Evaluation of Genetic Diversity and Pedigree within Crapemyrtle Cultivars Using Simple Sequence Repeat Markers

Xinwang Wang  
Texas AgriLife Research and Extension Center, Department of Horticultural Sciences, Texas A&M University System, Dallas, TX 75252

Phillip A. Wadl  
Department of Entomology and Pathology, University of Tennessee, Knoxville, TN 37996

Cecil Pounders  
USDA-ARS, Thad Cochran Southern Horticultural Laboratory, 810 Highway 26 West, Poplarville, MS 39470

Robert N. Trigiano  
Department of Entomology and Pathology, University of Tennessee, Knoxville, TN 37996

Raul I. Cabrera  
Texas AgriLife Research and Extension Center, Department of Horticultural Sciences, Texas A&M University System, Dallas, TX 75252

Brian E. Scheffler  
USDA-ARS, Genomics and Bioinformatics Research Unit, 141 Experiment Station Road, Stoneville, MS 38776

Margaret Pooler  
USDA-ARS National Arboretum, Floral and Nursery Plants Research Unit, 10300 Baltimore Avenue, Building 010A, Beltsville, MD 20705

Timothy A. Rinehart  
Texas AgriLife Research and Extension Center, Department of Horticultural Sciences, Texas A&M University System, Dallas, TX 75252

ADDITIONAL INDEX WORDS. ornamental breeding, molecular markers, cultivar diversity, germplasm, SSR

ABSTRACT. Genetic diversity was estimated for 51 Lagerstroemia indica L. cultivars, five Lagerstroemia fauriei Koehne cultivars, and 37 interspecific hybrids using 78 simple sequence repeat (SSR) markers. SSR loci were highly variable among the cultivars, detecting an average of 6.6 alleles (amplicons) per locus. Each locus detected 13.6 genotypes on average. Cluster analysis identified three main groups that consisted of individual cultivars from L. indica, L. fauriei, and their interspecific hybrids. However, only 18.1% of the overall variation was the result of differences between these groups, which may be attributable to pedigree-based breeding strategies that use current cultivars as parents for future selections. Clustering within each group generally reflected breeding pedigrees but was not supported by bootstrap replicates. Low statistical support was likely the result of low genetic diversity estimates, which indicated that only 25.5% of the total allele size variation was attributable to differences between the species L. indica and L. fauriei. Most allele size variation, or 74.5%, was common to L. indica and L. fauriei. Thus, introgression of other Lagerstroemia species such as Lagerstroemia limii Merr. (L. chekiangensis Cheng), Lagerstroemia speciosa (L.) Pers., and Lagerstroemia subcostata Koehne may significantly expand crapemyrtle breeding programs. This study verified relationships between existing cultivars and identified potentially untapped sources of germplasm.

There are more than 50 species of Lagerstroemia L. (Cabrera, 2004; Furtado and Montien, 1969), but L. indica and L. fauriei have been the most extensively used in horticultural breeding programs. Lagerstroemia indica, native to southeast Asia, is a medium to large multistemmed shrub with pink to brown bark and simple, glabrous, deciduous leaves that change from green to yellow, orange, or red in the fall. The flowers of L. indica range from 2 to 5 cm long. Lagerstroemia indica cultivars perform well as ornamental shrubs or trees in U.S. Department of Agriculture cold-hardiness Zones 7 through 9.
Lagerstroemia fauriei is found only on the Japanese island of Yakushima, has strong resistance to powdery mildew (Erysiphe lagerstroemiae E. West), is slightly more cold-hardy than \textit{L. indica}, and has an appealing exfoliating bark. Flowers are small, white, and blooming occurs only once per season (Creech, 1985). \textit{Lagerstroemia fauriei} was introduced into the U.S. National Arboretum breeding program in 1956 through seeds collected in Japan. When crossed, \textit{L. indica} and \textit{L. fauriei} produce various desirable combinations of ornamental traits such as interesting growth habits and bark colors; a wide range of flower colors, including white, pink, purple, and red; and resistance to powdery mildew and some insect pests. Over 200 crapemyrtle cultivars exist (Dix, 1999) with at least half of these available from wholesale and retail nurseries. There are 32 crapemyrtle cultivars that are protected by U.S. patents.

