Molecular Characterization and Genetic Relatedness Among Pecan Cultivars Based on RAPD Markers

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Introduction:

Understanding the genetic similarity of frequently used germplasm is vital to any breeding program attempting to increase the genetic diversity of new cultivars. An accurate knowledge of the origin and parentage of parental germplasm may also lead to a better understanding of the inheritance of important genetic traits. In many crops, this information is can be obtained by pedigree analysis. In pecan this is problematic because many cultivars were developed in the late 19th and early 20th centuries by nurserymen with sometimes inadequate record keeping and protection against cross pollination, resulting in many cultivars where only the maternal parent or neither parent has been established. In addition, most cultivars represent at most two generations of controlled crosses and many are seedling selections. Because of these limitations, it is often difficult to predict those crosses most likely to increase genetic diversity.

Genetic markers are a basic tool plant breeders use for cultivar identification, pedigree analysis, and assessing genetic diversity. In pecan, only two distinct genetic markers, a lace-leaf phenotype (Marquard, 1991b) and dichogamy type (Thompson and Romberg, 1985), have been found. Several isozyme systems have been developed for pecan (Marquard, 1987; 1989; 1991a; Marquard et al. 1995; Ruter et al. 1999) and have proven useful for studying genetic diversity in natural and cultivated germplasm collections (Ruter et al. 1999, Grauke et al. 1995, Wood et al. 1998), and determining outcrossing rates (Marquard, 1988). However, the relatively small number of isozyme markers reduces their utility in assessing genetic relationships and fingerprinting cultivars.

The development of PCR-based marker systems, especially Randomly Amplified Polymorphic DNA (RAPD) markers (Williams et al., 1990), has been a boon to plant breeders and geneticists in recent years. RAPD markers have the advantage of combining low technical input with almost unlimited marker numbers. Because of this, they are often employed by pomologists whose programs are hampered by a shortage of labor and money. RAPD markers have proven extremely useful in determining genetic relationships among breeding materials and fingerprinting cultivars in many woody plant crops.

In this study our objective was to use RAPD markers to estimate genetic similarity among a group of cultivars of importance to the breeding program. In addition, RAPD-based DNA-fingerprints were developed for each of the cultivars. These fingerprints will be a valuable means of identification in pecan where most cultivars are classified by their fruit which is often not produced until several years after the tree has been planted.

Materials and Methods:

Plant Material- 12 of the 43 cultivars ('Burkett', 'Colby', 'Evers', 'Giles', 'Green River', 'Major', 'Mohawk', 'Odom', 'Peruque', 'Podsednik', 'Riverside', and 'Success') examined in this study were obtained from the variety collection at the USDA-ARS Fruit and Nut Research Unit, Byron Ga. Leaf material from the 'Jenkins' cultivar was kindly provided by Dr. William Goff at Auburn University, and the remaining cultivars were obtained from the variety collections at the Coastal Plain Experiment Station in Tifton, Ga. Cultivars were selected based on their historical importance or their importance to the breeding program as potential parents.

RAPD Markers- DNA extraction was based on a procedure developed by Porebski et al. (1997) for plants containing high polysaccharide and polyphenol components. RAPD reactions were carried out in 25 uL volumes consisting of 10 mM Tris-HCl (pH=9.0), 50 mM KCl, 0.1% Triton X-100, 3 mM $MgCl_2$, 200 uM of each DNTP (Promega Inc., Madison WI), 0.6 uM primer (Operon Inc., Almeda CA) and 1.0 U Taq DNA polymerase (Promega Inc., Madison WI) and either 2 or 8 ng of DNA (8 ng in the original amplification and 2 ng in a separate replication). Amplifications were carried out using a Mastercycler gradient thermocycler (Eppendorf Sci., Westbury NY) programmed as follows: 1 cycle of 2 min at 94° C, followed by 40 cycles of 45 sec at 94° C, 1 min at 36° C, and 2 min at 72° C with a ramp speed of 0.3° C per sec between 36° C and 72° C. The last cycle was followed by a final incubation of 8 min at 72° C and PCR products were stored at 4° C until electrophoresis. The DNA amplification products were separated in 0.7 % agarose 0.35% synergel (Diversified Biotech, Boston MA) gels using 0.5´ TBE buffer. Gels were stained with ethidium bromide and visualized under UV light. Band sizes were calculated by comparison to a 100 bp DNA ladder (Promega Inc., Madison WI).

Data Analysis- RAPD bands were scored from digital pictures as either present (1) or absent (0) for all markers for all individuals in the study. From this data a similarity matrix was constructed by the NTSYS-pc version 2.02i (Rohlf, 1998) based on the Dice coefficient, also known as the similarity coefficient of Nei & Li (1979). Clustering analysis was conducted using the unweighted pair-group method with arithmetic averages (UPGMA) and a dendogram constructed. Similarity matrixes were compared using the Mantel matrix-correspondence test (Mantel, 1967).

