioactive DNA finger printing technique has been established. Simple repeat oligonucleotide probes have been identified which reveal loci exhibiting a high degree of polymorphism in chickpea. Alleles at many such loci can be detected simultaneously. This technique is presently used for genotype identification and will be used for genetic purity testing, and gene introgression. Further work will be conducted to expand the technique to barley and others applications.

RFLP Linkage Map:

Barley genome mapping is fairly advanced and ICARDA intends to explore the use of Restriction Fragment Length Polymorphisms (RFLP) as genetic markers. Emphasis will be on traits that are not easily selected for in current breeding programs or are very expensive to screen under field conditions. These traits include factors particularly relevant to the Mediterranean region, such as drought and cold tolerance, and traits introduced through alien genes. Present RFLP technology is not yet capable of efficiently handling the large number of individuals commonly dealt with by plant breeders, and alternative technology such as the polymerase chain reaction (PCR) will be considered.

Information obtained from such studies will be useful not only in conventional breeding programs, but will also be crucial in the identification and cloning of important genes and in the understanding of their interrelationships at molecular level.

Immunodiagnostics

Immunodiagnostic techniques have become an important component in viral and microbiological research at ICARDA. Polyclonal antisera have been used effectively for detection and field surveys for a number of plant viruses. ELISA kits are being prepared for several viruses, such as barley yellow dwarf virus, broad bean stain virus, broad bean mottle virus and bean leaf roll virus, and will be provided for use by national program scientists in the WANA region. Fluorescent antibody and ELISA techniques also allow investigations of critical areas of *Rhizobium* behaviour in soil and especially *Rhizobium*-plant interactions in the rhizospheres.

More recently, monoclonals have proven to be useful in identification of the PAV, RPV and MAV types of barley yellow dwarf virus. In collaboration with advanced institutions, research efforts will be pursued to develop monoclonal antibodies and molecular probes.

Interspecific Hybridization

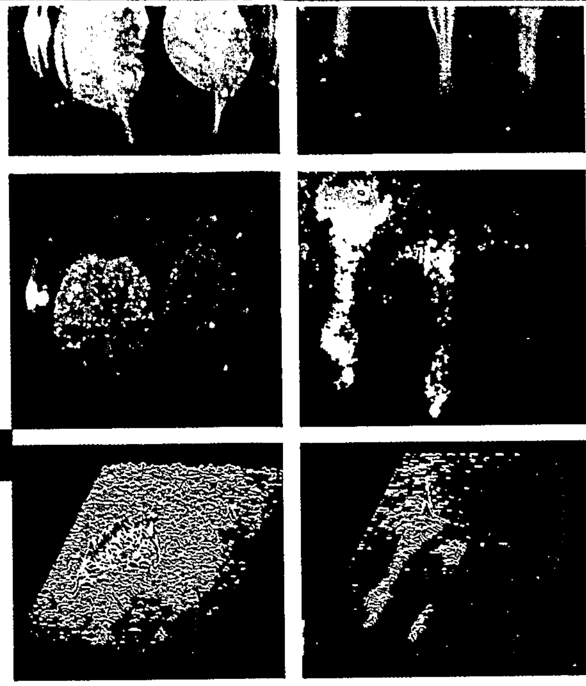
The gene pools of the wild relatives of crop species contain a large amount of genetic material that is potentially very useful for crop improvement. Standard hybridization procedures can be used to introduce this genetic material into a crop species from relatives with which it can form fertile hybrids. However, complex procedures are often required because of seed failures associated with embryo abortion and endosperm disintegration, sterility or poor fertility of the hybrid form.

Isolation and in vitro culture of embryos enable the removal of crossability barriers. At ICARDA, attempts are being made to establish an ovule-embryo rescue technique in order to cross the cultivated species *Lens culinaris* with *Lens nigricans*.

Gene Transfer

Several methods are now available to transform cultured tissues, cells, and protoplasts. Nevertheless, the production of transgenic plants of crop species, such as the cereals and grain legumes, is still rather difficult because the transformation methods are far from routine. In particular, regeneration of plants from single cells, a frequent pre-requisite for cellular and molecular manipulation, has proven to be especially difficult in ICARDA mandated crops. In fact, ICARDA activities are limited in this field. However, some experiments have been carried out using cocultivation of *Agrobacterium* and naked DNA with germinating chickpea, lentil and barley seeds as a noncell culture approach.

At present, only a few genes of agricultural importance are available and have been successfully transferred. These genes confer resistance to several herbicides, a number of destructive insects, and viruses. Most of the agronomically important genes have neither been identified at molecular level nor cloned. Recent initiatives in mapping through RFLP are a step in this direction.



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