A 'Novel Lace-leafed Pecan

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Abstract. More than 30 pecan [*Carya illinoensis* (Wangenh.) K. Koch] seedlings of 'Riverside' have exhibited an unusual leaf morphology described as lace-leafed. Seedlings have relatively low vigor, compound leaves, and leaflets with deeply serrated margins with higher length : width ratios than normal seedlings. Some leaves are both pinnate and palmately compound and some leaflets are lobed. The segregation ratio of lace-leafed seedlings for one gene controlling a polymorphic genetic marker supports the hypothesis that lace-leafed seedlings result from selfing of 'Riverside.' Nine percent (three of 33) of seedlings derived from selfing of 'Riverside' exhibited the lace-leafed morphology, suggesting a recessive trait controlled by one or two genes. The gene(s) controlling this phenotype in pecan is arbitrarily designated the *ll* gene(s).

Preservation of germplasm has become increasingly important for many crop species. Identification and conservation of germplasm with aberrant morphology, physiology, or biochemistry is useful in breeding and in the characterization of the plant genome (i.e., construction of linkage maps). Many unusual characteristics have been identified in some crop species. For example, =1200 genes are known to be associated with various mutations in tomato (Rick, 1986) and the use of some traits has made a significant impact on the development of superior cultivars.

The National Plant Germplasm System of the U.S. Dept. of Agriculture-Agricultural Research Service was established in the mid-1970s to maintain and preserve germplasm of many agronomic and tree crop species (White et al., 1989). The National Clonal Germplasm Repository for Pecan, Hickories, and Chestnut was established at Brownwood, Texas, in 1984 to preserve valuable germplasm. Occasionally, specimens possessing unusual morphologies are observed in pecan, including albinism, dwarfism, and a simple-leaved morphology.

A simple-leaved pecan originating from a single 'Riverside' seedling has been propagated and maintained in Brownwood (Jones, 1978). This variant grows more slowly, has relatively limber stems, shorter internodes, and duller green leaves than normal pecans (Jones, 1978) but has produced seedlings that appear normal. This report documents a second unusual leaf morphology in pecan and provides evidence that the trait is controlled by one or two recessive genes.

In 1987, a seedling grown from an open-pollinated seed of 'Riverside' produced a lace-leafed (LL) phenotype. That seedling quickly produced compound leaves with leaflets having deeply serrated margins and lacey fo-

liage (Fig. 1A). In subsequent years, LL pecans have been recovered in two ways. First, bulk plantings of open-pollinated seed of 'Riverside' are grown in the greenhouse after stratification. Open-pollinated seed was collected from two 'Riverside' trees growing within 35 m of each other. These two mother trees were in a 5-ha planting with tree spacing of 9.1 × 9.1 m. 'Riverside' represented 0.6% of the clones present in the 5-ha block. From bulk plantings, "normal" seedlings emerged after several weeks and were eventually decapitated to enhance the growth and emergence of less vigorous LL seedlings. Three LL and three normal seedlings were recovered from a single bulk planting in 1989 and were transplanted to 8-liter containers within 6 weeks of germination. Leaf characteristics from these six seedlings were measured and compared using the second leaf flush. Self-pollinations were also made by bagging female flower clusters of 'Riverside' before receptivity and injecting pollen onto stigmas at anthesis as previously described (Smith and Romberg, 1940). Fruit were collected in the fall, stratified, and planted to produce LL seedlings.

All lace-leafed seedlings were genotyped for three biochemical markers to help identify ploidy and paternal parents of these seedlings. Starch gel electrophoresis was used to genotype each seedling for malate dehydrogenase (*Mdh-1*), phosphoglucose isomerase (*Pgi-2*), and phosphoglucose mutase (*Pgm-1*) using leaf tissue extracts (Marquard, 1987, 1991).