Most cultivars selected before the latter part of the 20th century were \textit{L. indica} seedlings chosen for unique flower color or growth habit (Egolf and Andrick, 1978). Subsequent crapemyrtle breeding has been primarily limited to interspecific hybridizations between \textit{L. indica} and \textit{L. fauriei}. Wild-collected \textit{L. indica} germplasm from China is not readily available. Additional unique \textit{L. fauriei} germplasm may not exist because it is a rare species, possibly a single population, localized to one island in Japan. Therefore, most crapemyrtle breeding programs seek new genetic combinations by crossing existing cultivars to create new combinations of ornamental traits. Genetic diversity estimates are critical for crapemyrtle breeding, germplasm management, and conservation strategies because of inbreeding depression (Pounders et al., 2006).

Breeding for new crapemyrtles also incorporates pest resistance found in existing cultivars. Many cultivars with \textit{L. fauriei} in their pedigree show resistance to two common fungal diseases that affect crapemyrtles, powdery mildew and leaf spot (Cercospora lythracearum Heald & Wolf) (Hagan et al., 1998; Williams et al., 1998). \textit{Lagerstroemia fauriei} cultivars also appear to exhibit differential resistance to flea beetles [\textit{Altica} spp. Geoffroy (Coleoptera: Chrysomelidae)] and japanese beetle [\textit{Popillia japonica} Newman (Coleoptera: Scarabaeidae)], two of the most common insect pest of crapemyrtles (Cabrera et al., 2008; Pettis et al., 2004). Several \textit{L. indica} cultivars appear to have some resistance to the crapemyrtle aphid [\textit{Tinocalis kahawaluokalani} Kirkaldy (Hemiptera: Aphididae)] (Herbert et al., 2009).

In recent years \textit{L. subcostata}, \textit{L. limiti}, and \textit{L. spectiosa} have also been used in crapemyrtle breeding (Dix, 1999; Pounders et al., 2007a). \textit{Lagerstroemia subcostata} has large flowers but displays a more limited range of flower colors (lavender, pink, white) and growth habits than \textit{L. indica}. \textit{Lagerstroemia spectiosa} is a large tree species that exhibits desirable flowering performance and display. However, desirable traits from both of these tropical species must be introgressed into cold-hardy backgrounds because their usefulness as ornamental plants is limited to southern Florida, coastal California, and Hawaii. Conversely, \textit{L. limiti} has sufficient cold-hardiness and disease resistance to be grown in temperate regions, but it has small flowers and lacks ornamental appeal.

Interspecific hybrids between \textit{L. indica} and \textit{L. fauriei} show no apparent loss of fertility (Pounders et al., 2006). Chromosome number is not reported for \textit{L. fauriei}, but cross-compatibility with \textit{L. indica} suggests that it is consistent with the basic chromosome number of \textit{x = 8} for family Lythraceae J. St.-Hil. nomen conservandum (Raven, 1975; Tobe et al., 1986). Bowden (1945) and Guha (1972) report \textit{2n = 50} for \textit{L. indica}, whereas Ali (1977) reports \textit{2n = 48}. Repeated interspecific hybridizations among \textit{L. indica}, \textit{L. fauriei}, \textit{L. limiti}, and \textit{L. subcostata} indicate broad compatibility among species (Pooler, 2006a, 2006b). However, hybridizations between \textit{L. indica} \textit{\times} \textit{fauriei} ‘Tonto’ and \textit{L. spectiosa} only produced sterile progeny suggesting cytogenetic differentiation may interfere with some combinations (Pounders et al., 2007a). Chromosome number for \textit{L. spectiosa} is reported as \textit{2n = 50} by Bowden (1945) and \textit{2n = 48} by Guha (1972).

The first genetic diversity studies on \textit{Lagerstroemia} were conducted by Pooler (2003), in which the diversity of 12 \textit{L. fauriei} clones was revealed by amplified fragment length polymorphism (AFLP) and random amplified polymorphism DNA (RAPD) molecular markers. The objectives of this study were to compare pedigree information and parentage of selected cultivars released by the U.S. National Arboretum and other breeders using molecular diversity data; 2) to assess the genetic diversity between \textit{L. indica} and \textit{L. fauriei}; and 3) to evaluate share allele sizes using pedigree-based analyses. Alleles associated with important horticultural traits could be used to accelerate future crapemyrtle breeding through molecular marker-assisted selection (MAS).

SSRs were used to DNA fingerprint 93 crapemyrtle cultivars because they are ubiquitous for most eukaryotic genomes, are codominant, and provide useful assessments of genetic diversity (Goldstein and Pollock, 1997; Pollock et al., 1998; Rossetto, 2001; Wang et al., 1994). SSRs have been used to confirm inter- and intraspecific hybridization, verify parentage, and assign genetic distinctions among cultivars of woody ornamental landscape crops (Caetano-Anollés et al., 1999; Pounders et al., 2007a; Rinehart et al., 2006). Some of the SSR loci used in this study are expected to cross-amplify in other \textit{Lagestroemia} species for future research on species diversity (Pounders et al., 2007a).

