Development of Microsatellite Markers from Crape Myrtle (Lagerstroemia L.)

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Abstract. Lagerstroemia (Lagerstroemia) is an economically important woody plant genus with several deciduous flowering ornamental species. A wide range of flower colors, long flowering periods, growth habits ranging from miniature to tree sizes, and exfoliating bark characteristics provide horticulturists and nursery growers with a great deal of interest in the breeding and genetics of this genus. We report microsatellite marker development from a GT-enriched genomic library of the interspecific hybrid ‘Natchez’ (L. indica × L. fauriei Koehne). Twelve of 43 novel microsatellite loci were characterized on a collection of 33 Lagerstroemia cultivars and accessions. Four to eight alleles per locus (mean = 5.6 alleles) were detected. Allelic richness ranged from 3.9 to 7.2 with a mean of 5.3. The level of polymorphism detected (average gene diversity of 0.68) indicates moderately high genetic diversity within the selections of crape myrtle cultivars and accessions. The examined markers also exhibited high cross-species transferability to L. fauriei, L. limii Merr., and L. subcostata Koehne.

The genus Lagerstroemia L. contains shrubs or trees that are primarily native to tropical regions of southeastern Asia and Indo-Malaysia (Furtado and Srisuko, 1969), which are cultivated, and in some cases naturalized, in temperate and tropical regions worldwide (Pooler, 2007). Of the over 50 species in the genus (Cabrera, 2004; Furtado and Srisuko, 1969), two species, L. indica and L. fauriei, comprise the majority of the cultivated ornamental flowering types in the world. These species, their selections, and interspecific hybrids are collectively known as crape myrtles and are popular ornamental shrubs/trees in many temperate regions of the United States. Crape myrtle cultivars have many attractive features such as bright flower color (white, red, purple, and their combined variants), wide diversity of growth habits (miniature shrub to large tree), prolonged flowering (up to 120 d), and appealing exfoliating bark that exposes cinnamon- to gray-colored inner bark. Molecular tools are increasingly used for crape myrtle cultivar identification, parentage comparison, and interspecific hybrid analysis (Pooler, 2003; Pounders et al., 2007). We report the development of microsatellites from crape myrtle ‘Natchez’ (L. indica × L. fauriei) using a biotin enrichment protocol (Wang et al., 2007) and test their amplification in 33 L. indica cultivars (including interspecific hybrids) and accessions. These polymorphic markers were also tested for cross-species amplification in three other Lagerstroemia species (L. fauriei, L. limii, and L. subcostata). These microsatellites will be useful in assessing genetic diversity of cultivars and hybrids of L. indica and L. fauriei and other Lagerstroemia species.

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(BIO-RAD, Foster City, CA) using the following conditions: one cycle at 95 °C for 3 min, 35 cycles at 95 °C for 1 min, 50 °C for 1 min, 72 °C for 1 min, and one cycle at 72 °C for 1 min. PCR products were separated on 2% agarose gels and colonies were considered positive for an inserted microsatellite motif if a smear was present on the gel (Wang et al., 2007).

Ninety-six positive colonies were duplicated into a new 96-well plate containing 200 μL LB freezing medium and 100 μg·mL⁻¹ ampicillin (Sambrook et al., 1989). Plasmid DNA of the colonies was isolated using the PureLink™ 96 HQ Mini Plasmid DNA Purification Kit (Invitrogen) following the manufacturer’s instructions and sequenced with pJET sequencing primers following the standard BigDye 3.1 sequencing protocol. Products were resolved on an ABI3130XL genetic analysis system (Amersham, Foster City, CA).

Of the 96 positive colonies sequenced, 87 (91%) contained microsatellite repeats and 44 sequences either contained short repeats (less than five) or did not have sufficient flanking region to design optimum primers. These sequences and any duplicated sequences were discarded from the study. Consequently, 43 microsatellite primer pairs were designed using Primers3 (Rozen and Skaletsky, 2000) and synthesized by Integrated DNA Technologies.