Lace-leafed seedlings grown in the greenhouse were readily apparent even before leaf expansion exceeded 5 cm in length. Margins of even very young leaves are more deeply serrated and hypocotyls are more prominently red than leaves of normal seedlings. The frequency of LL seedlings recovered from our bulk plantings of open-pollinated 'Riverside' seed is ≈0.2%. More than 30 LL pecans have been recovered by this method, although some mortality is associated with low seedling vigor.

Lace-leafed pecan seedlings show considerable variability in length : width ratio, degree of leaflets terminating with a long bristle-

like extension, leaf pubescence, overall seedling vigor, basal adnation of leaflets, leaflet compounding (Fig. 1B), Leaflet lobing (Fig. 1C), and the ability of stems to support top growth of the seedling without bending over. Though variability exists among individuals, each clearly exhibits the lace-leafed phenotype.

Seedling total leaf area, leaves per seedling, percentage of single leaves produced, leaflet length : width ratio, and average leaf area were statistically different between normal and LL seedlings (Table 1).

One open-pollinated 'Riverside' seed produced two epicotyls (i.e., twins) of which one epicotyl produced leaves that exhibited the lace-leaf morphology. Twin seedling production can result in spontaneous haploid production (Dweikat and Lyrene, 1990). However, isozyme analyses confirmed these two epicotyls to be genetically different, diploid, and likely result from two embryos (Table 2). After 10 weeks of growth, seedling vigor and leaf dimensions from the seventh leaflet were measured from each twin. Although grown in the same pot and attached

Table 1. Vegetative characteristics from the second flush of "normal" and "lace-leafed" (LL) seedlings from open-pollinated 'Riverside' seed. Values represent the mean from three seedlings of each type of equal age and planting date.

Factor	Seedling type ^a	
	Normal	LL
Simple leaves (%)	74	50
Leaves (no.)	9	6
Total seedling leaf area (cm ²)	765	231
Individual leaf area (cm ²)	82	39
Leaf margin		
Sinus ht (mm)	0.49	1.18
Sinus depth (mm)	0.11	0.44
Leaflet length : width ratio	2.1	3.6
Midstem caliper (mm)	3.7	2.7

^aAll characteristics are statistically different between normal and lace-leafed seedlings using a *t* test, *P* ≤ 0.05.

Table 2. Seedling vigor and leaflet factors from two seedlings produced from a single open-pollinated seed of 'Riverside' that bore one normal and one lace-leafed morphology. Seedlings were 10 weeks old and leaf factors were evaluated from the seventh leaflet.

Factor	Seedling type	
	Normal	LL
Ht (cm)	32	22
Total no. leaves	11	10
Distance between nodes 3-9 (cm)	14	7
Stem caliper		
1/3 up from root-collar (mm)	3.6	2.8
2/3 up from root-collar (mm)	3.0	2.3
Total leaf area (cm ²)	133	62
Terminal leaflet		
Area (cm ²)	94	8
Length (cm)	18	10
Width (cm)	7.5	1.6
L : W ratio	2.4	6.1
Mid-rachis diam (mm)	1.45	0.85
Isozyme genotype		
<i>Mdh-1</i>	aa	ab
<i>Pgi-2</i>	aa	ab
<i>Pgm-1</i>	ac	aa

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Table 3. Segregation of lace-leaf and normal open-pollinated seedlings of 'Riverside' for *Pgm-1* that were grown in bulk. 'Riverside' has an *ac* genotype for *Pgm-1*.

Seedlings of each <i>Pgm-1</i> genotype (no.)					Seedling phenotype
Genotype					
<i>aa</i>	<i>ab</i>	<i>ac</i>	<i>bc</i>	<i>cc</i>	
13	0	15	0	4	Lace-leaf pecan seedling ^z
120	13	110	10	1	Normal O.P. 'Riverside' seedlings ^y

^z χ^2 statistic ($\chi^2 = 5.19$, $df = 2$, $0.05 < P < 0.1$) for difference between lace-leaf pecan genotypes and expected ratio for selfed 'Riverside' (1*aa* : 2*ac* : 1*cc*).

