Isozyme and Randomly Amplified Polymorphic DNA (RAPD) Analyses of Cherokee Rose and Its Putative Hybrids 'Silver Moon' and 'Anemone'

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ABSTRACT. Two banding patterns were revealed by phosphoglucosyltransferase (PGM) isozyme analysis of 24 accessions of Cherokee rose (*Rosa laevigata* Michx.) from eight southeastern states, based on the presence (in 5 accessions) or absence (in 19 accessions) of an additional slow-migrating band. RAPD analysis of these accessions showed a corresponding division into the same two groups determined by PGM analysis, except for two accessions with unique RAPD phenotypes. Field-grown accessions showed distinguishing morphological characters corresponding to the groupings from the isozyme and RAPD analyses. Those in the predominant isozyme and RAPD groups, as well as the two with unique RAPD phenotypes, exhibited smooth lateral stems, while those in both nonpredominant groups exhibited markedly bristly laterals. These results suggest that the 24 accessions are ramets of two major clones with one clone predominating and that, contrary to long-standing belief, the Cherokee rose has not naturalized by reseeding in the southeast. PGM and RAPD analyses of putative Cherokee rose hybrids 'Anemone' and 'Silver Moon' showed that 'Anemone' is likely to be such a hybrid but that 'Silver Moon' is not. Historical records revealed that widespread vegetative propagation of the Cherokee rose was initiated in 1820-21 and that L. Wiesener, not J.C. Schmidt, was the originator of 'Anemone'.

The Cherokee rose (*Rosa laevigata* Michx.) was believed to be a U.S. native when it first came to the attention of plant enthusiasts in the late 18th and early 19th centuries (Lindley, 1820). This belief persisted into the 20th century and is so stated in the resolution adopting it as the Georgia state flower (General Assembly of the State of Georgia, 1916). Although it is now acknowledged to be a native of China, the circumstances of its entry into the United States remain obscure. It has long been reported to be naturalized widely in the southern states (Bailey, 1925; Radford et al., 1968; Torrey and Gray, 1838-40). Confusion between this and another Chinese native, the Macartney or Chickasaw rose (*R. bracteata* J.C. Wendl.), may have influenced this belief. However, personal observation since 1972 has revealed no evidence of reseeding of the Cherokee rose as if it were native, suggesting that it is not naturalized in the sense of Jackson (1928) but is persistent at dwellings and other sites of deliberate planting. In contrast, reseeding of the Macartney rose has infested ≈201,000 ha in Texas, and much effort has been devoted to its control (Garoian et al., 1984).

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Attempts to incorporate Cherokee rose genes into other roses have met with limited success (Basye, 1986; Van Fleet, 1916). One reported hybrid, 'Silver Moon', is grown widely and is available

Table 1. Accession number and collection location/source of Cherokee roses used in isozyme and RAPD analysis.

Accession no.	Location/source ²
1	Macon, Ga.
2	Slidell, La.
3	Bryan, Texas
4	Rocky Mount, N.C.
5	Natchez, Miss.
6	Murrell's Inlet, S.C.
7	Savannah, Ga.
8	Macon, Ga.
9	Macon, Ga.
10	Monroe County, Ga.
11	Tuskegee, Ala.
12	Opelika, Ala.
13	Camden, S.C.
14	Berrien County, Ga.
15	Slidell, La.
16	Slidell, La.
17	Opelousas, La.
18	Macon, Ga.
19	Lake Wales, Fla.
20	Lakeland, Fla.
21	Charleston, S.C.
22	Dixiana, S.C.
23	Columbia, S.C.
24	Chatham County, Ga.
25	Sebastopol, Calif.
26	Wilmington, N.C.
27	Wilmington, N.C.

²Except for the Slidell, La. (2, 15, 16), and Wilmington, N.C. (26, 27), accessions, multiple collections in an area were widely separated and gave no indication of a common origin.

from a number of nurseries in the United States and abroad (Dobson and Schneider, 1996). It was bred by Walter Van Fleet (1916), who said that it was the result of "Cherokee Rose pollen on the stigmas of a cross between [*Rosa*] *Wichuraiana* and *Devoniensis*..." However, 'Silver Moon' lacks prominent features of the Cherokee rose, most notably its spiny hypanthium and solitary flowers. 'Anemone' (*R. × anemonoides* Rehder), another reported hybrid, has been attributed to J.C. Schmidt (McFarland, 1930).