Designed primer pairs were tested for polymorphisms using 30 cultivars (including interspecific hybrids) and three accessions from L. indica species (‘Apalachee’, ‘Arapaho’, ‘Arkansas Red’, ‘Byers Standard Red’, ‘Carolina Beauty’, ‘Centennial Spirit’, ‘Gamad I’ (Cherry Dazzle®), ‘Cheyenne’, ‘Christiania’, ‘Dallas Red’, ‘Houston’, ‘Moned’ (Chica Red®), ‘Monia’ (Majestic Orchid®), ‘Musgo-kee’, ‘Natchez’, ‘New Orleans’, ‘Orlando’, ‘Ozark Spring’, ‘Peppermint Lace’, ‘Monhid’ (Petite Orchid®), ‘Piroksla’, ‘Queen’s Lace’, ‘Whit V’ ( Tight-wad®), ‘Tonto’, ‘Twilight’, ‘Velma’s Royal Delight’, ‘Weeping Red’, ‘Wichita’, ‘William Toovey’), NA63872, NA67620, and NA69850). The polymorphic markers were also tested for cross-species amplification in L. fauriei (‘Kiowa’), L. limii, and L. subcostata (NA40181). Microsatellite amplification was performed using the following PCR conditions: 10-μL reaction containing 1 ng genomic DNA, 1 × GoTaq green reaction buffer (Promega), 2.5 mM MgCl₂, 0.2 mM dNTPs, 0.25 μM of each primer, 0.2 U GoTaq DNA Polymerase (Promega), and sterile water. The PCR profile was conducted on a MyCycler Thermal Cycler (BIO-RAD) consisting of 3 min at 95 °C, 35 cycles of 95 °C for 40 s, 55 °C for 40 s, 72 °C for 4 min.

Twelve of the 43 microsatellites primer pairs successfully amplified genomic DNA and showed polymorphisms on 3% Metaphor® agarose gels (Lonza, Rockland, ME). These PCR products were also analyzed on a QIAxcel Capillary Electrophoresis System (QIAGEN) using a 25-bp DNA step ladder to allow consistent allele size calling for all loci and samples tested. All loci were polymorphic and produced clear and reproducible alleles within the expected size range (Table 1). Linkage disequilibrium and deviations from the Hardy-Weinberg equilibrium (HWE) for 12 loci were calculated using GENEPOP Version 4.0.10 (Rousset, 2008). All loci showed significant deviations from HWE. These deviations probably resulted from the small population size and the absence of nonrandom mating resulting from the selection process used to sample the cultivars tested in this study. Linkage disequilibrium was not significant (P < 0.05) for all pairwise comparisons of the 12 loci tested. The allele numbers per locus (A) and allelic richness (R̄) (El Mousadik and Petit, 1996) were computed using Fstat 2.9.3.2 software (Goudet, 2001). The number of alleles per locus detected ranged from four to eight with an average of 5.58. Allelic richness for all samples tested ranged from 3.94 to 7.17 with an average of 5.25. Observed heterozygosity (gene diversity) ranged from 0.54 to 0.75 with a mean of 0.68 and expected heterozygosity ranged from 0.06 to 1.00 with an average of 0.43. This moderately high genetic diversity indicated that there is genetic variation in the limited genetic germplasm of crape myrtle breeding programs. Results of this study indicate that the 12 microsatellite loci characterized here have potential in genetic diversity analyses across the genus Lagerstroemia. The 30 cultivars and three accessions from L. indica (including 10 of their interspecific hybrids) and another three Lagerstroemia species (L. fauriei, L. limii, and L. subcostata) used in this study represent a broad spectrum of ornamental and horticultural traits found in crape myrtle and the genetic diversity is reflected in the polymorphic content of some loci (for example, LID020, LID031, LID032, and LID038). These loci will be useful for genetic linkage mapping, population and genetic studies as well as for future crape myrtle breeding programs. Cross-species amplification results indicate that the L. indica microsatellite markers

