

Global Citrus Germplasm Network (GCGN)

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Orlando, Florida, USA

Working Group Reports

Working Group of Germplasm Characterization

Introduction to Working Group of Germplasm Characterization

Working Group Leader Dr. Fred Gmitter, CREC, USA

Our emphasis in the network should center on identification of potential cooperative activities. We also need to emphasize that market limitations on what citrus is used commercially does not mean that we are not using all the different citrus germplasm in research and breeding programs.

Presentation on Revised Citrus Descriptors

Christian Vernière, INRA-CIRAD, France

The updated, IRGRI Citrus descriptors list was described. Several individuals, worldwide, contributed editorial comments. The system is now very robust and comprehensive. It can be viewed at http://www.ipgri.cgiar.org/publications/pubfile.asp?/10_PUB=359.

Presentation on Molecular Markers Databases

Francois Luro, INRA-CIRAD, France

It is very useful and necessary to develop a uniform system to identify citrus genomes. Such a system can be used for establishing genetic relationships, identifying useful genes for breeding and transformation work, and for establishing the genetic identity of patented cultivars. To this purpose, currently the system using micro-satellite markers appears to be the most promising.

Question: Are we going to build one inclusive database, containing all citrus?

Answer: This working group is doing this now, characterizing collections with micro-satellite markers. Using the markers that Mike Roose at University of California, Riverside, worked with, a project to identify up to 100 markers is underway and appears feasible. Work in Brazil to develop micro-satellite markers is occurring also.

There needs to be a reasonable amount of effort to optimize the test conditions so that several laboratories can get the same reproducible results. We really need to continue with what has been established using the various markers. We need to have a posting place where new markers, as they are developed, can be published, perhaps on a web-site. We do not want to end up with duplicate markers during development, but rather an extensive library of complimentary markers. If we do not establish a standard marker system, we won't be able to tell if our assessments are properly identified. Standardization can be achieved by a system to deposit sequences and their amplification on a web-site database.

Question: What form is the data in that you are generating now?

Answer: Data is for a genotype, and then for each genotype there will be a list of the alleles that it has, so it will be a 0-1 matrix. If you have a locust with 30 alleles then you have 30 columns in the matrix.

Question: Have any of the results gone into the GRIN system?

Answer: Gene markers have not been incorporated into the GRIN database. Currently, these markers should not be considered to be fool-proof as laboratory methodology may not be standardized sufficiently to get reproducible results.

Further problems while sharing these markers may develop. The system needs to be examined cautiously.

Without standardized laboratory procedures, allele size is not going to come out exactly the same in all of those systems. To establish standardized conditions, common assessments should be used to establish that everybody can get the same results.

Question: How much plant material do you need and is plant age a consideration?

Answer: Budwood from a single source, perhaps from the USDA-Riverside or a similar collection. From the point of quarantine, it might be easier to have DNA samples sent to each lab. That would at least give everyone a core of samples, that everyone could use to establish standard lab procedures that provide the same marker identification patterns.

Question: How would everyone deal with the concern of people wanting to be the first to report something new?

Answer: Immediately after or during publication, there should be no problem in sharing markers. It is natural to be trying to do something ahead of everyone else. The different labs will help build the marker systems in their individual published work. They will find innovative ways to use the system in their germplasm characterization and breeding programs. Having all the laboratories represented and working toward a core goal of characterizing the citrus genome is a worthy effort for the network. Resolving different results that the laboratories may get with the same material will speed the process of obtaining reliable marker sequences.

Question: Is posting these marker sequences on a web-site the best way to handle the exchange of information? Could the exchange be through an e-mail list of the working group? Do we need to create a web-site for the network?

Answer: If someone provided a format for how the information should be posted then it could be done on a web-site. If some direction as to the format and standards could be agreed on for posting, everyone could provide data in the same way and it would be easy to read. This would speed-up exchange compared to waiting for published results.

There may be other alternatives, but basing the web-site at the Citrus Research and Education Center of the University of Florida is a viable option. A general homepage for the Global Citrus Germplasm Network could be established with sub-pages for general work, working group activities, future meetings, methodology format to post new markers, marker posting, citrus germplasm conservation issues, and other items.

* It was proposed that we have a GCGN web-site and that there be sub-sections for

all the different working group areas.

In posting primer information, the primer, the amplification, the system, and genotypes for the subset should be posted. Not the results of a collection, but an agreed on set of standard genomes should be used.

Question: What would be the standard materials that we would all use to describe a given set of primers, or a given marker, and so on?

Answer: Suggested standard genotypes that could be posted are in the following group: Buddha's Hands, Flying Dragon, Pummelo, Duncan, Denson or Oari Satsuma, 'Washington' navel, and Cleopatra mandarin. Marker X - alleles from this group would be used to standardize on a global basis. It was not decided on how many of this group should be used. Seven genotypes for each marker set may not be necessary.

Initially, several attendees agreed that different DNA samples could be provided. An extract of a genotype would establish one sample that will be sent to everyone interested in the project. This process would validate the technique in several labs and provide the stepping-stone for expanded. If a DNA sample is sent out, it isn't going to last forever, hopefully the procedures can be validated with reasonable assurance can you establish that you have in your collection the same source of the DNA provided. The local collection could then be used in the future. For the long-term, budwood could be obtained for any mislabeled or absent genotypes within the standard group. This is really the first step, otherwise everyone is using their own untested material, and doing their own procedure in the laboratory without any knowledge of reproducibility of results. Michael Roose and Robert Kruger are willing to extract and distribute DNA samples from the USDA, University of California, Riverside collection as long as their funding allows it. Everyone should be aware that genotypic characterization in general takes a lot of resources. China indicated that they will participate in micro-satellite characterization. Japan was not sure whether or not they will be able to participate in micro-satellite identification. Other countries did not respond during the meeting but Brazil and France are likely participants along with the USA.

There may be commercial value to this work in the future for the grower who wants to be sure he is growing the cultivar he paid for. But for now, we are concerned with the germplasm that would be used by breeders, not so much any commercial aspects. This is technology in principle, that we should be able to transfer between labs easily. For now results are too limited to compare an unknown with standard samples and get useful information.

Several subjects were brought up and discussed by the group. In the near future, in order to provide consent to licence cultivars and protect patented cultivars we will need a global agreed upon procedure to identify cultivars. Fingerprinting for cultivars of citrus may evolve from this work. Although it is potentially a very hard problem, we are applying and developing techniques that will allow us to distinguish some of those things. The current molecular techniques for characterizing citrus will not allow cultivar fingerprinting now or in the near future. We do not have the technology to target that detailed a requirement. The best current techniques, can only place cultivars into groups.

In order to move ahead, a general agreement was reached, to identify locations that are working in this area and are willing to work on common procedural standards and share marker information to allow coordinated work on citrus genome sequencing. The micro-satellites technique is the agreed procedure. Dr. Albrigo agreed that a web-site would be developed. There would be a basic link to FAO's homepage, it will have a specific address that you can go to directly, and it should be linked to all other related web-sites. We should not lose sight

of potentially easy methodology that would find some kind of DNA pattern that would allow identification of cultivars as this would have financial potential.