This paper reports 1) the results of the genetic comparisons,

using isozymes and RAPD analysis, among accessions of Cherokee roses collected in the southeastern United States; 2) comparisons among 'Silver Moon', 'Anemone', and those accessions; 3) a rediscovered account of the true origin of 'Anemone'; and 4) an early widespread vegetative distribution of the Cherokee rose.

Materials and Methods

PLANT MATERIAL. Accessions of Cherokee rose were either

Table 2. Polymerase chain reaction primers used for RAPD analysis, molecular weight of polymorphic bands, and phenotypic profiles of 27 Cherokee rose accessions.

Primer	Molecular wt (kb) ^z	RAPD phenotypic profile ^y			
		1 ^x	2 ^w	3 ^v	4 ^u
OPA-01	0.80	0	+	0	0
OPA-04	0.92	0	+	0	0
OPA-04	0.40	+	0	0	+
OPA-06	0.65	0	+	+	0
OPA-07	1.55	+	0	+	+
OPA-07	0.88	0	+	0	0
OPA-08	1.70	+	0	0	0
OPA-08	1.05	0	+	0	0
OPA-08	0.88*	+	0	*	*
OPA-08	0.52	0	+	+	+
OPA-08	0.50	+	0	0	0
OPA-14	1.22	+	0	+	+
OPA-14	0.91*	0	+	*	*
OPA-20	1.30	0	+	+	0
OPB-07	1.64	+	0	+	+
OPB-07	0.89	+	0	+	0
OPB-07	0.80	0	+	+	0
OPB-10	1.10	+	0	+	+
OPB-10	0.80*	+	0	*	*
OPB-11	1.90	0	+	+	0
OPB-11	0.40	0	+	+	+
OPB-12	1.51	0	+	+	0
OPB-12	0.66	+	0	+	+
OPB-12	0.45	0	+	0	0
OPB-15	1.50	+	0	+	+
OPB-15	1.45	0	+	0	0
OPB-15	0.83	0	+	0	0
OPB-15	0.61	+	0	+	+
OPB-18	0.88	+	0	+	+
OPC-05	1.42	+	0	+	0
OPC-05	0.91	0	+	0	0
OPC-05	0.59	+	0	+	0
OPC-05	0.50	0	+	+	+
OPC-08	0.75	0	+	+	+
OPC-09	1.47	0	+	+	0
OPC-09	1.30	+	0	+	+
OPC-09	1.00	+	0	+	0
OPC-09	0.84	+	0	+	+
OPC-09	0.80	0	+	+	0
OPC-09	0.72	0	+	+	0

^zkb = kilobase.

^y(+) = band present, 0 = band absent. Polymorphisms labeled with an asterisk (*) were confirmed only in the accessions (1, 2, 3, 5, 6, 7, 8) studied in RAPD Expt. 1 and could not be resolved in the remaining ones (4, 9 through 27) under the PCR conditions of Expt. 2.

^xProfile of 20 accessions (3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 17, 18, 19, 20, 23, 24, 25, 26, 27).

^wProfile of five accessions (1, 2, 8, 15, 16).

^vProfile of accession 22.

^uProfile of accession 21.

collected by C.A. Walker, Jr., from Bibb, Chatham, and Monroe Counties, Ga., or donated by persons from other geographical locations (Table 1). The provenance of each accession was determined to the extent possible. Except for the Slidell, La., and Wilmington, N.C., collections, either only one plant was present at a site or the donor collected material from one conveniently located plant. Only one plant each of 'Silver Moon' and 'Anemone' was donated by commercial nurseries.

