

DEVELOPMENT OF HIGHLY RELIABLE MOLECULAR MARKERS FOR WALNUT

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ABSTRACT

Utility of molecular marker analysis in walnut has been constrained in the past because the marker systems used have either been technically cumbersome (i.e. RFLP), limited in number (i.e. isozyme), or somewhat irreproducible (i.e. RAPD). Our project will overcome these limitations when we complete development of a microsatellite (a.k.a. SSR) marker system for the Persian walnut. We are working with a USDA Forest Service unit in Indiana to develop a special set of SSR markers that will differentiate and identify all of the walnut varieties used in California. We will publish the SSR fingerprints, primer sequences, and reaction conditions for each of the markers on the Internet, where they will be freely available to the nursery industry and whoever else may want to use it. This data will serve as a benchmark for the genetic identification of commercial walnut germplasm. Our fingerprints will be based on trees in the UC Davis Walnut Improvement Program, since those trees are arguably the most authentic specimens for each variety. In addition to developing this database for industry, our project will provide a major resource for other walnut research efforts, including Vito Polito's studies of pollen flow in walnut as related to Blackline disease, Gale McGranahan's walnut breeding program, Dan Potter's *Juglans* taxonomy research, and Chuck Simon's *Juglans* germplasm research.

OBJECTIVES

- I. Develop a microsatellite marker system for walnut. This is the primary objective of this project, as it will be needed to accomplish most of our other objectives. This objective will encompass several phases of research, as noted below under 'procedures'.
- II. Develop a database of DNA fingerprints for walnut cultivars. This is the objective that most immediately concerns the walnut industry. We will publish this database openly on the internet, where it will be useful to nurseries and others who wish to confirm the genetic identity of their germplasm.
- III. Develop a repository of frozen walnut DNA reference samples. To facilitate the use of the database as a tool for validating the authenticity of the genetic identity of germplasm, we will establish an archive of the DNA samples used to create the database. Aliquots of these samples will be made available to service labs and others who wish to use them for reference.
- IV. Develop a linkage map of the walnut genome. This is a very valuable byproduct of developing this marker system, as it will offer utility to future walnut breeding efforts and other genomic research in walnut. This effort will also result in a large mapping population that will be suitable for future walnut studies.

PROCEDURES

Four genomic libraries were purchased from a company in San Diego who enriched them for specific prescribed SSR motifs. Isolated clones from one of the libraries are being sequenced at a sequencing facility and primers for the flanking regions of the SSR regions are being synthesized. The primers are then tested on walnut DNA to see how well they amplify in PCR, and how polymorphic they are.

Since we want to develop markers that will be readily usable by anyone who wishes to use them, we are making every effort not to complicate any aspect of our developmental research. Therefore, we intend to use only well-established and universally available procedures. DNA is isolated from walnut varieties, from members of the mapping population, and from other walnut germplasm by a standard CTAB isolation method. PCR is performed on primer sets without a great deal of special customization, as it is our goal to develop primer sets that are quite straightforward to work with. SSR fragment analysis is performed on our analyzer by the standard methodology for this device. Because this instrument is rather expensive and not widely available, for the cultivar database, we will generate fingerprints using the more common polyacrylamide gel electrophoresis methodology in addition to the set generated using our automatic analyzer.

AFLP mapping is being performed by standard AFLP methodology, and the results are analyzed on our automatic analyzer. The mapping population was generated by selfing the variety 'Serr', which is purported to be a direct offspring of a cross between the variety 'Payne' by the wild 'PI 159568' from Afghanistan. We have a large F2 population of this 'Payne' x 159568 consisting of 45 trees that range in age from 4-9 years, and approximately 200 new seedlings that are not yet a year old. Morphological data from the older trees has been collected by Chuck Leslie using descriptors and methods published on the GRIN database, www.ars-grin.gov/npgs.

RESULTS AND DISCUSSION

- I. **Funding.** As mentioned in last year's proposal, funding for this project was being sought separately from four sources. Efforts to secure this funding were completely successful, resulting in a very healthy first year budget for this project.
- II. **DNA Isolation and Purification.** DNA was extracted and purified from 421 walnut genotypes, including the mapping population parents and F1, 45 older mapping trees, 140 mapping seedlings, 108 cultivars and breeding lines from the new 'Stuke' block, and 125 species accessions from the repository collection. This last category includes 2-5 accessions from each of the 19 repository species, and a few accessions of the related genera *Pterocarya* and *Carya*, which will serve as outliers for our genetic studies. This DNA is in stable storage in our freezers.
- III. **AFLP Mapping.** Restrictive-ligation reaction, preamplification, and selective amplification of DNA samples from six selected F2 individuals and parents were standardized to generate optimal results. Matrix standards and internal size standards have been established. Our ABI 310 DNA analyzer has been optimized to obtain maximum

reproducibility. All 64 AFLP primer combinations have been tested and optimized. Levels of polymorphism and segregation patterns are currently being analyzed with the ABI software 'Genotyper'. We hope to have a preliminary linkage map for these markers on this population by the time of this meeting.

- IV. Collaboration. As announced in last year's oral report (but not in the written proposal, as it had not yet happened at the time of the writing), we have formed a very productive collaboration on this project with a USDA Forest Service group in Indiana. They have agreed to contribute toward this project several of the initial portions of SSR cloning and primer design.
- V. SSR Primer Development. As this is written, the Indiana group is testing 450 primer sets from a walnut GA library for their potential application toward this project. We have sent them DNA from our mapping population parents, and they have tested 40 primer sets against them. Of the 40, ten have shown polymorphism in the parents, which is a very promising beginning. The Indiana group plans to have all 450 sets screened on this DNA by the time of this meeting, and we also hope to have a few of the more promising markers on the preliminary map.
- VI. Phylogenetic Analysis of *Juglans*. Ancillary to this specific project, we have made good use of the expertise of the new staff member who is performing this project by having him work part-time on a project Dan Potter started several years ago. The new scientist, Dr. Malli Aradhya, used his experience in analysis of specific regions of chloroplast genes to help fortify a phylogenetic study Dan is doing on the walnut genus. That work is now nearly ready for publication.