Avocado Plant Improvement Planning Meeting: Taking advantage of what we learned from Avocado Brainstorming 2011

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Drs. Arpaia and Smith organized a meeting to discuss the status of avocado genetics and breeding worldwide which was held in Riverside on January 11 – 12, 2012. This meeting was followed by a grower field day at the University of California South Coast Research and Extension Center in Irvine.

The Riverside meeting was attended by 14 individuals including J. Chaparro (Univ. of FL), David Kuhn (USDA-ARS Miami), Luis Herrera (Mexico), Vered Irihimovitch (Israel), Neena Mitter (Australia), Fernando Pliego (Spain), Stefan Köhne (South Africa) and Zelda Van Rooyen (South Africa). M.L. Arpaia, H. Smith, G. Douhan, V. Ashworth, J. Patrona, E. Focht attended from the University of California Riverside. Representing CAC was J. Dixon and R. Hofshi. The grower meeting had 98 participants. A tour was provided to the participants of the variety and rootstock programs, the Gwen mapping population and the CAS variety collection. The tour was followed by a sponsored lunch where each of the international researchers gave growers a brief overview of their research programs.

Currently, there are several major advances being made in the area of avocado plant genetics and overall plant improvement. At the September 2011 Avocado Brainstorming meeting in New Zealand participants received an update on the sequencing of the avocado genome project headed by Dr. Luis Herrera and his team as well as the SNP chip being developed by Dr. Ray Schnell and Dr. David Kuhn of USDA-ARS. Meeting participants also learned of recent advances in plant transformation in avocado as well as progress in culturing avocado by Dr. Fernando Pliego (Spain) and Dr. Neena Mitter (Australia). Several discussions also occurred at this meeting about collaboration and sharing of information.

M. L. Arpaia and H. Smith proposed to have several of the key researchers involved in these activities visit with scientists working on avocado plant improvement in California to discuss areas of mutual interest including sharing of information, deciding which cultivars, species to sequence and developing accessible databases for all. We also proposed that we would organize a 3-hour grower seminar to present some of this current information.

We proposed that this meeting would be held just prior to the annual PAG (Plant and Animal Genome Conference) which is held in San Diego each year. The dates of the 2012 Plant and Animal Genome XX Conference were January 14-18, 2012. The meeting to discuss the status of avocado genetics and breeding worldwide was held in Riverside on January 11 – 12, 2012. This meeting was followed by a grower field day at the University of California South Coast Research and Extension Center in Irvine on January 13, 2012.

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International avocado genetics, genomics and plant breeding working group

The White Paper – DRAFT

January 2012

<u>Summary</u>

Avocado is an economically important fruit crop grown worldwide. The Hass avocado variety currently dominates the global market. Although Hass produce good quality fruit, new varieties that complement Hass are needed to expand production and increase consumption. Market and production constraints are moving avocado production to a worldwide monoculture based on a single scion a limited number of rootstocks. This makers the industry highly vulnerable to pandemics caused by new pathogen strains. Traditional avocado breeding programs have been successful in producing new varieties with favorable characteristics. However, in practice, these breeding programs are a time consuming and a costly endeavor. The high cost of traditional breeding program is attributed to the fact that avocados have a long juvenile period and has large space requirements. This is compounded by the fact that the majority of the trees that are selected are eventually discarded after being cultivated for 5-10 years. Therefore, approaches to identify favorable genotypes at an early stage of tree development would significantly reduce the breeding costs and increase the number of favorable varieties produced by the breeding program. Marker-assisted selection is aimed at reducing the costs the breeding programs by using molecular markers that predict favorable traits. The ability to predict what parental combinations and/or progeny will have favorable traits will allow breeders to better plan breeding programs and select superior trees at the seedling stage. At least three F1 segregating populations have been established. Extensive germplasm collections also exist worldwide with the most diverse populations being found in Mexico and Israel. To exploit the genetic diversity available in avocado breeding programs and germplasm collections, genomics based approaches are needed to characterize the diversity and understand its function. A marker assisted selection tool kit will be developed using the DNA polymorphisms associated with horticultural traits. To effectively characterize the genetic diversity of avocado and develop the molecular tools necessary to improve the efficiency of avocado genetics and breeding, a working group of researchers from Mexico, Israel, South Africa, Australia, Spain and the US have formed a collaborative group to establish a worldwide genomics-breeding group.

Introduction

Avocado (*Persea americana*) is major fruit crop produced throughout the world. In 2007, avocado world production reached 3.4 million metric tons. Currently, the international avocado market is dominated by the Hass variety. Although the Hass trees produce high quality fruit, the need for improved varities is essential to expand production. Optimal avocado varieties would display drought and salinity tolerance phenotypes to reduce the cost for watering and allow for the use of reclaimed water in areas where irrigation water is limited or of poor quality. Semi-dwarfed varieties that display a high apical dominance potential would allow for the desired growth habit needed for high density planting. In order to meet optimal production, new varieties must display a low alternate bearing potential as well as high fruit quality and marketable fruit size. Lastly, varieties with different maturity seasons would allow growers to

extend avocado production over a longer harvest window. Traditional breeding programs are not effective at creating varieties with multiple favorable traits. However, implementation of marker-assisted selection will allow breeders to develop new varieties containing multiple favorable traits that will ultimately increase production for avocado growers.