ISOZYME ANALYSIS. The 24 accessions, 'Silver Moon', and 'Anemone' were surveyed using malate dehydrogenase (MDH), glucose phosphate isomerase (GPI), catalase (CAT), and phosphoglucumutase (PGM). Isozymes were characterized on starch gels using histidine citrate pH 5.7 (MDH, PGM), morpholine-citrate pH 6.1 (GPI), or lithium-borate/tris-citrate pH 8.3 (CAT) and previously described electrophoretic techniques (Mowrey et al., 1990a), except for CAT, which was studied using procedures described by Werner (1992). Multiple loci, as well as putative alleles at a polymorphic locus, were numbered in accordance with the relative anodal migration speeds of their associated isozyme bands, with 1 denoting the fastest.

RAPD ANALYSIS (EXPT. 1). DNA polymorphism analysis was conducted in two experiments using RAPD. In the first, DNA was extracted from young, unexpanded leaves (100 mg) of accessions 1, 2, 3, 5, 6, 7, and 8 (Table 1), 'Anemone', and 'Silver Moon' using the cetyltrimethylammonium bromide (CTAB) procedure (Bousquet et al., 1990). High amounts of interfering polysaccharides were removed by adjusting the dissolved DNA preparations to 1.5 M NaCl, chilling at 4 °C for 1 h, centrifuging at 10,000 g_n at 4 °C on a microfuge for 30 min, and discarding the clear, gelatinous pellet. DNA was reprecipitated with an equal volume of isopropanol, dried, and dissolved in TE buffer (10 mM tris-HCl, 1 mM EDTA, pH 8.0).

These genomic DNA preparations proved suitable for RAPD analysis and were amplified using 16 different decanucleotide primers (Operon Technologies, Alameda, Calif.) (Table 2). Polymerase chain reaction (PCR) conditions consisted of 10 mM tris-HCl (pH 9.0), 50 mM KCl, 2.5 mM MgCl₂, 0.1% Triton X-100, 1 unit of *Taq* DNA polymerase (all from Promega; Madison, Wis.), 0.08% nonacetylated bovine serum albumin (BSA), 100 μ M each of dATP, dTTP, dGTP, and dCTP, 0.2 μ M primer, and 5 ng genomic DNA in a final volume of 15 μ L. The reaction mixture was placed in the well of a 96-well microtiter plate, overlaid with 40 μ L of mineral oil, and placed in a thermal cycler (model PTC 100; MJ Research, Inc., Watertown, Mass.) programmed for 41 cycles of 1 min at 94 °C, 1 min at 35 °C, 2 min at 72 °C, and then held at 4 °C until recovery. 'Redhaven' peach was included as a control. Amplified DNA fragments were resolved by electrophoresis on 1.5% agarose gels in 1 $\times$ TBE buffer (0.090 M tris-borate, 0.002 M EDTA), visualized by ethidium bromide staining, and photographed in ultraviolet light. Fragment size was estimated using a molecular weight marker (1 kb DNA ladder; Bethesda Research Labs). Using the photographs, polymorphic bands were scored as + (band present) or 0 (band absent), and accessions were placed into pro-

file groups based on the resulting patterns (Table 2).

The similarity of 'Anemone' and 'Silver Moon' to Cherokee rose was expressed as the percentage of RAPD bands that were shared with the accessions. Because the accessions exhibited polymorphism, any band present in the putative hybrids was considered shared if it was found in any accession.

