BIOTECHNOLOGICAL APPROACHES TO CROP IMPROVEMENT IN THE DRY AREAS AT THE INTERNATIONAL CENTER FOR AGRICULTURAL RESEARCH IN THE DRY AREAS

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ABSTRACT

At the International Center for Agricultural Research in the Dry Areas (ICARDA) biotechnology is included in the crop improvement and genetic resources program. Emphasis is given to the identification and exploitation of genetic resources of improved stress resistance, particularly improved water use efficiency. Non-radioactive DNA technology is being utilized for fingerprinting genetic resources. Numerous molecular-marker systems have also been used for genome mapping and gene-tagging. Markers have already been identified, to be linked with traits of agronomic importance. The technology available for using these markers for marker-assisted selection (MAS) has also greatly improved. Fluorescent-labeled allele-specific markers are being developed and can be used with automated sequencers to allow the screening of thousands of lines within a short period as required by breeding programs. The ability to use MAS to pyramid genes will make this technology an essential tool for breeders. Besides gene tagging and genome mapping, there is considerable effort to characterize the pathogen populations and to develop geographical distribution maps. These maps will allow the deployment of effective host-plant resistance genes. *In vitro* techniques are being used to overcome species barriers to introgress agronomic traits of wild species into adapted cultivars. Embryo- and ovule rescue techniques are being used for inter-specific and generic hybridization programs. Somaclonal variation is exploited from regenerants of *Lathyrus* explants to reduce neurotoxins in the plant and seed tissue. Doubled haploid breeding is being used when rapid solutions are required. Anther- and isolated microspore culture systems are being used for the development of doubled haploid lines for barley and wheat. DH breeding for the barley program is used to develop mapping populations for drought tolerance. DH breeding for the wheat programs is used specifically to introgress Hessian fly resistance for North Africa and yellow rust resistance into adapted germplasm. When variability for key traits is low, genetic engineering is being used to incorporate new genes into plant materials. Fungal and abiotic stress resistance is being engineered in chickpea in cooperation with the University of Hannover, Germany and insect and abiotic stress resistance is being engineered in lentils in cooperation with the Center for Legumes in Mediterranean Agriculture (CLIMA), Australia.

Key words: SSR markers, doubled haploids, genetic engineering, drought

APPROCHES BIOTECHNOLOGIQUES A L'AMELIORATION DES RECOLTES DANS LES ZONES SECHES AU CENTRE INTERNATIONAL DE RECHERCHE AGRICOLE DANS LES ZONES SECHES (INTERNATIONAL CENTER FOR AGRICULTURAL RESEARCH IN THE DRY AREAS)

RESUME

FRENCH

Au Centre international de recherche agricole dans les zones sèches (ICARDA), la biotechologie fait partie du programme d'amélioration des récoltes et des ressources génétiques. L'accent est mis sur l'identification et l'exploitation des ressources génétiques en tant que source de résistance améliorée aux pressions, notamment une amélioration dans l'utilisation de l'eau. La technologie ADN non radioactive est utilisée pour établir des systèmes de sélection basée sur des marqueurs. Des techniques in vitro sont utilisées pour surmonter la barrière des espèces afin d'introduire des caractéristiques agronomiques d'espèces sauvages dans des cultivars adaptés. La reproduction diploïde est utilisée lorsque des solutions rapides sont nécessaires. Lorsque la variabilité pour les caractéristiques clés est faible, le génie génétique est utilisé pour incorporer de nouveaux gènes dans les matières végétales.

Mots clés: marqueurs SSR, diploïdes, génie génétique, sècheresse

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INTRODUCTION

ICARDA, as a member of the CGIAR (Consultative Group for International Agricultural Research), was established with headquarters in, Aleppo, Syria, in 1977 and charged with global responsibility for the improvement of barley, lentil and faba bean. ICARDA also has regional responsibility for the improvement of bread wheat, durum wheat, Kabuli chickpea and pasture and forage crops. Within this broad mandate, ICARDA aims to promote improved and more productive agriculture through research and training activities conducted in cooperation with national and regional research institutions. Specifically, the objectives of ICARDA's Crop Improvement Program are summarized as follows:

- Development of crop varieties with stable and substantially high yields under limited rainfall (200mm to 600mm);
- Identification and development of superior germplasm with tolerance to abiotic stresses (drought, cold, heat and salt);
- Genetic crop improvement to biotic stresses (virus, fungi, insects and nematodes).
- Maintenance and improvement of the nutritional quality of food crops;
- Development of agronomic practices; and
- Training scientists and technicians to improve the scientific capability and food production capacity within the region.

