Mediterranean project. Improvement of the citrus sector in the Mediterranean by setting up common conservation strategies for the free exchange of healthy citrus plant genetic resources

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2nd Part

MEDITERRANEAN PROJECT

Improvement of the citrus sector in the Mediterranean by setting up common conservation strategies for the free exchange of healthy citrus plant genetic resources

Project background

- The Mediterranean Citrus Network (MECINET) is a body operating under the aegis of the Food and Agricultural Organization of the United Nations (FAO) to promote technical cooperation between all Mediterranean countries. MECINET is presently coordinated by Turkey through the University of , Adana. Within MECINET four working groups have been established and approved by the Coordination Board made by country representatives, officially appointed by governments. These working groups are dealing with the major topics for citrus improvements in the Mediterranean :
  - Genetic evaluation and utilization of citrus and its relatives.
  - Planting material improvement
  - Citrus fruit quality improvement
- The Mediterranean Network on Certification of Citrus (MNCC), is a sub-network of the Mediterranean Networks on Integrated Pest Management (MNIPM) promoted by the Centre International de Hautes Etudes Agronomiques Méditerranéennes (CIHEAM), coordinated by the Mediterranean Agronomic Institute of Bari (MAIB) to address problems affecting citrus by the specific research on the most important Mediterranean diseases and by the harmonization of the certification protocols in the Mediterranean countries.
- Within MNCC a protocol for the production, maintenance and utilization of healthy citrus selections in the Mediterranean was set up and some member countries have been surveyed to assess the sanitary status of citrus and to select citrus stocks of native ecotypes. Within the MNIPM a Study is ongoing on "Production and exchange of virus-free plant propagating material in the Mediterranean region". The output of this study will represent the first step towards the creation of the Mediterranean free-trade area for agricultural products.
- MNCC and the MECINET working group on "Genetic evaluation and utilization of citrus and its relatives" prepared the project proposal on the "Improvements of the citrus sector in the Mediterranean by the setting up of common conservation strategies for the free exchange of healthy citrus plant genetic resources". This programme will enhance the citrus sector by a sustainable utilization of healthy citrus genetic resources, in order to meet the industry needs and to protect the environment.
- The project proposal was discussed at two technical joint meetings of MNCC and MECINET members which were held in Algiers, 4-6 October, 2000, and Cairo, 26 October 1 November, 2001. At the meetings participants were: 21 network members coming from 10 countries,

representatives of FAO, MAIB/CIHEAM, GTZ, CFC, the Italian Embassy in Algiers and in Cairo, Algerian and Egyptian authorities, researchers, technicians and nurseryman).

In addition to the meetings, technical visits in the citrus growing areas of Algeria and Egypt and a short-course on "Advanced techniques for germplasm localization, collection, sanitation, conservation and on virus detection" were organized for supporting project activities.

A Project Commission was appointed under the coordination of FAO and CIHEAM and the followings were approved:

- project coordination under the umbrella of CIHEAM and FAO
- technical guidelines and financial country proposals
- legislative proposal regulating the exchange of citrus genetic resources
- government official approvals
- project submission to the international organizations (OEPP, IPGRI, ICARDA, IFAD, Arab Centre for the Studies of Arid Zones and Dry Lands)
- project presentation to different donors for co-financing with participating Governments (CFC, MEDA, EU etc.)
- relations with other existing Citrus networks (IACNET) and (GCGN)
- training on project activities

Until now, 10 countries (Albania, Algeria, Egypt, Jordan, Iran, Italy, Lebanon, Malta, Morocco, and Turkey) have officially joined the Mediterranean programme envisaging their co-financing, and to his aim they have appointed their representatives within the two networks.

Project justification

Citrus is the most widespread fruit crop in the Mediterranean region, where favourable soil and climatic conditions occur.

The threats of genetic erosion of citrus diversities in the Mediterranean, even if they are not alarming yet, represent the major priorities to be faced as a guarantee of the citrus improvement in this area.

Many reashave been identified as major cause for their decline:

- the farmer's choice, which plays a vital role for their survival. In fact, nowadays, the number of historic citrus varieties and clones is decreasing, whereas some varieties tend to be "almost omnipresent", like the navels etc.

- the lack of awareness, on the part of most Mediterranean governments about the healthy status, care and maintenance of various Citrus species, which is resulting in the alarming increase of cultivar vulnerability to environmental changes, new pests and diseases.
- the damage caused by pests and diseases, which is still the major constraint for a successful maintenance and management of citrus gene banks.

Citrus graft-transmissible diseases cause very important economic losses in most Mediterranean countries, such as decline, loss of vigour and shortening of the commercial life of the grove, low yields and poor fruit quality. These diseases also restrict the use of many rootstocks.

