Working Group on Utilization of Citrus Germplasm

Introduction to Working Group on Utilization of Citrus Germplasm

Dr. Luis Navarro, INIA, Valencia, Spain

The primary uses of citrus genetic resources are direct use for commercial propagation and use in breeding programs. The primary limitations to use of citrus genetic resources for commercial propagation are pathogen infection and problems and restrictions on registered or patented cultivars. One of the most successful approaches to solving problems related to pathogen infection of citrus genetic resources is to employ shoot tip grafting in vitro. Countries where citrus shoot-tip grafting has been used for producing disease free budwood are Spain, France, Italy, Greece, Cyprus, Turkey, Israel, Morocco, South Africa, Argentina, Brazil, Colombia, Uruguay, Chile, Venezuela, Cuba, Mexico, and the USA. Recommended biological indexing methods for citrus sanitation and Quarantine Programs require the use of 8 different indicator plants. Biotechnological methods described for indexing include Serological, sPage, Hybridization, PCR, Culturing, and EM techniques.

There are limitations to use of citrus genetic resources in breeding programs. Citrus has a complex reproductive biology that includes extensive polyembryony, that masks herozygosity, a extensive juvenility period, lack of knowledge of the inheritance of most traits, and poor characterization of most genotypes. Some new tools for citrus breeding that are helping to break these traditional barriers include use of molecular markers, somatic hybridization, triploid embryo rescue, and genetic transformation.

Presentations about Disease-free Nursery Tree Certification Programs

Presentation on the Cuban certification programme

Dr. Maria del Carmen Pérez, IICT, Cuba

Cuba's production system for certified citrus planting material was initiated in the late 1960s (see Appendix 8 for full text). Prior to the start-up of the National Comprehensive Program there were no certified budwood sources in Cuba. The background of the planting material was based on selections of superior trees from commercial groves in different regions of the country. This was the first plant material for the creation of the Germplasm collection. Due to their high production, these budwood sources had a high probability of being healthy and were selected for mother trees. The improvement of the National Certification Program was needed for the massive development of citrus that occurred starting in the 1970s. In the 90s a diversification project was started to replace the citrus trees in the industry on sour orange rootstock. Development of new indexing techniques for virulent tristeza isolates was accomplished because of the appearance of Toxoptera citricida. A sanitary program was initiated to clean up the commercial selections of interest. This process included indexing of mother trees, cleaning of the material by shoot tip grafting in vitro, indexing using appropriate procedures, and maintaining healthy plants. The Citrus Germplasm Collection is now an extensive collection of commercial cultivars, rootstocks, and selections of related material. A quarantine program has been initiated to permit the import of foreign budwood while avoiding any introduction of diseases. This is an important process with very rigorous regulations. Cuba is building a high-security post-entrance center. This allows for an increase in the number of the introductions and diversification of the sources available for the Cuban citrus industry. The current certification program is aimed at producing certified budwood for propagating by the commercial nurseries, for germplasm collection, and for export. The program is mandatory and is governed by a National Certification Commission.

This national organization is concerned with maintaining a protected germplasm collection, providing plants of immediate commercial interest, and plant material of future interest. The industry includes 88 basic production units, 41 agricultural cooperatives with citrus, and 27 service and credit cooperatives associated with citrus production. The complete cycle of time from budwood clean up through commercial nurseries to grafted trees is 3.8-4.6 years. The evaluation plots are used to check for desired genetic characters. A visual inspection and 2% annual sampling for CTV is routine. Other aspects of the program are registered seed fields with routine sampling of these registered trees. Protected production is maintained by a technological system for supervision and control of pests and disease to guarantee the healthiness of the citrus plants. There has been the creation of capacities for the biological and biochemical assays needed for testing. One of the main results of this effort is the protection of Cuban citriculture. As there were in Cuba, there are constraints for disease free planting material production at the regional level. There are severe limitations that Cuba would like to contribute to solving. Regional projects for production of disease free planting material have been proposed and funding requested.

Presentation on the Argentinean certification programme
Dr. Catalina Anderson, INTA, Argentina

Some of the major citrus industry problems in Argentina include fruit fly that causes severe damage to citrus production and psorosis, which is the most important disease killing approximately 5% orange and grapefruit trees each year. Moreno et al. (1990) estimated that bud transmitted diseases cause production losses from 15 to 25% worldwide. Argentina has most of these diseases in its commercial orchards. The Concordia Introduction and Sanitary Center (CUIS) is located at Concordia, Entre Rios (NEA) and is part of Procitrus. It is responsible for the selection of high quality trees in local orchards, introduction of new varieties from breeding programs or from citrus producing areas. The indexing strategy includes indicator plants for tristeza and psorosis, molecular analysis for exocortis and cachexia-psorosis, and serology for tristeza, CVC, and citrus canker.

The Catamarca Foundation Block Center is located in Catamarca. The mother tree plot includes only commercial cultivars and rootstocks demanded for the different regions. At least 4 trees of each variety are maintained. This center is responsible for gathering information about the performance of each mother tree including tree size. The mother trees will be inspected annually to detect any genetic changes or health problems. The Budwood Multiplication Centers (CIR) increase budwood produced (CPMC, Catamarca). In conclusion, Procitrus is the propagation source for the Argentinean Certification Program and now has 117 virus-free cultivars available. A production increase of 15% is expected in orchards using propagation material from Procitrus. Not only will Procitrus result in higher production, but also premium fruit quality. The control of citrus nurseries will be done with a certification system implemented by INIA.