\textbf{Materials and Methods}

\textbf{Plant material.} Fifty-one \textit{L. indica} cultivars, five \textit{L. fauriei} cultivars, and 37 interspecific hybrid cultivars were used in this study (Table 1). \textit{Lagerstroemia limiti} and \textit{L. subcostata} were included as outgroups for genetic analyses. To guard against plant mislabeling, leaf samples were collected from multiple sources for most cultivars. Duplicate samples from multiple sources produced 100% identical genotype data, as expected for vegetatively propagated plants, and only one sample was analyzed further. Uneven sample sizes and deviations from Hardy-Weinberg equilibrium (HWE) resulting from selection did not alter genetic comparisons, which are based on genetic distance estimates.

\textbf{Sample processing.} SSR development has been described by Wang et al. (2010) and the PCR protocol is the same as was used in Rinehart et al. (2006) and Waldbieser et al. (2003). Briefly, SSR-enriched libraries were made from genomic DNA of \textit{L. indica} ‘Whit IV’ and \textit{L. indica \textit{\times} fauriei} ‘Tonto’ using the SSR motifs GA, AAG, ATG, and CAG. From 684 potential SSR loci that were identified, 96 polymorphic loci were tested on all samples (Table 2). DNA was extracted from 1 \times 1-cm sections of fresh leaf tissue using a Plant Mini Kit (Qiagen, Valencia, CA) and polymerase chain reaction (PCR) amplification was performed using a three-primer protocol in 96-well plates using a thermocycler (Tetrad; Bio-Rad).
Table 1. Characteristics of 93 *Lagerstroemia* cultivars used this study, including growth habit and flower color, which are the two most important horticultural traits for consumers.

| Cultivar                  | Genetic background | Tissue source | Growth habit | Flower color    |
|---------------------------|--------------------|---------------|--------------|----------------|
| Acoma                     | *L. indica* 3/4    | SHLcp         | Semidwarf    | White          |
|                           | *L. fauriei* 1/4   |               |              |                |
| Apalachee                 | *L. indica* 1/2    |               | Intermediate | Light lavender |
|                           | *L. fauriei* 1/2   |               |              |                |
| Arapaho                   | *L. indica* 11/16  | SHLcp         | Tree         | Dark red       |
|                           | *L. fauriei* 1/16  |               |              |                |
|                           | *L. limii* 1/4     |               |              |                |
| Basham’s Party Pink       | *L. indica* 1/2    | SHLcp         | Tree         | Lavender pink  |
|                           | *L. fauriei* 1/2   |               |              |                |
| Baton Rouge               | *L. indica*        | SHL            | Dwarf        | Deep red       |
| Biloxi                    | *L. indica* 1/2    | SHLcp         | Tree         | Light pink     |
|                           | *L. fauriei* 1/2   |               |              |                |
| Bourbon Street            | *L. indica*        | AUB            | Dwarf        | Watermelon red |
| Bradberry’s Wine          | *L. indica*        | SHL            |              |                |
|                           | *L. fauriei*       |               |              |                |
| Byers Wonderful White     | *L. indica*        | SHLcp         | Tree         | White          |
| Caddo                     | *L. indica*        |               | Semidwarf    | Bright pink    |
|                           | *L. fauriei*       |               |              |                |
| Carolina Beauty           | *L. indica*        | SHLcp         | Tree         | Deep red       |
