New Zealand and Australia Avocado Grower's Conference '05. 20-22 September 2005. Tauranga, New Zealand. Session 4. New germplasm and global breeding programmes. 8 pages.

AVOCADO GERMPLASM PRESERVATION AND BREEDING PROGRAM IN CALIFORNIA

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SUMMARY

The avocado breeding and germplasm preservation projects in California may ultimately provide the industry with consistently heavier producing, high-quality avocado varieties, better pollinizer varieties, and improved rootstock hybrids. The program will rely on the continued evaluation of new material generated through traditional selection techniques and the introduction of new material from other countries, as well as refined genetic techniques used to increase the efficiency of crossing and selection.

Selections from open pollinated progeny; the main approach of the breeding program for over 60 years, will probably not yield great progress without the help of modern genetic tools. Most modern plant breeding programs incorporate new molecular genetic methods. Methods that provide a systematic analytical approach to predictability will be essential to breeding progress in the future.

Keywords: avocado breeding, scion, rootstock, molecular markers, germplasm

INTRODUCTION

Avocados were introduced to California at the turn of the last century and ever since growers, enthusiasts and researchers have been hunting for improved varieties. A search through the industry's foremost annals, in particular the California Avocado Society Yearbook, reveals that many new selections of avocado were made in the industry's infancy and over subsequent years but few had commercial significance. By the 1950's around 25 different varieties of avocados were being commercially packed and shipped in California, with 'Fuerte' accounting for more than two-thirds of the production (Anon, 1957). Even though 'Hass' was discovered in the early 1930's and patented by Rudolph Hass in 1935, it was not until large-scale industry expansion occurred in the late 1970s that 'Hass' replaced 'Fuerte' as the leading California variety.

The first controlled avocado breeding programs were started in 1937 by J. W. Lesley at

UC Riverside, and in 1939 by W.E. Lammerts at UCLA (Lammerts,1943). However, it was not until Bob Bergh took over the UC Riverside breeding program in 1956 that sufficient numbers of seedlings were planted to yield a reasonable chance for finding a variety of commercial success (Bergh, 1957). Twenty years after planting and screening around 15,000 seedlings from promising parents, the first varieties with real potential for commercial success, 'Gwen', 'Whitsell' and 'Esther' were released to the industry in 1982 (Bergh and Whitsell, 1982). In a second wave, about 60 000 seedlings, predominantly derived from 'Gwen', 'Whitsell', 'Hass' and 'Pinkerton' hybrids, were planted on private properties across the industry. These massive plantings yielded only a handful of selections with commercial potential including the named varieties 'Lamb Hass', 'Sir Prize', 'Harvest', and 'GEM'.

What is most important to consider is that, up until the very recent growth in 'Lamb Hass' acreage, every successful commercial variety, including 'Fuerte' and 'Hass', was the result of selections made by private growers and avocado enthusiasts from chance seedlings. As a result this has added relatively little to our knowledge of avocado genetics because all arose from open pollination and even the maternal parent was unknown! Even the most promising breeding program introductions, like 'Gwen' and 'Lamb Hass', have been slow to gain commercial standing in the industry.

Rootstock breeding has a more recent history. While the search for improved rootstocks began in earnest after the discovery of avocado root rot (*Phytophthora cinnamomi* Rands), it was only with the commercialization of clonal rootstock propagation methods in the late 1970s that it became possible to economically reproduce clonal rootstock material and therefore introduce improved rootstocks to the industry. Clonal avocado rootstock production was first described by Frolich and Platt in 1971, and introduced commercially by W. H. Brokaw in 1977 with the first planting of 'Hass', 'Pinkerton' and 'Bacon' on clonal 'Duke 7' rootstock.

Today, the majority of new generation avocado trees planted in California are on clonal rootstocks. The California grower has an increasing selection of rootstocks to choose from and we are beginning to better understand the environmental adaptation and influence of the rootstock on the scion's productivity and growth habits.

As we move forward with rootstock and scion breeding, molecular marker-guided methodology may substantially help us accelerate the breeding of avocado. One advantage of the molecular approach is that it potentially eliminates the step of growing seedlings to mature trees: instead, seedlings could be screened for the presence of markers that correlate closely with desirable traits. To employ this approach, however, it will be essential to characterize the correlation between molecular markers (microsatellites or simple sequence repeat [SSR] loci) and productivity-related traits (Ashworth et al., 2004).