RAPD ANALYSIS (EXPT. 2). In this experiment, an additional 17 accessions (4, 9–24) (Table 1) were examined using the same 16 Operon primers (Table 2) as in Expt. 1. Accessions 1, 3, and 6 were included as controls representing the two Cherokee rose profile groups revealed in Expt. 1. Because of the difficulty experienced in routinely extracting polysaccharide-free DNA in Expt. 1, an alternative procedure was adapted from Guillemaut and Maréchal-Drouard (1992). Young unexpanded leaves (100 mg) were ground in liquid N₂ in a microfuge tube then mixed with 750 μ L of extraction buffer (100 mM NaOAc, pH 4.8, 50 mM EDTA, pH 8.0, 500 mM NaCl, 2% PVP40, and 1.4% SDS, with 60 mM β -mercaptoethanol added immediately before use; adjusted to pH 5.5). Tubes were incubated at 65 °C for 15 min and centrifuged at 10,000 g_n . The supernatant was mixed with one-third its volume of 3 M KOAc, mixed by gentle inversion, and incubated on ice for 15 min. Tubes were centrifuged at 10,000 g_n at 4 °C for 15 min to pellet the protein. The supernatant was removed and centrifuged again at 10,000 g_n for 15 min to remove all traces of debris. The supernatant was removed and mixed with 0.6 its volume of isopropanol, incubated at 4 °C for 30 min, and centrifuged at 10,000 g_n for 20 min to pellet the DNA, which was then washed twice with 50% ethanol, dried, and dissolved in 100 μ L TE. Resuspended pellets were treated with RNase A, and DNA concentrations were estimated by comparison to known λ DNA standards on mini-gels. These DNA preparations showed less evidence of polysaccharide contamination than those prepared with CTAB. PCR conditions differed from those in Expt. 1 in that *Taq* DNA polymerase and PCR reaction buffer (2.0 mM tris-HCl (pH 8.0), 0.1 mM DTT, 10 mM EDTA, 10 mM KCl, 2.5 mM MgCl₂) were obtained from United States Biochemical (Cleveland). All other conditions were unchanged.

MORPHOLOGY. Cherokee rose accessions were established in plots at the Horticultural Field Laboratory in Raleigh, N.C., and were examined for discriminating morphological characters after two growing seasons.

LITERATURE SEARCH. Records from the 18th and 19th centuries were searched for information on Cherokee rose and its cultivation. Depictions of 'Silver Moon' were retrieved from the literature and compared with the rose now grown under that name to determine if discrepancies existed in its identification.

Results and Discussion

ISOZYME ANALYSIS. Cherokee rose accessions showed no differences in banding patterns with MDH, CAT, or GPI; but, with PGM, they resolved into two groups (Fig. 1) based on the presence

Fig. 1. Phosphoglucumutase (PGM) banding pattern revealing two Cherokee rose phenotypes. Letters denote lanes, and numbers indicate Cherokee rose accessions (Table 1). Left to right: A = 4, B = 9, C = 'Anemone', D = 10, E = 1, F = 11, G = 12, H = 'Thornless Fortuniana', I = 13, J = 25, K = 14, L = 15, M = *R. $\times$ fortuniana*, N = 16, O = 2, P = 17, Q = 6, R = 'Anemone', S = 18, T = 8. Accessions not shown (3, 5, 7, 19 through 24) matched the pattern of lane A. *Pgm 2-1* and *Pgm 2-2* refer to the two putative *Pgm2* alleles in the Cherokee rose accessions. Origin at bottom of photograph.

Fig. 2. Morpholine-citrate (GPI) banding pattern of two Cherokee rose accessions and other rose cultivars. Because of poor resolution at *Gpi1*, only *Gpi2* was scored. Left to right: A = *R. banksiae lutea*, B = *R. ×fortuniana*, C = 'Thornless Fortuniana', D = *R. laevigata* seedling from South America, E = 'Silver Moon', F = Cherokee rose accession 3, G = Cherokee rose accession 4, H = *R. banksiae banksiae*. All other Cherokee rose accessions matched the pattern of lanes F and G. Although not pictured on this gel, 'Anemone' showed the same banding pattern as lane C, 'Thornless Fortuniana'.

or absence of an additional slow-migrating band, suggesting the existence of more than one clone. One group consisted of the five accessions (1, 2, 8, 15, 16) with the additional band, and the other group contained those lacking it. PGM is a monomer (Weeden and Gottlieb, 1980). Previous studies in *Prunus* L. (Rosaceae) revealed the presence of two PGM loci (Chaparro et al., 1987; Mowrey et al., 1990b) with which the banding pattern of Cherokee roses in this study is consistent. The presence of the fast-moving band in all 24 accessions suggests coding by a putative locus *Pgm1*, which is fixed for one allele in all the accessions. The presence of two additional bands in accessions 1, 2, 8, and 15 but only one of these bands in the remaining accessions suggests coding by a second putative locus, *Pgm2*, with alleles *Pgm2-1* and *Pgm2-2*. The two-banded phenotypes are interpreted as heterozygotes and the one-banded phenotype as homozygous for *Pgm2-1*. 'Anemone' appears homozygous at both PGM loci, because it shared both bands with the apparently homozygous accessions, but 'Silver Moon' shared none with any accession. CAT and MDH were uninformative, being monomorphic for all the roses.