Avocado Genomics

In avocado, laboratories focused on the evolution of floral patterning and plant genomes (Floral Genome Project and the Ancestral Angiosperm Genome Project) have generated approximately 16,600 expressed sequence tags (EST) as well as thousands of sequences derived from 454 pyrosequencing. Dr. David Kuhn at the USDA-ARS in Miami, Florida, has developed an avocado single nucleotide polymorphism (SNP)-array based on polymorphisms in the coding regions of Hass, Bacon, Simmonds and Tonnage. This array contains 6,000 SNPs that have been positioned on the avocado genetic map. In addition, Dr. Luis Herrera's research group at the Centro De Investigacion y Estudios Avanzados, CIEA, Mexico is continuing to work on the sequencing, assembling and annotation of the avocado genome. Using the initial genome sequence as a reference, additional avocado genotypes are being targeted for sequencing. The information generated by this sequencing project will generate a SNP database that can be used for mapping horticultural favorable traits. The genomic information, tools, and technology being makes it possible to fully analyze F1-segragating populations and harness the diversity present in the germplasm collections.

Avocado Breeding Populations and Germplasm collections

The research strategy of this working group is to utilize a quantitative genetics and genomics-based approach to map horticultural traits in avocado. An avocado F1-mapping population, consisting of 204 individuals was created approximately ten years ago at the University of California, Riverside (UCR) Agricultural Operations (AgOps) and the University of California South Coast Research and Extension Center (SCREC) in Irvine, CA. We refer to this population as the MC (Mike Clegg)-population. The population was generated using the avocado cultivar Gwen as the maternal parent in an open pollinated experiment. Paternity analysis of the progeny indicated that the paternal pollen parents were Fuerte, Bacon, Zutano and pollen donor(s) of unknown identity. Fuerte, Bacon and Zutano each comprise about 25% of the population. The cultivars Gwen, Fuerte, Bacon, Zutano and all the clones of the 204 genotypes were grafted onto the clonal Duke 7 rootstock. Each seedling genotype is replicated twice at each site to differentiate genetic and environmental effects on the phenotypes. Heritability analysis of growth rates and fruit set demonstrates that this population can be used for artificial selection. The population is segregating for multiple shoot architecture phenotypes including fasciated shoots, "knotted"-like stems, and variation in the size of the inflorescence, fruit size, fruit shape, peel color and peel texture (data not shown).

Two mapping populations have been established by Dr. David Kuhn in Miami, FL. The first F1 population was derived from a cross between Simmons and Tonnage. The second F1 mapping population was derived from a cross between Hass and Bacon. Distinct genotypes in these populations display variation in phenotypes associated with tree architecture, leaf and cold tolerance. The UC also has a fairly extensive germplasm collection at the SCREC.

In addition to the mapping populations, a diverse collection of genotypes exist in Mexico and Israel germplasm collections. The phenotypic diversity displayed by the genotypes in these

populations can also be used to map horticultural traits in conjunction with the F1-breeding populations.

The end goal of this working group is not only identify and study the genes that control horticultural favorable traits but to also utilize the polymorphic data and develop a marker assisted selection tool box that can be used to identify genotypes that will express multiple desired traits.

Scientific Goals:

The primary goal of this working group is to map horticultural traits that would benefit growers around the world.

I. Mapping avocado horticultural traits using the F1-breeding populations (David Kuhn and Vanessa Ashworth)

a. It is likely that a majority of horticultural traits are influenced by environmental conditions. Therefore, one of the goals of this project is to replicate these three breeding populations in different parts of the world, such as Israel, Australia, Mexico, Spain. In addition, the Florida mapping populations can be replicated in California and vice versa. In addition, the working group has decided that it would be beneficial to develop two additional breeding populations.

b. The next goal is develop a standardized phenotyping approach for each trait and begin the initial phenotyping in Florida and California. A standardized phenotyping will be essential for determining the expressivity of each trait under different environmental conditions across the world.

c. As stated in the introduction, Dr. David Kuhn's research group has developed an avocado SNP-array. DNA has been isolated from the individuals in the mapping populations in Florida. DNA is currently being isolated from leaf tissue from the MC-population. Lastly, the working group has decided to select specific genotypes, varieties and races to be included in this analysis. Once the DNA has been collected from F1 mapping populations and germplasm collections, the samples could be sent to Illumina who will perform the hybridization and SNP analyses.

II. Mapping avocado horticultural traits using races in the germplasm collections (David Kuhn, Vanessa Ashworth, Vered Irihimovitch and Alejandro Barrientos-Priego)

a. To complement the F1-mapping populations, the working group has proposed to identify genotypes in the germplasm collections in Florida, Israel and Mexico. It is likely that Mexican, Guatemalan and West Indian races will be a rich source of genetic variation that can be exploited for mapping horticultural traits in avocado. Therefore, the first goal of this objective is to identify "X" number of Mexican, Guatemalan and West Indian races.

b. The next step is to replicate these genotypes in different parts of the world to examine environmental effects on horticultural phenotypes.

c. Initial phenotyping can be performed in the regions where these genotypes are established.

d. Once identified, DNA will be isolated from the genotypes and used in the SNP array analysis to develop SNP map for each of the genotypes.

III. Avocado Genomics (Luis Herrera Estrella)

Once the avocado genome sequence is finished, selected genotypes can be sequenced for a fraction of the cost and in a short period of time using the reference genome. Sequencing of selected genotypes will uncover additional SNPs that can be utilized in the mapping studies. In addition, transcriptome profiling studies performed on specific tissues and at distinct developmental time points will provide the necessary framework to help predict the function of a gene. This will likely aid in identifying genes that is associated with specific horticultural traits.

IV. Avocado transformation (Neena Mitter and Fernando Pliego Alfaro)

The ability to use transformation technology will be essential for determining the function of genes/proteins identified in mapping analyses. The laboratories of Neena Mitter and Fernando Pliego Alfaro are focused on developing these technologies.