Most existing citrus trees in the Mediterranean are grafted on sour orange rootstock that is extremely susceptible to one of the most devastating citrus pathogens, citrus tristeza virus.

Differently from the other major fruit crops, citrus is affected by many destructive diseases, which can severely cripple the industry of any country. Witches' broom, greening, severe Capao Bonito or the Australian stem pitting strains of CTV, the new Turkish citrus chlorotic dwarf disease, citrus badnavirus mosaic in India, transmissible psorosis in Argentina and Uruguay, canker as occurring in the Maldive islands, exocortis in Belize are examples of such diseases. In addition, new diseases are popping up all of the time.

Most of these diseases are fortunately not present in the Mediterranean area the major threat for the citrus industry being "tristeza" whose agent (CTV) occurs in several countries (Albania, Cyprus, Egypt, Israel, Italy, Libya, Lebanon, Morocco, Palestine, Spain, Tunisia, Turkey) and the new Turkish citrus chlorotic dwarf disease, which is reported only from the country of origin.

The absence of certification and quarantine programmes in several Mediterranean countries has enabled these graft-transmissible pathogens to spread through germplasm exchange.

The cultivation of Citrus fruit crop, as any other irrigated crop of the Mediterranean, is prone to one of the largest problem in the Region: water scarcity. A physiological assessment in terms of water use efficiency (or water productivity) of the major citrus varieties, then, will allow a strong link between "genetic" and "field" issues for a more complete description of the Citrus biodiversity in the Mediterranean and a subsequent more sound decision-making process by farmers.

Considering:

- the social and economic importance of citriculture in the Mediterranean agricultural production
- the necessity of maintaining and free exchange citrus populations for any possible use among the Mediterranean
- the need of harmonizing the existing regulations
- the future role of the Mediterranean as a free trade area

it is compulsory to carry out operations to safeguard and free exchange citrus germplasm

Efforts in this sense are given within two established networks which worked with few resources to disseminate information, create awareness, organize workshops: (i) MNCC since 1995 has been able to support surveys for the assessment of the sanitary status of citrus and for the monitoring of quarantine pathogens, and to propose a technical protocol for citrus certification in the Mediterranean. (ii) MECINET, since 1993 has been able to favourite activities for the conservation of citrus genetic resources and for the improvement of plant material and fruit quality.

Objective

The present proposal is part of a long-term strategy for the improvement of the citrus sector by a sustainable conservation and utilization of healthy citrus genetic diversities in order to meet the industry needs, to protect the environment and to favorite free exchange of these resources in the Mediterranean.

The objective is to promote the establishment of a Mediterranean programme for the citrus germplasm identification, physiological assessment in terms of water use efficiency, sanitation, conservation and free exchange. This would preserve the Mediterranean from the dissemination of diseases through citrus germplasm exchange and would favourite citrus improvement and management maintaining the existing citrus genepool for traits devoted to citrus breeding and citrus productivity and would accelerate the process of Barcelona Declaration to establish a free-trade area in the Mediterranean.

The project will also aim to:

- the harmonization of protocols for germplasm characterization, pathogens detection and sanitation and conservation
- the introduction of new technologies low costs, easy to be applied and reliable
- favourite the establishment and the harmonization of the certification programmes
- the gaining of knowledge and the upgrading of technical skills

Operative steps of the project

All participating countries should carry out the operative steps for the inventory, conservation, characterization and sanitary assessment of citrus germplasm. Therefore a Citrus Germplasm Conservation Centers (CGCC) should be established, in each country or for a group of countries, for the sanitation, *in vitro* and *in vivo* conservation and pomological traits assessment of healthy citrus germplasm.

Standardized protocols will be included in each operative step considering their characteristics: user friendly, reliable, low costs and low environmental impact

Country activity

Inventory of citrus germplasm

Each country should carry out the prospection and inventory of the citrus germplasm using the MECINET information system. The germplasm will be selected for pomological characters and observed for diseases. A field data base (GPS) will be used for location and GIS system for mapping the selected trees.

In situ conservation of the inventoried citrus germplasm

All inventoried germplasm will be maintained in the original country in a field collection for characterization and sanitary traits.

Identification of citrus germplasm (morphological, cytological characteristics, and molecular markers)

The identification of citrus germplasm will be carried out by the use of:

- Traditional techniques

  The inventoried germplasm will be analysed for agronomical, morphological, physiological, phenological characters.
  These data will be important for the use and management of the genetic material, the establishment of a standard type of language and lack of advanced technology.

- Molecular techniques
  - RAPD's
  - AFLP
  - Microsatellite analysis

Assessment of the physiological water use efficiency of major Citrus varieties associated to main biodiversities

The major determinations will regard: photosynthetic assimilation, stomatal conductance, leaf interior CO_2 partial pressure and photosynthetic water use efficiency. Through these determinations, the Citrus characterization will account for some of the most important tree-water relationships.