It is essential that the world industry make a serious commitment to avocado germplasm preservation. Avocado germplasm resources could provide a source of unique genes that may carry the desired characteristics for future varieties. Whether the desired character is insect resistance, cold hardiness, a unique flavor, or productivity, the great diversity observed in avocado and its relatives may hold the genes for nearly every possible commercial purpose. The integration of unique genes from this

germplasm pool may provide the genetic material for the new varieties of our lifetime. Current land clearing practices in centers of avocado evolution, along with changes in climate will likely result in tremendous loss of avocado diversity. Predictions for the future are that this trend will continue, and it is the avocado community's responsibility to collect and preserve this biodiversity before it is lost.

The California avocado breeding and germplasm preservation program is run in four related modules each as separately administered projects:

1. Conventional scion breeding: selection and evaluation of improved varieties
2. Screening and evaluation of new rootstocks with resistance to *Phytophthora cinnamomi* and other desirable traits
3. Application of molecular markers to avocado improvement
4. Germplasm preservation

CONVENTIONAL SCION BREEDING: SELECTION AND EVALUATION OF IMPROVED VARIETIES

The goal of the avocado scion breeding program, run by Dr. Mary Lu Arpaia and staff (University of California, Riverside), is to produce new avocado varieties, superior to 'Hass' with consistent high production, optimal fruit size distribution, good handling and eating quality, and an extended long season of harvest maturity. The project is also looking for better pollinizer varieties (this aspect is described in a separate paper by Dr. Mary Lu Arpaia at this meeting). The goals of this project will be achieved through continued evaluation of new material generated through traditional selection techniques, collaboration with Dr. Clegg and others as they develop refined techniques to increase the efficiency of selection, and introduction of new material from other breeding programs.

In recent years this project has concentrated on collecting seed material from open-pollinated flowers from cultivars of maternal interest. These are grown and then planted out at the University of California South Coast Research and Extension Center in Irvine, CA. Seed are being collected from 'Marvel' (BL516), 'Nobel' (BL667), 'GEM', 'Gwen', 'Lamb/Hass', and 'Sir Prize', as well as from isolation blocks at UC Riverside: Thille x GEM and BL516 x GEM. Additional isolation blocks have been established at a commercial cooperator property and include the following pairings; ('Gwen' x 'Gwen'; 'GEM' x 'Lamb Hass', 'Lamb Hass' x 'Nobel', 'Lamb Hass' x 'Thille', 'Reed' x 'Stewart', and 'Reed' x 'Lamb Hass'). 'Hass' is not being used as a parent in breeding blocks based on advice and experience of others including Dr. B. Bergh.

A major emphasis of this part of the breeding program is to establish new seedling populations in the field for evaluation and screening. About 1,000 plants will be established in the field in 2005 to augment the approximately 1,200 plants already established, and this number is expected to ramp up each year in coming seasons.

Fruit evaluations are ongoing of existing varieties, 'new' varieties, as well as recent selections. Fruit of 'Marvel', 'Nobel', 'GEM', 'Harvest', 'Hass', 'Lamb Hass', and 'Sir Prize' are still subject to ongoing evaluation from representative sites across the

industry. Material of some of these selections has been shared internationally with interesting results. For instance, the Harvest shows greater promise as a variety in South Africa and Israel than California.

Fruit evaluations from the breeding program have also begun on more several promising new selections. The program is also evaluating 'Hass'-like selections introduced from other industries including Mexico and Chile.

SCREENING AND EVALUATION OF NEW ROOTSTOCKS WITH RESISTANCE TO *PHYTOPHTHORA CINNAMOMI* AND OTHER DESIRABLE TRAITS

Dr. Greg Douhan (University of California, Riverside) has replaced Dr. Menge (recently retired) in the UCR Plant Pathology Department, and the administration of the avocado rootstock breeding program. We believe that there will be a seamless transition and that Dr. Douhan will continue the work with as much enthusiasm and productivity as we saw from his predecessor.

Aside from programs in South Africa and Australia – there are no other concerted rootstock-breeding programs in the world. This project now has more than 30 field plots testing 55 rootstocks with potential root rot resistance.