<table>
<thead>
<tr>
<th>Locus</th>
<th>Sequence (5'-3')</th>
<th>GenBank acc. no.</th>
<th>Tm (°C)</th>
<th>Repeat</th>
<th>Sₑ (bp)</th>
<th>Sₒ (bp)</th>
<th>A</th>
<th>Hₑ</th>
<th>Hₒ</th>
<th>Rₛ</th>
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<tr>
<td>LID001</td>
<td>F-AGAGAAGAGGAGGAGCGGAGATT R-ACCTCTCCCCAATTAACCC</td>
<td>GQ402050</td>
<td>55</td>
<td>(GT)⁶GC(GT)⁶</td>
<td>213</td>
<td>186–214</td>
<td>4</td>
<td>0.22</td>
<td>0.68</td>
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<tr>
<td>LID004</td>
<td>F-ACGTTCGCTACGCTTTGGCGACT R-GGACATGCCTTGCAATTAATCC</td>
<td>GQ402053</td>
<td>55</td>
<td>(GT)⁷</td>
<td>181</td>
<td>150–184</td>
<td>5</td>
<td>0.06</td>
<td>0.66</td>
<td>4.44</td>
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<tr>
<td>LID009</td>
<td>F-GTGGGAGATGTCAACAAGCGAAG R-ATGGATTGCCTGTAGGGTTCCTT</td>
<td>GQ402058</td>
<td>55</td>
<td>(TG)⁶</td>
<td>161</td>
<td>119–175</td>
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<td>0.56</td>
<td>0.66</td>
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<td>LID019</td>
<td>F-GGGAGGATAAACGCTTGTAGCGAG R-GTAAGTACGGACACCTGGTATGC</td>
<td>GQ402068</td>
<td>55</td>
<td>(TG)⁸</td>
<td>183</td>
<td>170–196</td>
<td>5</td>
<td>0.36</td>
<td>0.69</td>
<td>4.99</td>
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<tr>
<td>LID020</td>
<td>F-GTGGGAGATTGGCTTGGTACCC R-ATACCCGGAGCATTTACACCA</td>
<td>GQ402069</td>
<td>55</td>
<td>(TG)⁹</td>
<td>219</td>
<td>196–244</td>
<td>8</td>
<td>0.69</td>
<td>0.71</td>
<td>7.17</td>
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<tr>
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<td>F-CCCTCTACCTTTCCTTACGGTCA R-ATCCTTTGAAAAACTTTCTTCTC</td>
<td>GQ402073</td>
<td>55</td>
<td>(AT)³</td>
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<td>92–224</td>
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<td>0.18</td>
<td>0.63</td>
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<td>LID031</td>
<td>F-CATGGAGCATTTCCTTGCGTGA R-CCACAATCCAGAAGGAGGACCAAG</td>
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<td>55</td>
<td>(GT)¹⁹</td>
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<tr>
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<td>55</td>
<td>(TA)⁶</td>
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<td>55</td>
<td>(GT)⁸</td>
<td>207</td>
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<td>0.39</td>
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<td>LID038</td>
<td>F-ACTGGGATTCCTGTGTTTTCCTT R-CAGGGATCTGTTTTCTTCTTT</td>
<td>GQ402087</td>
<td>55</td>
<td>(AC)⁶</td>
<td>160</td>
<td>144–178</td>
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<td>0.39</td>
<td>0.72</td>
<td>5.69</td>
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<td>LID039</td>
<td>F-GCTACTTCTCTTGATCTCTCTG R-GTGGTGCTTCTGTTGCTGCTG</td>
<td>GQ402088</td>
<td>55</td>
<td>(GT)⁹</td>
<td>188</td>
<td>157–196</td>
<td>5</td>
<td>0.81</td>
<td>0.69</td>
<td>5.00</td>
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<tr>
<td>LID042</td>
<td>F-GCTTAGTTGTTCTTCTTCGTCG R-GGACATGGTGCTCCAATTACCC</td>
<td>GQ402091</td>
<td>55</td>
<td>(GC)⁶</td>
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<td>185–208</td>
<td>4</td>
<td>0.11</td>
<td>0.54</td>
<td>3.94</td>
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</tbody>
</table>

Mean 5.58 0.43 0.68 5.25

Table 1. Characterization of 12 microsatellite loci isolated from crape myrtle ‘Natchez’ (Lagerstroemia indica × L. fauriei) based on 33 Lagerstroemia cultivars and accessions.

F = forward primer sequence; R = reverse primer sequence; Tm = annealing temperature; Sₑ = expected allele size; Sₒ = observed allele size; He = expected heterozygosity; Ho = observed heterozygosity; A = allele numbers per locus; Rs = allelic richness.

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are likely to be informative in other related Lagerstroemia species such as L. fauriei, L. limii, and L. subcostata.

Literature Cited