Assessment of the sanitary status of the identified citrus biodiversities

The characterized material will be submitted to a mass diagnosis for the main graft transmissible pathogens using serological (ELISA and DTBIA) and molecular techniques (Dot blot).

Sampling of the germplasm for sanitation

The explant types of the germplasm to sent for sanitation are represented by flowers and *in vitro* cuttings. This mode of sampling guarantees the safe movement of germplasm between participating countries and the CGCC, if it is established abroad.

Activities of the Citrus Germplasm Conservation Center (CGCC)

Sanitation of identified citrus germplasm

The identified germplasm will be sent to the CGCC for sanitation by *in vitro* shoot-tip grafting (STG) combined or not with thermotherapy. *In vitro* somatic embryogenesis by style culture (SE), a new technique very promising for the sanitation of infected citrus genotypes, will also be used and validated during the project.

Assessment of the sanitary status of the germplasm submitted to sanitation

The complete assessment of the sanitary status of the germplasm submitted to sanitation must be carried out in the CGCC. Serological, molecular and biological assays will be conducted only for the pathogens infecting the starting material.

Ex situ conservation of the sanitated germplasm: in vitro and in vivo

The healthy germplasm should be maintained *in vitro* for the numerous

advantages of this technique in the easy management and movement of the plant material, with a low risk of germplasm variability.

The *in vivo* conservation of the core collection must be carried out under isolated conditions (insect-proof screenhouse or field), in areas free from quarantine pathogens to prevent possible reinfections.

Assessment of pomological traits in the healthy germplasm in vivo maintained

This is an important step for the assessment of the trueness-to-type of the sanitated germplasm and for the evaluation of their potential interest as cultivars. To value at best the work done all knowledge will be available through the MECINET information system.

Legislation for the free exchange of citrus germplasm

The harmonization of the existing regulations for citrus germplasm exchange among participating countries will be carried out by drafting a common legislation. Genotypes as well as related information will be available respecting the rights of the breeders.

Training and meetings

An important part of the project is devoted to training and transfer activities for gaining knowledge on project techniques and upgrading technical skills. Training will be an integral part of the project activities. Most of the methodologies are available except those newly introduced.

Duration of the project

The project is a long-term programme which can be divided in two phases:

A 4 year period is needed in the first phase in order to:

- train the projects operators
- well develop the programme for the operative steps to carry out in each participating country, facilities included (inventory, conservation, characterization, mass diagnosis and sampling for sanitation of citrus germplasm)
- harmonize the legislation for the free exchange of citrus germplasm
- establish facilities for the CGCC
- establishment of the agreements among countries to favourite the free exchange of citrus germplasm
- introduce collected information in the database

A 6 year period is needed in the second phase for

- continuing training
- the activities to conduct in the CGCC (sanitation, sanitary assessment, *in vitro* and *in vivo* conservation and assessment of pomological traits of citrus germplasm)
- introducing the overall information in the database
- writing a publication

In some countries the second phase of the project can start within the activities of the first phase.

It will depend from the:

- existing germplasm in collection or in the field
- presence of certification programmes (facilities and competences)

A continuous publicity of the project activities and results will be carried out through seminars, meetings, publications during all the project steps.

Partners

Being CIHEAM/IAMB and FAO two International Organizations devoted to develop startegies for a sustainable agriculture in the Mediterranean, and the promoters of the two Mediterranean networks, MNCC and MECINET, the project activities will be carried out under their coordination and management.

Partner institutions, which will be in quite different and complementary situations, will be identified in each participating countries within MNCC and MECINET members. Their participation will depend from the political will of Mediterranean Governments to co-finance the project.

Proceedings

1993. Proceedings of the FAO-expert meeting to promote inter-country cooperation on citrus production improvement in Mediterranean countries, Adana (Turkey).