For GPI, all the roses showed two zones of activity (Fig. 2), suggesting the presence of two putative loci, consistent with studies in other species (Gottlieb, 1981). Resolution at the *Gpi1* locus was poor, but at *Gpi2* 'Silver Moon' showed one band and 'Anemone' showed three; none of these four matched the one band shared by all accessions. 'Silver Moon' and the accessions have different single bands and thus appear to be fixed for different *Gpi2* alleles. Because GPI is a dimer (Gottlieb, 1977), the three-banded phenotype of 'Anemone' suggests that it is heterozygous at *Gpi2*.

Fig. 3. RAPD banding patterns in 6 representative gels (A–F) of 7 Cherokee rose accessions (Table 1), 'Silver Moon', 'Anemone', and 'Redhaven' peach control. Lane 1 = accession 1, lane 2 = accession 2, lane 3 = accession 3, lane 4 = 'Silver Moon', lane 5 = accession 5, lane 6 = accession 6, lane 7 = accession 7, lane 8 = accession 8, lane 9 = 'Anemone', lane 10 = 'Redhaven peach' control, lane 11 = 1 kb molecular weight ladder. Reproducible polymorphic bands among the Cherokee rose accessions are labeled with approximate molecular weight in kilobases. Primers used: A = OPA-14, B = OPB-07, C = OPB-10, D = OPB-11, E = OPB-15, F = OPC-09.

The provenances of accessions 1 and 8 (from Macon, Ga.) were traced to Thomasville Nurseries, Thomasville, Ga. Paul Hjort, the owner, does not know the source of the Cherokee rose stock that was at the nursery when he started to work there in the late 1940s, but he replenished it about 25 years ago from nearby Greenwood Plantation (P. Hjort, personal communication). Accessions 2, 15, and 16 came from Slidell, La., and have not been traced further. However, Tammia and Tranquility Nurseries were once located close to the collection sites (W. Carroll, personal communication). Tammia Nursery exchanged camellia budwood with Thomasville Nurseries (P. Hjort, personal communication), but this exchange appears to have taken place after the Cherokee roses were present in Slidell and does not seem to be the means by which the roses arrived in this area.

RAPD ANALYSIS (EXPT. 1). RAPD analysis of accessions 1–3 and 5–8 with the 16 primers revealed 40 reproducible polymorphisms

(Table 2). These accessions resolved into two RAPD phenotypic profile groups (Fig. 3), one (P1) consisting of those four accessions (3, 5, 6, 7) lacking the additional PGM band and the other (P2) consisting of those showing it (1, 2, 8).

Based on the number of RAPD bands resolved in 'Anemone' (98) and 'Silver Moon' (90), their respective similarities to Cherokee rose were 69.4% and 22.2%, suggesting a Cherokee rose parentage for 'Anemone' but arguing against one for 'Silver Moon'.

RAPD ANALYSIS (EXPT. 2). Analysis of accessions 1, 3, 4, 6, and 9–24 with the 16 primers revealed 37 polymorphisms. The different PCR conditions of this experiment failed to detect three of the polymorphisms revealed in the first. Accessions resolved into either P1 or P2 as before, except for 21 and 22, whose RAPD phenotypes differed from each other and from those of P1 and P2 (Table 2).

MORPHOLOGY. The two accessions of Cherokee rose traced to Thomasville Nurseries (1, 8) and the three from Slidell (2, 15, 16) all had markedly bristly lateral stems (Fig. 4), while those of all others were smooth—a prominent distinction present throughout the year. No additional differences were noted.

Among-test results were consistent; accessions lacking the extra PGM band had smooth laterals and were in P1, except for 21 and 22, which had smooth laterals but unique RAPD phenotypes. Similarly, those with the extra band were in P2 and had bristly laterals. These results suggest that most of the accessions studied comprise ramets of two major clones.

Three additional accessions were also studied: 25, outside the geographical range of the study (California), underwent all three analyses; 26 and 27, from Orton Plantation near Wilmington, N.C., were acquired after isozyme analysis had been conducted. All three had smooth laterals and were in RAPD group P1.