^yFrom the frequency of *b* alleles in the normal seedlings, the probability of not seeing a *b* allele in the lace-leaf seedlings is $P = 0.048$, suggesting significant differences in pollen parents among the two groups of seedlings.

Fig. 1. (A) Typical leaf margin serrations found on lace-leafed (LL) pecan seedlings, (B) LL pecan leaf showing variation in compounding (both pinnately and less commonly palmately), and (C) leaflets from normal (left) and LL seedlings showing lobing, degree of serration, length:width ratio, and variability in leaf shapes observed on LL types.

to the same carbohydrate source (i.e., attached cotyledons), the lace-leafed seedling was smaller than the normal seedling for all measured characteristics (Table 2). Clearly, LL pecans are less vigorous than "normal" seedlings (Tables 1 and 2).

'Riverside' has been genotyped for three biochemical markers known to be simply inherited, including a gene controlling *Pgm-1*. 'Riverside' has an *ac* genotype for *Pgm-1*. The *c* allele occurs infrequently (<5%) among named clones. Presumably, LL pecans are homozygous recessive for one or more genes, and a likely parental scheme to produce LL pecans is from self-pollinated 'Riverside'. This hypothesis is supported from isozyme analysis for *Pgm-1* of 32 LL pecans. The observed segregation pattern and genotypes of *Pgm-1* in LL pecans do not deviate significantly from the expected ratio for selfed 'Riverside' (Table 3). Open-pollinated "normal" seedlings also grown in bulk were genetically more diverse, and the pollen parents of these two groups of seedlings were statistically different (Table 3).

The second method to recover LL pecans has been from controlled crosses and selfing of 'Riverside.' The frequency of LL types recovered from selfed fruit was three in 33

seedlings. The ratio of 1:11 suggests either one or two genes are responsible for the LL morphology. Presumably, 'Riverside' is heterozygous for the relevant gene(s) and selfing produces some homozygous recessive individuals. The observed frequency after self-pollination (three of 33) has a low ($P = 0.021$) and relatively high ($P = 0.85$) probability of occurring for a one- and two-gene model, respectively. Though the frequency of observed LL types is lower than expected for a single gene model, reduced seedling survival, vigor, and seed germination may explain the lower-than-expected frequency of LL types after self-pollination.

This lace-leafed phenotype in pecan has rarely been observed, suggesting that the recessive allele(s) carried by 'Riverside' is un-

common. At least one gene controls the lace-leafed morphology, and it is arbitrarily designated as the *ll* gene in pecan. Currently, heterodichogamy is the only morphologic trait in pecan known to be under single gene control (Thompson and Romberg, 1985). The lace-leafed, phenotype may be the second morphologic trait identified in pecan that is monogenic.

Literature Cited

- Dweikat, I.M. and P.M. Lyrene. 1990. Twin seedlings and haploids in blueberry (*Vaccinium* spp.) *J. Hered.* 81:198-200.
- Jones, R.W. 1978. A simple-leaved pecan. *Pecan South* 5:110-112.
- Marquard, R.D. 1987. Isozyme inheritance, polymorphism, and stability of malate dehydrogenase and phosphoglucose isomerase in pecan. *J. Amer. Soc. Hort. Sci.* 112:717-721.
- Marquard, R.D. 1991. Inheritance of phosphoglucose mutase in pecan. *HortScience* 26(9):1213-1214.
- Rick, C.M. 1986. Tomato mutants: Freaks, anomalies, and breeders resources. *HortScience* 21:918.
- Smith, C.L. and L.D. Romberg. 1940. Stigma receptivity and pollen shedding in some pecan varieties. *J. Agr. Res.* 60:551-564.
- Thompson, T.E. and L.D. Romberg. 1985. Inheritance of heterodichogamy in pecan. *J. Hered.* 76:456-458.
- White, G.A., H.L. Shands, and G.R. Lovell. 1989. History and operation of the national germplasm system. *Plant Breeding Rev.* 7:1-56.