ICARDA'S STRATEGY IN BIOTECHNOLOGY

Biotic and abiotic stresses are major limitations to yields of cereal and legume crops in the West Asia and North Africa region. The objective of the crop improvement program is to increase yield and yield stability under variable arid and semi-arid conditions. Under these conditions crop yields are generally low and vary greatly from year to year. To improve the efficiency and effectiveness of the crop improvement program, a biotechnology strategy addressing some of the most severe limitations to crop improvement in WANA has been developed. The implementation of the biotechnology strategy is incorporated within ICARDA's Medium Term Plan.

Activity 1. Crop Germplasm Enhancement

Pre-breeding and biotechnology: Emphasis will be given to the identification and exploitation of genetic resources for sources of improved stress resistance, particularly improved water use efficiency. Non-radioactive DNA technology will be utilized to establish marker assisted selection systems. *In vitro* techniques will be used to overcome species barriers to introgress agronomic traits of wild species into adapted cultivars. Doubled haploid breeding will be used when rapid solutions are required. When variability for key traits is low, genetic transformation will be employed to incorporate new genes into plant materials: this work is being carried out in collaboration with advanced institutes: for chickpea with the University of Hannover, Germany, for lentil with the Center for Legumes in Mediterranean Agriculture (CLIMA),Australia and in barley with the University of Hamburg, Germany.

Activity 2. Germplasm Collection and Conservation

Biodiversity conservation at ICARDA responds to the Leipzig Global Plan of Action for the Conservation and Sustainable Utilization of Plant Genetic Resources of National Agricultural Research Systems (NARS) and to the CGIAR stripe review of plant genetic resources. ICARDA holds the largest gene bank in the Mediterranean region, holding approximately 20% of the germplasm in CGIAR centers. In the continued exploration for genetic resources, emphasis will be given to the collection of plant germplasm from low rainfall temperature and rangeland areas. However, overall, the shift from collection and *ex situ* conservation of plant germplasm to its characterization, evaluation, documentation and will be accelerated to exploit the biodiversity held at ICARDA. The adoption of DNA-marker technology and the formation of "core collections" will improve the efficiency of germplasm collection management and use. Increased attention will be given to the techniques of *in situ* conservation through promoting improved resource management in the dry areas.

The concept of 'core' collections was initially proposed by Sir O. Frankel (1), and later elaborated by other authors to promote the use of germplasm collections. The idea is to select a small subset of gene bank accessions, which would represent maximum genetic diversity of the entire collection. Such a 'core' collection is widely distributed to researchers of different profile to obtain comprehensive evaluation data on the identical germplasm.

Selected Biotechniques used at ICARDA

I. Tissue-culture techniques

1. Doubled haploid breeding
2. Exploitation of somaclonal variation in *Lathyrus*

II. Molecular marker techniques

1. DNA marker for fingerprinting diseases: *Ascochyta rabiei*
2. Mapping host plant resistance traits related to dry land agriculture

III. Genetic transformation

IV. Training

I. TISSUE-CULTURED TECHNIQUES

1. Doubled haploid breeding

The value of doubled-haploid (DH) line production for breeders is the reduced time required to obtain homozygous populations. Regenerated haploid plants of hybrids after colchicine doubling comprise a completely homozygous population. Furthermore, with the introduction of DNA-marker technology in plant breeding for gene tagging and genome-mapping, DH lines represent the ideal plant material for the application of this technology.

Anther- and isolated microspore culture systems are being used for the development of doubled haploid lines for barley and wheat at ICARDA [2]. DH breeding for the barley program is used to develop mapping populations for drought tolerance. DH breeding for the joined CIMMYT/ICARDA spring bread wheat program is used specifically to introgress Hessian fly resistance for North Africa [3] and yellow rust resistance [4] into adapted germplasm. DH breeding for the joint Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT)/ ICARDA facultative and winter wheat program is being used to introgress yellow rust resistance.

2. Use of somaclonal variation in Lathyrus

Consumption of *Lathyrus sativus* seeds by humans and animals has been limited by the presence of a neurotoxin known as ß-N-Oxalyl-L-, α- ß-Diaminopropionic Acid (ß-ODAP) in the seeds, which when taken in large quantity can lead to "lathyrism" a disease causing paralysis of the limbs. By making use of somaclonal variation plants can be developed with a low concentration of the neurotoxin, and thus make consumption by humans and animals in larger amounts possible. Existing protocols for explant culture of *L. sativus* have been used at ICARDA. Somaclones showed high variation for morphological traits as well as for ß-ODAP [5].