Results and Discussion:

Cultivar fingerprinting- All 42 cultivars in this study could be separated based on the RAPD fingerprints with one or more primers. Seven cultivars: 'Giles', 'Colby', 'Evers', 'MoneyMaker', 'Elliot', 'Wichita', and 'Sumner', could be identified through the presence or absence of a single RAPD band. All other cultivars required at least two bands to be scored in order for an identification to be made. RAPD markers have good potential for use in fingerprinting pecan cultivars. Judicious use of a few primers that produce multiple bands will provide a relatively high degree of certainty that the cultivar is correctly identified.

Genetic relationships among samples- The cultivars analyzed in this test represent a wide range of germplasm consisting of cultivars developed in breeding programs, cultivars selected from seedling orchards, and cultivars selected from native stands from a wide geographical range (Table 1). The two most genetically similar cultivars in this test group were 'Schley' and 'Mahan', with a similarity coefficient of 0.91. The two most dissimilar cultivars with 'Elliot' and 'Barton', with a similarity value of 0.46. The average similarity over all cultivars in this test group was 0.66. When only parent-offspring values were averaged, the average genetic similarity increased to 0.80.

A dendogram constructed from the similarity data shows relatively indistinct groupings among the different cultivars (Figure 1). However, a few prominent groupings could be discerned. 'Success' and 'Pabst', were selected from the same seedling orchard (KenKnight, 1970) and may have a similar pedigree. These two cultivars are grouped with 'Desirable' and 'Forkert', both of which have 'Success' as the maternal parent. The largest group consists of 'Schley' and its likely progeny 'Mahan', along with 'Cape Fear', 'Kiowa', 'Moreland', 'Sioux', 'Oconee', 'Mohawk', and 'Wichita', all of which have 'Schley' or 'Mahan' as a parent. Other smaller clusters such as 'Evers', 'Osage', and 'Shoshoni' also represent parent cultivars and their progeny. The cophenetic correlation coefficient was relatively low at only 0.691. The coefficient was most likely reduced because of the presence of several cultivars such as 'Forkert', 'Kiowa', and 'Pawnee' that are progeny of two cultivars that are not closely related. This forces the progeny to be grouped with only one of the parents, reducing the overall correlation coefficient.

However, most progeny were grouped with at least one of the parents, supporting the accuracy of the similarity coefficients.

Pedigree analysis- A large number of pecan cultivars are of unknown or questionable pedigree (Table 1). This is because many were selected from seedling orchards where only the maternal parent or neither parent was known, or they were produced early in the century before efficient means of pollination control of this wind-pollinated species were established (Sparks, 1992). We were therefore interested in using the information gathered in this study to examine the putative origins of several cultivars.

'Mahan' is a well-known older cultivar that has been widely used in pecan breeding (Sparks, 1992). The cultivar originated from a seed planted in about 1910 by J.M. Chesnutt (KenKnight, 1970). Thompson and Romberg (1985) proposed 'Schley' as a parent of 'Mahan' based upon the inheritance of unnamed characters. They also suggest that 'Mahan' may be a self of 'Schley' because 'Mahan' is homozygous dominant *PP* for heterodichogamy. This is a rare genotype since most crosses are between protogynous (*PP* or *Pp*) and protandrous (*pp*) genotypes. The high level of similarity between 'Schley' and 'Mahan' (0.91) provides good support that 'Schley' is the parent of 'Mahan'. The presence of three RAPD bands in 'Mahan' but not in 'Schley' (data not shown) does not support the hypothesis that 'Mahan' resulted from a self of 'Schley' since RAPD bands are inherited in a dominant manner.

The 'Sumner' cultivar originated as a seedling tree identified in Tift County GA in about 1932. No record exists as to the possible pedigree of this cultivar, but the nut shape is similar to 'Schley' and it is occasionally sold as "Jumbo Schley" (Sparks, 1992). The genetic similarity between 'Schley' and 'Sumner' is 0.82, providing strong evidence that 'Schley' may be a parent of 'Sumner'. The only other cultivar with a comparable level of similarity is 'Moreland', but 'Moreland' originated after 'Sumner' was developed, and is likely a half-sib of 'Sumner', with 'Schley' as the common parent.

The 'Moreland' pecan was propagated from a sprout originating below the graft of a tree purchased from the Bass Pecan Company around 1945 (O'Barr et al., 1990). Because of its origin and its similarity in appearance to 'Schley', 'Schley' has been proposed as a probable parent. 'Moreland' is genetically most similar to 'Schley' (0.79), 'Mahan' (0.80) and 'Sumner' (0.81) in this group of cultivars. Of these three, 'Sumner' is the least likely to have been a parent because although the tree was discovered in 1932, it was not widely disseminated until recently (Sparks, 1992).