| Catawba                   | *L. indica*        | SHLcp         | Intermediate | Violet purple  |
| Centennial Spirit         | *L. indica*        | SHLcp         | Intermediate | Dark red       |
| Cheyenne                  | *L. indica* 11/16  | unknown       | Semidwarf    | Bright red     |
|                           | *L. fauriei* 1/16  |               |              |                |
|                           | *L. limii* 1/4     |               |              |                |
| Chickasaw                 | *L. indica* 5/8    | AUB            | Dwarf        | Pink lavender  |
|                           | *L. fauriei* 3/8   |               |              |                |
| Chocolate Mocha (Delta Jazz) | *L. indica*       | MSU            | Tree         | Pink           |
| Choctaw                   | *L. indica* 3/4    | MSUcp         | Tree         | Light pink     |
|                           | *L. fauriei* 1/4   |               |              |                |
| Christiana                | *L. indica*        | SHLcp         | Intermediate | Deep red       |
| Comanche                  | *L. indica* 3/4    | MSUcp         | Intermediate | Coral pink     |
|                           | *L. fauriei* 1/4   |               |              |                |
| Cordon Blue               | *L. indica*        | AUB            | Dwarf        | Lavender pink  |
| Country Red               | *L. indica*        | SHLcp         | Intermediate | Dark red       |
| Dodd #1                   | AUB                |               | Tree         |                |
| Dodd #2                   | *L. indica*        | AUB            |               |                |
| Dwarf Red                 | *L. indica*        | AUB            | Semidwarf    | Watermelon red |
| Dwarf Windmill            | *L. indica*        | SHL            |              |                |
| Fantasy                   | *L. fauriei*       | SHLcp         | Tree         | White          |
| Firebird                  | *L. indica*        | AUB            | Tree         | Deep red       |
| GAMAD I (Cherry Dazzle)   | *L. indica*        | MSU            | Dwarf        | True red       |
|                           | *L. fauriei*       |               |              |                |
| GAMAD II (Raspberry Dazzle) | *L. indica*      | MSU            | Semidwarf    | Raspberry red  |
|                           | *L. fauriei*       |               |              |                |
| GAMAD III (Snow Dazzle)   | *L. indica*        | MSU            | Dwarf        | White          |
|                           | *L. fauriei*       |               |              |                |
| GAMAD IV (Ruby Dazzle)    | *L. indica*        | MSU            | Dwarf        | Red            |
|                           | *L. fauriei*       |               |              |                |
| GAMAD V (Dazzle Me Pink)  | *L. indica*        | MSU            | Dwarf        | Bright pink    |
|                           | *L. fauriei*       |               |              |                |
| Geronimo                  | *L. indica*        | FLW            | Deep red     |                |
| Glendora White            | *L. indica*        | AUB            | Intermediate | White          |
| Grey’s Red                | *L. indica*        | AUB            | Intermediate | Watermelon red |
| Hardy Lavender            | *L. indica*        | AUB            | Tree         | Medium lavender|
| Hopi                      | *L. indica* 3/4    | MSUcp          | Intermediate | Medium pink    |
|                           | *L. fauriei* 1/4   |               |              |                |
| Kiowa                     | *L. fauriei*       | SHLcp          | Tree         | White          |