II. THE USE OF MOLECULAR MARKERS FOR CROP IMPROVEMENT

A number of techniques i.e. microsatellites or simple sequence repeat (SSR)-based DNA and amplified fragment length polymorphism (AFLP) DNA markers are used for fingerprinting genetic resources in plants at ICARDA. Expressed-sequence tags (EST) databases provide opportunities for gene discovery, such databases may also provide a novel source of microsatellites (SSRs) that are physically associated with coding regions of the genome (EST-derived SSRs). Genomic SSRs [6-8] as well as EST-derived SSRs [9,10] are currently being used to genotype germplasm collections.

Numerous molecular-marker systems have also been used for genome mapping and gene-tagging. Markers have already been identified to be linked with traits of agronomic importance. Besides the efforts of mapping and identifying host-plant resistance, there is considerable effort to characterize the pathogen populations. If pathogenes can be characterized by DNA markers and diagnostic markers be developed, geographical distribution maps will allow the deployment of effective host-plant resistance genes.

The technology available for using these markers in marker-assisted selection (MAS) has also greatly improved. Fluorescent-labeled allele-specific markers can be used in automated systems such as automated sequencers to allow the screening of thousands of lines within a short period as required by breeding programs. The ability to use MAS to pyramid genes will make this technology an essential tool for breeders.

1. DNA markers for fingerprinting diseases: *Ascochyta rabiei* in Syria

Ascochyta rabiei (Pass.) Labr. is the most severe fungal disease limiting chickpea production, especially in the winter-grown chickpea areas of the Mediterranean region. Conventionally, the population structure of the pathogen is determined by pathogenicity surveys (pathogenic variability) based on reaction on a set of differential cultivars. Such a study in Syria revealed the occurrence of three pathotypes for *A. rabiei*. Additionally, a set of microsatellite and Random Amplified Polymorphic DNA (RAPD) markers were also used which lead to the identification of suitable RAPD markers, allowing a more precise determination of the pathotypes. All the surveys revealed the predominance of a single genotype (genotype-H) in all the chickpea-growing regions [11]. The genotype (pathotype III) is increasing its frequency in all the chickpea-growing regions of Syria.

Despite thousands of lines having been screened for resistance to all three pathotypes, resistance is only available to pathotype I and II and some tolerance to pathotype III. However, with suitable packages of integrated disease management (planting date, fungicide sprays) is the successful cultivation of chickpea possible. Furthermore, the availability of markers for pathotype I and II allow the monitoring of the pathotype distribution and to give recommendation for the planting of suitable chickpea cultivars.

2. Mapping host plant resistance traits in ICARDA mandated crops

DNA molecular marker techniques allow construction of linkage maps for crops. Together with statistical techniques these linkage maps can be used to locate and estimate phenotypic effects of quantitative trait loci (QTL) and the genes responsible for the expression of agronomic traits. For a homozygous population derived from a cross with parents contrasting in response to, for example, water, QTL analysis reveals the approximate map location of loci associated with performance under dry land conditions. This is then amenable to marker-assisted selection using DNA markers flanking the identified QTLs.

Genetic improvement of barley in stressful environments within WANA is rather slow due to the frequency, timing, duration and severity of a number of climatic stresses [12,13]. Additionally, powdery mildew (caused by *Erysiphe graminis* DC. Ex Mérat f.sp. *hordei* Em. Marchal) and scald (caused by *Rynchosporium secalis* (Oud.)J.J. Davies are important foliar diseases in this region. In a population of random recombinant inbred lines of Tadmor/Sel160 gene tags have been identified for Powdery mildew, scald as well as for characters adapted to dry land [14]. In the cross of barley variety 'Arta' with *H. spontaneum,* the objective was to combine the yield potential of Arta with the drought tolerance, earliness, acceptable cold tolerance, and the ability to maintain plant height under drought of *H. spontaneum* [15]. In the recombinant inbred population of this cross, QTLs for grain yield, biological yield, plant height, and days-to-heading were located on chromosome 3H. The plant height alleles of *H. spontaneum* at this location directly influence biological yield and grain yield. There is good correlation between plant height and root length, which might be an important factor imparting drought tolerance in the recombinants derived from the above crosses [16]. Closely linked markers to disease resistance genes are being used to transfer resistance genes in backcross programs. Markers identified for other agronomic traits are also being tested for routine use and selection of better alleles in germplasm collections.