The project has four main areas of research:

1. Collection and Selection of Germplasm – They are actively investigating and importing foreign germplasm for Phytophthora root rot resistance as well as conducting an active search in southern California for trees which have survived root rot epidemics.

2. Breeding Program – Using small "breeding blocks," they are making crosses between desirable root-rot tolerant/resistant parents. Last year they tested 2016 seedlings from these blocks, but have the capacity to ramp-up to as many as 12,000 per year.

3. Screening and greenhouse evaluation of rootstocks -Initial screening is carried out by germinating seeds in the greenhouse and infesting them with three different isolates of *Phytophthora cinnamomi.* After 8-10 weeks seedlings are evaluated and those with a high percentage of surviving roots are transplanted to UC potting-mix with active *P. cinnamomi* inoculum. Rootstocks surviving this test are then planted and grown in *P. cinnamomi*-infested soils in the field (described below).

4. Field Evaluation -Rootstocks that performed well in the screening and greenhouse evaluations are further tested under field conditions. All trials include at least 20 replicate trees on each clonally-propagated rootstock. All trials include known susceptible and resistant varieties. Trials for each rootstock are conducted in at least three other locations in naturally-infested commercial fields. Trees in field trials are assessed for health, growth, and yield. Assessment for Dothiorella canker and Citricola canker are also made. Soil analysis and leaf nutrient analysis of field trees is done on most trials. Notes on soil types, *Phytophthora* pressure, phosphorus acid applications, fertilization and other grower practices are made on each trial.

Through their very active and organized approach to rootstock selection this part of the breeding program has yielded important new rootstocks for commercial application.

Results indicate that ‘Dusa’ (an introduction from South Africa), which was released two years ago, is still doing well. Other varieties which are doing well include ‘Evstro’ (another South African selection), ‘Uzi’, ‘Steddom’ and ‘Anita’. ‘Zentmyer’, an extremely hardy rootstock emerging from trials, is yielding better than expected and the fruit are consistently the largest from any rootstock. However it is susceptible to salt damage. ‘Dusa’, ‘VC 801’, ‘VC 218’, ‘Day’ and ‘Rio Frio’ appear to have good salt tolerance.

Three varieties appear to be consistently slow growing and might be useful for new high density plantings. They are ‘VC 241’ (an introduction from Israel), ‘Witney’ and ‘Frolic’. The ‘Erin’ rootstock appears to dwarf ‘Hass’ avocado. If this dwarfing is not caused by a serious incompatibility, ‘Erin’ may be useful for potted avocado or extreme high density plantings.

APPLICATION OF MOLECULAR MARKERS TO AVOCADO IMPROVEMENT

This part of the California research program is conducted by Dr. Michael Clegg and staff (University of California, Irvine) and has five objectives: (1) to develop a large number of microsatellite or simple sequence repeat (SSR) markers; (2) to map the avocado genome with at least 100 SSR loci; (3) to establish an experimental population for quantitative genetic analysis; (4) to analyze marker-trait associations in this experimental population as a means of identifying major quantitative trait loci (QTLs) of potential commercial value; and (5) to initiate a program of marker assisted selection to accelerate the genetic improvement of commercial avocado.

The researchers in this part of the program have developed a total of 127 microsatellite markers (so far) and are now focusing their efforts on the collection of data from the following established experimental population: Organized plots consisting of 200 genotypes of open pollinated ‘Gwen’ progeny grafted to ‘Duke 7’ rootstock have been established at two locations (UC South Coast Research and Extension Center, and UC Riverside Agricultural Operations). There are two replicates of each genotype planted in a randomized design at each site; 800 trees in all were planted in these two plots.

The group is collecting data in each of four trait categories: (1) tree height and canopy diameter, (2) trunk girth, (3) presence/absence of fruit, fruit productivity per tree, fruit size, dry weight, and quality, and (4) flowering data, including bloom stage, bloom intensity, bloom opening time and bloom drop, and fruit set. They are also starting observations on some other commercially interesting traits, such as tree shape and branching patterns.

GERMPLASM PRESERVATION

An important ongoing part of the California program is to maintain and improve the California Avocado Society variety block and the *Persea* and *Persea*-relatives germplasm block located at the UC South Coast Research and Extension Center, Irvine, California.