Discussions and Identification of Potential Cooperative Activities

Certification programs are being established in more and more countries each year. However, there are few programs in most of the Caribbean and Asian countries. Technologies are viable. People can be trained in Spain, Italy, California, Cuba, Argentina and other locations.

Citrus utilization through certification programs and using the new tools in breeding and biotech techniques can allow us to bypass some of the past limitations in citrus. It will be much more important in the future that we get the characterization of the species so that we can

recognize were there are unique genes to be used in citrus improvement.

Dr. D'Onghia stated that the network activities should give some concern for indexing for certification. The network should evaluate testing methods which are commercially available and report on their performance. A goal should be to simplify the testing procedures so that much simpler techniques can be introduced in the protocol. Virus-free should be distinguished from virus-tested. Virus-free is an incorrect term, only virus-tested and virus undetected should be used.

In this kind activity of a cooperative network, we should try to speak the same language and use the same proven techniques to identify pathogens in plants. Through the network there is a possibility to harmonize the procedures. Certification programs are different, partially for overall procedures and goals, but partially for testing procedures. It would be good for a web-page of the network to have information regarding recommended testing and identification procedures.

Disease Indexing Methods

Dr. D'Onghia stated, it is difficult for different countries to use the same technology. I think the network is the right place to do comparative trials. Dr. Navarro stated that maybe that can be done with other new technologies.

Dr. Albrigo stated we should be sure that everyone is aware of all the methods that can be used. FAO has a section oriented to plant protection. Richard Lee at CREC is very aware of what information FAO can offer; who is the resource for what methodology. Individuals can make a comparison of the tests for themselves, or rely on previous reports. Dr. Navarro stated that FAO has an excellent book published on indexing of citrus (he is a co-author). Dr. D'Onghia stated that it includes easy to apply testing procedures for indexing. Dr. Navarro states that the information on the FAO web-page can be useful (http://www.ecoport.org/). Technical guidelines have changed since the FAO book was written in 1989. FAO needs to make a new edition of the indexing reference book. Dr. Navarro stated that with some new methodologies, information might not be freely exchanged. These new procedures also may not have the proper evaluation.

Use of Citrus Germplasm

We lack a lot of information about the characterization of citrus germplasm. Available information is extremely difficult to find and very expensive. Putting together all of the information would be a very valuable tool. Dr. Albrigo stated that a large concern is that reported holdings are often based on a collection made many years ago, which may represent only two or three plants, and this is common for most of the citrus relatives. We have little idea for most of the Citrus relatives, what diversity exists. Also, for many of the Citrus species that are basically clonally propagated due to polyembryony, we may not have much diversity collected in that germplasm as well. Likely except for a few species, perhaps a very small part of the citrus genome has been protected and preserved. Certainly most citrus species have not been tested in a proper situation (area of origin) as to what diversity exists of exploration and germplasm characterization. Those of you that are working in this area, I think need to be thinking about that. For the ultimate utilization, emphasis should be placed going back to origin areas to start exploring for citrus genetic diversity. Some of the recent work described (Australia-Vietnam, Japan-Asia) may be addressing these concerns. A big problem is access to new material. An agreement may be made to get new germplasm for programs such as discussed earlier. The

resulting cultivars may carry joint propriety with the country of origin to genetic information. Research is an easier transfer agreement. If we want to expand a breeding program with access to new material from another country, there is information regarding Material Transfer Agreements on the FAO web-site. At the very least, agreements to explore and catalog Citrus genetic diversity where it exists in-situ should be developed and pursued.

We appear to have two choices. Either work with the countries where the material is, with agreements to protect their rights to that genetic material if it exists, or with an agreement that there be the right to develop it for a fee. The evaluation work needs to be done in the country. If you help them develop the technology to evaluate the germplasm, there may be some kind of agreement that they are not the only beneficiaries. Or, eventually every country will develop their own capability. They will then be able to evaluate their native germplasm. They will have the technology and the remaining germplasm, but by then much of the diversity may be lost due to development. What will happen? The country of diversity will develop a naval orange with new disease resistance and sell it to everyone else. Maybe that is appropriate, but it will occur from a reduced germplasm pool.

Dr. Albrigo further stated that global network cooperation in trying to locate and identify citrus germplasm, where there is still native material, needs to be a high priority. Those areas may get developed if this process is delayed. Clearly there is a need to identify areas and evaluate plant material in some critically vulnerable areas. Another big issue to accomplishing this is money. There do not appear to be international funds readily available to do this in spite of the talk and agreements to protect biodiversity. I don't think that we can do much as a network regarding the access or lack of access to citrus germplasm in areas of origin. But offers by countries that can offer training in genome identification and disease indexing could be taken advantage of in cooperative agreements. This training could be used to develop or maintain citrus germplasm in areas of origin with the possibility of some access arrangement. We will publicize this information on the web-site. It is not ambitious but is realistic. It was agreed to post a list of the countries that offer training in different areas of citrus disease indexing and genome identification with their contact information on the web-site. Countries, institutions, or people with problems can find the experts on that particular problem in order to contact them and seek training or advice.