'SILVER MOON'. In contrast to certain other antique rose cultivars whose identities are doubtful (Walker, 1990), the rose currently grown as 'Silver Moon' appears to be correctly identified. None of the rose literature examined to date has revealed any disagreement concerning its identity. New (Scanniello and Bayard, 1994) and old illustrations (McFarland, 1922; Nash, 1917; Nicholas, 1930b) are consistent with the currently grown plant. For each primer used in the RAPD analysis, 'Silver Moon' showed few

bands in common with Cherokee roses (22.2%), providing strong evidence that it is not a *R. laevigata* hybrid. Neither the source nor the clone of the Cherokee rose used by Van Fleet is known, but it is unlikely that it would have accounted for the differences exhibited by 'Silver Moon' in this study. His discussion of *R. lae-*

Fig. 4. Differences in lateral stem morphology representative of Cherokee rose accessions. (left) Smooth (group P1 and accessions 21, 22). (right) Bristly (group P2).

vigata, *R. bracteata*, and other species of roses (Van Fleet, 1916) suggests that he was breeding with a correctly identified plant of the Cherokee rose. Thus, either he reported the parentage incorrectly or an error was made in pollen transfer. He left no records of his work (Nicholas, 1930a), so corroboration of the parentage from other sources seems improbable.

'ANEMONE'. A search of French horticultural literature revealed that 'Anemone' was raised by L. Wiesener, who reported having obtained seeds from E. Bretschneider, who in turn had collected them in the mountains north of Peking, China, where he was serving as the Russian embassy physician. Wiesener planted them in 1884, and the one surviving plant flowered for the first time in 1889 (Carrière and André, 1889). Although J.C. Schmidt introduced this rose into commerce in 1896 (Mottet, 1901), Wiesener is clearly its originator. RAPD analysis supports the conjecture that this rose is a hybrid of *R. laevigata*, with which it shares many bands (69.4%).

SEARCH OF THE EARLY LITERATURE. A large volume of material relating to the Cherokee rose was discovered in 19th century literature, some of it apparently unseen by previous writers on this rose. Of particular relevance to the present study is an 1820 letter to the *American Farmer* from C.E. Rowand, who had been using it for hedging on his plantation near Charleston, S.C., since about 1808 and highly recommended it to his fellow planters, offering to send "any quantity of the cuttings" free of charge, except freight (Rowand, 1820). In 1826, a list of 50 recipients of Rowand's largesse of 1820–21 was published in the *American Farmer*, revealing that material had been sent to Delaware, Georgia, Louisiana, Maryland, Massachusetts, North Carolina, Pennsylvania, South Carolina, Virginia, and Matanzas, probably in Florida, as well as to Edinburgh, Scotland (Skinner, 1826). General John Joor of Wilkinson County, Miss., began experimenting with the Cherokee rose as a hedge plant about 1822. Only 30 years later, Benjamin Wailes (1852) estimated that >1000 miles of Cherokee rose hedges existed in the Mississippi counties of Adams and Wilkinson alone. Given these circumstances, it is not difficult to see how later writers would interpret ubiquity as naturalization.

Two early records reveal the presence in the southeast of Cherokee rose plants with smooth laterals like the predominant group in this study. One plant, grown by William W. Anderson (1820) of Stateburg, S.C., had an "oblong-ovate hispid germ seated upon a short smooth peduncle," apparently indicating a smooth flowering stem. Another plant was sent by James Wilson from Savannah, Ga., to the Glasgow Botanic Garden, where it flowered in 1828 for what was said to be the first time in the British Isles. A depiction of it shows smooth laterals (Curtis, 1828). This trait, together with the origin of the plants, suggests that these two plants belong to the predominant group in this study. Lindley (1820), after having examined only two herbarium specimens of it, described as *R. hystrix* a form of *R. laevigata* with bristly laterals. Results of this study suggest that a high percentage of the Cherokee rose plants now growing in the southeast are ramets of the same clone and may have been propagated from the cuttings distributed by Rowand from Charleston in 1820–21.

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