Recent improvements include the development of a database and accompanying plot maps for the variety and germplasm blocks at the facility. This way any changes to the plantings are recorded and updated in the master database maintained by Dr. Arpaia’s staff. It has been historically difficult to find funding for this part of the program and so

volunteers have been instrumental in maintaining these blocks. The volunteers maintain the existing trees and graft new and/or historical varieties on an on-going basis.

DISCUSSION

With the great diversity observed within avocado, it is not hard to imagine that in the future our breeders will develop varieties superior to 'Hass'. Varieties with consistent and higher production, better fruit size distribution, and excellent handling and eating qualities. Varieties span an extended season of harvest maturity so that all markets are abundantly supplied with high quality fruit. However, so far avocado breeding through a systematic organized conventional plant-breeding program has had limited success in California. Still today the leading cultivars from around the world originated as chance seedlings without even the female parent being known.

This seems likely to change significantly as molecular procedures can now provide the parentage of both male (pollen) and female (ovule) contributions to the embryo, making breeding and the predictability of outcomes considerably more efficient. The use of molecular procedures in avocado genetic research is likely to markedly increase the chances for obtaining superior new cultivars in our organized breeding program (Ashworth and Clegg, 2003).

While secure funding for the molecular marker work by Dr. Clegg's group has been difficult to maintain, it is hard to imagine a credible breeding program that does not incorporate these modern genetic tools. Ad hoc selection of desirable phenotypes from open pollinated progeny; the main approach of the breeding program for over 60 years, has been akin to playing the lottery and would probably continue to yield only limited progress without the help of such modern genetic methods. Methods that introduce a systematic analytical approach to predictability, such as those being developed by the Clegg group are essential.

There are basically two obstacles to the genetic improvement of avocado: (1) the absence of molecular research on avocado or other related plant taxa that can be directly leveraged into an avocado improvement program limits options; and (2) the long generation times required for avocado breeding, the lack of control of the breeding system, and the high cost of land for experimental populations imply high costs per generation.

For these reasons, Clegg and his team have tried to use what appear to be the most cost efficient and predictable approaches to the problem of avocado genetic improvement. The genetic populations they have established in the field show great promise and they will have completed the first phase of the quantitative genetics work by the middle of next year. They hope that this will provide a kind of road map for work directed towards fruit quality and other useful traits in the future. Under any circumstances it will be desirable to maintain these unique field populations for future genetic research.

The great utility of microsatellite markers rests on using them for marker assisted selection of important trait loci. The long term strategy is to do within-family selection on the Gwen progeny population, selecting for valuable traits that show a strong correlation

with particular microsatellite markers. This could be accomplished at very early seedling stages and thus allows the field testing of materials that have been pre-selected, both increasing the intensity of selection and reducing field costs. Because the selection is within the Gwen seedling population, linkage disequilibrium is high and markers moderately linked to trait loci of interest will be strongly correlated with the desired trait loci in transmission. Thus they may not require thousands of markers as is the case in other applications.

This combination of markers and quantitative genetics provides a cost effective and systematic approach to avocado improvement. There are, of course, other more sophisticated molecular tools out there, but none can be realistically pursued on a modest budget --nor is any other approach much more likely to yield more substantive progress.

It is very important to consider that this is also likely the last opportunity for a tree fruit industry anywhere in the world to do this type of research. The cost of crossing, propagating, planting and maintaining a population of trees like the 'Gwen' progeny plots is already close to prohibitive. In the authors' opinion, this is probably our one shot to establish this baseline genetic information which could then be used over a long period to improve the crop.

It is also worthwhile to note that this type of research is now central to the work of the big breeding companies working on field crop like corn and soybean. The reasons are (1) most economically desirable phenotypes are complex (determined by several or many genes) and this requires efficient programs of selection. We simply do not know enough about the genetic basis of complex phenotypes to use any other approaches. And, (2) marker assisted selection is viewed as conventional breeding and therefore does not incur the regulatory costs of transgenic making the issue of public acceptance mute.

Increasing the genetic diversity of varieties, both scion and rootstock may decrease the risk of major pest and disease invasions on what has rapidly become a susceptible monoculture in California – certainly from a scion standpoint.

Lastly, the investment of combined global industry resources into germplasm exploration, collection, maintenance, and evaluation will yield dividends to the avocado industry as it has for other major crops. The cost of avocado germplasm preservation is moderately high and should not be bourn by individual industries alone.

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