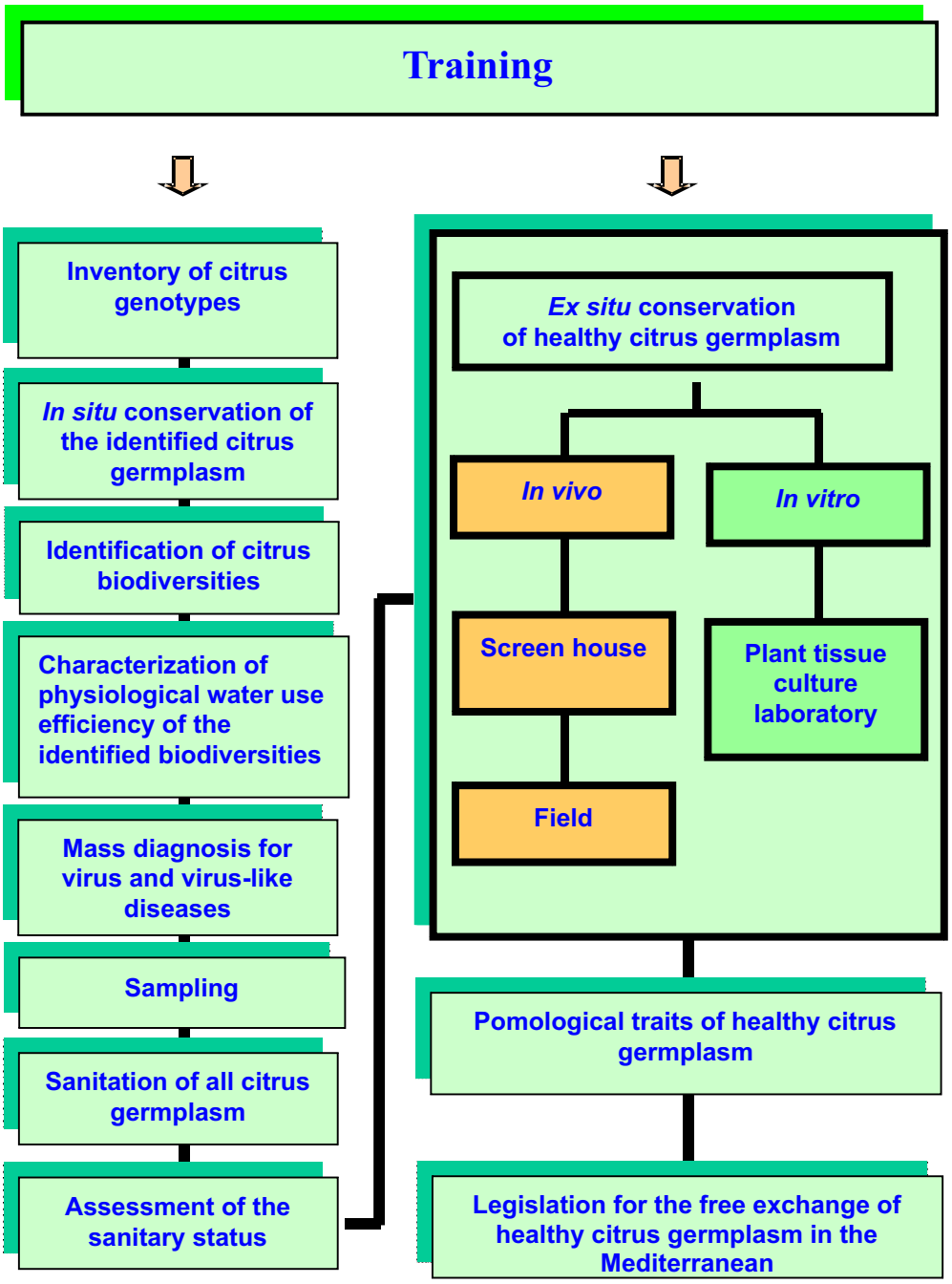
1997. Proceedings of the sectoral meeting of the Mediterranean citrus network (MECINET) on global cooperation for citrus germplasm conservation and use, Acireale (Italy)

1998. Proceedings of the Mediterranean Network on Certification of Citrus: 1995-1997. CIHEAM/IAMB publications Options Mèditerranéennes, series B, 21: studies and research

1999. Proceedings of the second general meeting of Mediterranean Citrus Network (MECINET), Adana (Turkey)

1999. Mediterranean selected fruits inter country network (MESFIN), Adana (Turkey)

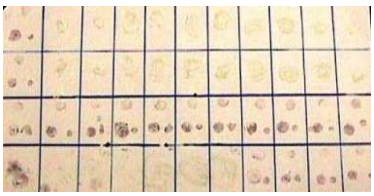
Project activities



Citrus Germplasm Conservation Center

Inventory of citrus genotypes

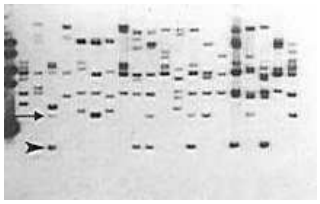
Mass sanitary diagnosis



***In situ* conservation**

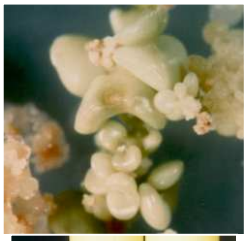
Sampling

Identification of citrus biodiversities



Regional Country

Sanitation of all citrus germplasm



Assessment of the sanitary status

Pomological traits

***Ex situ* conservation of healthy citrus germplasm**

In Vitro

In Vivo

Legislative agreement between regional countries in the Mediterranean