'Kiowa' was selected from a cross between 'Mahan' and 'Odom' made in 1953 by L.D. Romberg of the U.S. Pecan Field Station in Brownwood, Texas. Isozyme analysis later indicated that this parentage was incorrect because both of the putative parents express the *bb* genotype for the isozyme Mdh-1 and 'Kiowa' has an *ab* genotype at this locus (Marquard, 1987). The authors proposed that 'Mahan' was the maternal parent based upon the similar morphology of the leaves. 'Desirable' was proposed as a likely paternal parent based upon similarity of nut size and shape and isozyme genotype. Our results provide additional support for this inheritance given the high genetic similarity between 'Kiowa' and 'Mahan' (0.84) and 'Desirable' (0.80). The only other cultivar in the test group with an equally high similarity to 'Kiowa' is 'Schley' (0.80), which is the maternal parent of 'Mahan'.

'Gloria Grande' originated as a selection from a South Carolina seedling orchard (Worley, 1974). 'Stuart' has been suggested as a possible parent of 'Gloria Grande' due to similarities in tree form and nut characteristics. The wide-spread planting of 'Stuart', and the high level of genetic similarity between these two cultivars (0.86) provides additional support for this conclusion.

The results of this study clearly indicate the utility of RAPD markers for the detection of genetic variation in pecan. RAPD markers have good potential for identifying pecan cultivars, and would be especially useful in identifying young trees that have not yet begun to fruit. The genetic similarity values developed in this study provide breeders with a starting point for increasing the genetic diversity in their crosses. For the most part, these estimates show good agreement with known pedigrees, but little is known about the origins of many popular pecan cultivars. That many prior conclusions on the pedigree of these cultivars based upon physical similarities have been supported by this study is a testament to prior researchers keenness of observation and familiarity with the plant material.

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Table 1. Parentage and origin of pecan cultivars used in this study.

Cultivar	Parentage[a]	Origin[b]	Source Date[c]
Barton	Moore ´ Success	TX, Brownwood	1937
Burkett	Native	TX, Callahan Co.	1900
Caddo	Brooks ´ Alley	GA, Philema	1922 or 1923
Candy	Seedling	MS, Ocean Springs	1913
Cape Fear	Scley Sdlg.	NC, Willard	1912
Cheyenne	Clark ´ Odom	TX, Brownwood	1942
Colby	Native	IL, Clinton Co.	ca 1940
Curtis	Turkey Egg Sdlg.	FL, Orange Heights	1886
Desirable	Success ´ Jewett	MS, Ocean Springs	early 1900's
Elliot	Seedling	FL, Milton	1912
Evers	Seedling	Nut from Mex. or S. TX	before 1950
Forkert	Success ´ Schley	MS, Ocean Springs	ca 1913
Giles	Native	KS, Chetopa	ca 1927
Gloria Grande	Seedling	SC, Elloree	1923
Green River	Native	KY, Henderson	ca 1911
Jenkins	Seedling	MS, Rena Lara	1977
Kiowa	Mahan ´ Desirable?	TX, Brownwood	1953
Mahan	Seedling	MS, Kosciusko	1910
Major	Native	KY, Henderson	1908
Mohawk	Success ´ Mahan	TX, Brownwood	1946
Moneymaker	Seedling	LA, Mound	ca 1885
Moreland	Seedling	LA, Powhatan	ca 1945
Oconee	Schley ´ Barton	TX, Brownwood	1956
Odom	Seedling	MS, Ocean Springs	1923
Oklahoma	Native	OK, Ardmore	ca 1912
Osage	Major ´ Evers	TX, Brownwood	1948
Pabst	Seedling	MS, Ocean Springs	ca 1875
Pawnee	Mohawk ´ Starking H.G.	TX, Brownwood	1963
Peruque	Native	MO, St. Charles	before 1918
Podsednik	Seedling	TX, Arlington	unknown
Riverside	Seedling	TX, Big Valley	unknown
San Saba Improved	San Saba Sdlg.	TX, San Saba	1895
Schley	Stuart Sdlg. ?	MS, Scranton	ca 1881
Shoshoni	Odom ´ Evers	TX, Brownwood	1945
Sioux	Schley ´ Carmichael	TX, Brownwood	1943
Success	Seedling	MS, Ocean Springs	ca 1890
Starking H.G.	Native	MO, Brunswick	1950
Stuart	Seedling	MS, Pascagoula	ca 1874
Sumner	Seedling	GA, Tifton	ca 1932
Western	San Saba Sdlg.	TX, San Saba	1895
Wichita	Halbert ´ Mahan	TX, Brownwood	1940
Woodard	Seedling	GA, Tift Co.	before 1954

[a] Parentage of the cultivar. Seedling denotes trees planted by man where one or both parents are unknown. Native indicates trees idetified from a natural stand. Adapted from Thompson and Young, 1985 and Sparks, 1992.

[b] State, and town or county where original tree was grown.

[c] Year tree was identified, nut planted, or cross made.