Fusarium wilt is economically the most destructive disease of lentil (*Lens culinaris* Medik) and can cause up to 100% yield loss. Cold tolerance is an important trait for winter lentil cultivation at high elevations. Different DNA marker systems including Restriction Fragment Length Polymorphism (RFLP), RAPD and Amplified Fragment Length Polymorphism (AFLP) were used to construct a genetic linkage map of Lens sp [17]. F6-derived F8 recombinant inbred lines were genotyped with 257 morphological, RFLP, RAPD and AFLP markers[18]. The linkage map was exploited to identify markers linked to *Fusarium* wilt resistance and radiation-frost tolerance [19, 20]. The population was evaluated for two seasons for radiation-frost injury and three seasons for *Fusarium* wilt. Both traits were monogenically inherited. Four RAPD markers linked to the Fusarium wilt resistance locus were identified and located in the present map. Likewise, one RAPD marker was linked with the radiation-frost tolerance locus. Fine mapping is required to develop more closely linked markers. If developed, traits such as *Fusarium* resistance and radiation-frost tolerance will be transferred to other adapted lines in backcross programs.

Chickpea yields can also be increased considerably in low rainfall areas of West Asia and North Africa by winter sowing instead of the traditional spring sowing [21]. With improved cold tolerance, chickpea can be planted in early spring or winter to better utilize winter rainfall. Earlier sown chickpea escapes the terminal drought and heat stresses in spring. For tagging host plant resistance genes in chickpea, sequence-tagged-microsatellite-site (STMS) markers have been developed and tested for genetic diversity analysis [9,11,22] and genome mapping [23]. Host-plant resistance for Ascochyta blight is being mapped in several populations and genetic backgrounds [24, 25]. Aim of the tagging programs is the to identify markers for marker-assisted selection [26].

III. TRANSFORMATION OF PLANTS WITH GENETICALLY ENGINEERED STRESS RESISTANCE GENES

The Center is exploring the possibility of genetic transformation to achieve improved tolerance to drought and other stresses in ICARDA mandated crops. In order to use regional expertise available for genetic transformation, ICARDA has entered into a cooperation agreement with the Agricultural Genetic Engineering Research Institute (AGERI) in Cairo, Egypt to exploit transformation systems for the improvement of ICARDA mandated crops. In cooperation with the Center for Legumes in Mediterranean Agriculture, Australia lentil lines are being transformed with several different constructs for fungal resistance and for improving drought tolerance. In cooperation with the University of Hannover, Germany, and ICARDA, a chickpea transformation system was developed that allows incorporation of resistance genes. The protocol is being used at Hannover, AGERI and ICARDA to transform chickpea lines with two types of fungal resistance genes and with genes that improve drought tolerance. Transformations have been confirmed by PCR analysis and Southern hybridization [27].

A major transcription system that controls abscisic-acid-independent gene expression in response to dehydration and low temperature has been described [28]. The system includes the *DRE/CRT* (dehydration-responsive

element/C-repeat) cis-acting elements and its DNA binding protein, *DREB/CBF* (*DRE*-binding protein/C-repeat binding factor), which has an *AP2* domain. Over-expression of the cDNA encoding *DREB1A* in transgenic *Arabidopsis* plants activates the expression of many genes and results in improved tolerance to drought, salt loading, and freezing. The *DREB* constructs are being used to test the effect of the *Arabidopsis* transcription factors on ICARDA mandated crop plants.

SUPPORT TO REGIONAL PROGRAMS IN BIOTECHNOLOGY

ICARDA aims to assist breeders in West Asia and North Africa use up-to-date molecular biology tools, such as tissue culture techniques, DNA marker technology, and genetic engineering strategies, to develop superior cultivars with increased and stable yield. The training program includes individual non-degree training at ICARDA headquarters, training in specialized training courses at headquarter or as an in-country training course as well as MSc. or PhD training.

Through the project "DEVELOPMENT OF BIOTECHNOLOGICAL RESEARCH IN THE ARAB STATES", funded by the ARAB FUND FOR SOCIAL AND ECONOMIC DEVELOPMENT (AFSED), ICARDA has supported the development of infrastructure for biotechnology research in the national programs of the Arab countries (1998-2001). The project supported existing biotechnological efforts by transferring established techniques, and through training of scientists. The project also directly supported activities related to DH production, molecular marker applications, and genetic transformation. The project has also helped to support regional activities for the "Developing and harmonizing biosafety regulations for countries in West Asia and North Africa". A number of regional workshops and meetings are organized that help to promote the development of national or regional biosafety frameworks [29].

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