*continued next page*
Table 1. Continued.

| Cultivar*                      | Genetic background  | Tissue source  | Growth habit | Flower color          |
|-------------------------------|--------------------|---------------|--------------|-----------------------|
| Lipan                         | *L. indica* 5/8    | MSUcp         | Intermediate | Reddish lavender     |
| Low Flame                     | *L. indica*        | MSUcp         | Semidwarf    | Pinkish red           |
| Majestic Beauty               | *L. indica*        | AUB           | White        |                       |
| McFadden’s Pinkie             | *L. indica* 1/4    | SHL           | Semidwarf    | Light pink            |
|                              | *L. fauriei* 1/4   |               |              |                       |
|                              | *L. subcostata* 1/2|               |              |                       |
| Miami                         | *L. indica* 5/8    | MSUcp         | Tree         | Dark pink             |
| Monia (Majestic Orchid)       | *L. indica* 1/2    | SHL           |              |                       |
|                              | *L. speciosa* 1/2  |               |              |                       |
| Muskogee                      | *L. indica* 1/2    | MSUcp         | Tree         | Lavender–pink         |
| NA54981                       | *L. indica* 3/4    | MSU           |              |                       |
| NA58483                       | *L. indica* 5/8    | MSU           |              |                       |
|                              | *L. fauriei* 1/4   |               |              |                       |
|                              | *L. amabilis* 1/8  |               |              |                       |
| Natchez                       | *L. indica* 1/2    | MSUcp         | Tree         | White                 |
|                              | *L. fauriei* 1/2   |               |              |                       |
| Near East                     | *L. indica*        | AUB           | Intermediate | Pink                  |
| New Orleans                   | *L. indica*        | AUB           | Dwarf        | Purple                |
| Nivea                         | *L. indica*        | SHL           | Semidwarf    | White                 |
| Okmulgee                      | *L. indica*        | AUB           | Semidwarf    | Dark red              |
| Orbyrn Atkins                 | *L. indica*        | AUB           | Tree         | White                 |
| Osage                         | *L. indica* 1/2    | SHLcp         | Intermediate | Medium pink           |
|                              | *L. fauriei* 1/2   |               |              |                       |
| Pecos                         | *L. indica* 1/2    | MSUcp         | Semidwarf    | Medium pink           |
|                              | *L. fauriei* 1/2   |               |              |                       |
| Peppermint Lace               | *L. indica*        | AUB           | Intermediate | Rose pink edged with white |
|                              | *L. fauriei*       |               |              |                       |
| PI237884                      | *L. fauriei*       | SHLcp         |              |                       |
| Pink Ruffles                  | *L. indica*        | MSUcp         | Semidwarf    | Medium pink           |
| Pocomoke                      | *L. indica* 5/8    | SHL           | Dwarf        | Deep rose–pink        |
|                              | *L. fauriei* 3/8   |               |              |                       |
| Potomac                       | *L. indica*        | SHLcp         | Tree         | Medium pink           |
| Powhatan                      | *L. indica*        | SHLcp         | Semidwarf    | Medium purple         |
| Prairie Lace                  | *L. indica*        | AUB           | Semidwarf    | Pink with white       |
| Red River                     | *L. indica*        | SHLcp         |              |                       |
|                              | *L. fauriei*       |               |              |                       |
| Red Velvet                    | *L. indica*        | AUB           | Intermediate | Red                   |
| Regal Red                     | *L. indica*        | SHLcp         | Intermediate | Dark pink             |
| Rosea Grassi                  | *L. indica*        | SHL           |              | Light pink            |
| Rosea Nova                    | *L. indica*        | SHL           |              |                       |
| Rubra Compacta                | *L. indica*        | MON           | Semidwarf    | Watermelon red        |
| Sacramento                    | *L. indica*        | SHL           | Dwarf        | Deep red              |
| Sarah’s Favorite              | *L. indica* 1/2    | SHLcp         | Intermediate | White                 |
|                              | *L. fauriei* 1/2   |               |              |                       |
| Seminole                      | *L. indica*        | MSUcp         | Intermediate | Medium pink           |
| Sioux                         | *L. indica* 3/4    | MSUcp         | Intermediate | Clear medium pink     |
|                              | *L. fauriei* 1/4   |               |              |                       |
| Splash of Pink                | *L. indica*        | SHLcp         | Intermediate | White and pink bicolor |
| Tonto                         | *L. indica* 3/4    | SHLcp         | Semidwarf    | Fuchsia red           |
|                              | *L. fauriei* 1/4   |               |              |                       |
| Townhouse                     | *L. fauriei*       | SHLcp         | Tree         | White                 |
| Tuscarora                     | *L. indica* 3/4    | SHLcp         | Tree         | Dark coral pink       |
|                              | *L. fauriei* 1/4   |               |              |                       |

continued next page
Table 1. Continued.

| Cultivar* | Genetic background* | Tissue source* | Growth habit | Flower color |
|-----------|---------------------|----------------|--------------|-------------|
| Tuskegee  | L. indica 3/4       | MSUcp          | Tree         | Dark pink   |
|           | L. fauriei 1/4      |                |              |             |
| Twilight  | L. indica           | SHLcp          | Tree         | Dark purple |
| Velma’s Royal Delight | L. indica       | SHLcp          | Dwarf        | Magenta purple |
| Violacea  | L. indica           | SHL            | Violet       |             |
| Whit I (Raspberry Sundae) | L. indica      | SHLcp          | Intermediate | Dark pink edged with white |
| Whit II (Dynamo)    | L. indica           | SHLcp          | Tree         | True red    |
| Whit III (Pink Velour) | L. indica     | BAN            | Intermediate | Hot pink    |
| Whit IV (Red Rocket) | L. indica        | SHLcp          | Tree         | Cherry red  |
| Whit VI (Burgundy Cotton) | L. indica     | MSUcp          | Intermediate | White       |
| Whit VII (Siren Red)  | L. indica           | SHLcp          | Intermediate | Dark red    |
| Whit VIII (Rhapsody in Pink) | L. indica    | ECO            | Intermediate | Soft pink   |
| White Chocolate | L. indica           | SHL            | Semidwarf    | White       |
| Wichita    | L. indica 5/8       | MSUcp          | Tree         | Lavender    |
|           | L. fauriei 3/8      |                |              |             |
| William Toovey | L. indica         | SHLcp          | Intermediate | Pink red    |
| Woodlander’s Chocolate | L. fauriei      | SHLcp          | Tree         | White       |
| Soldier    |                    |                |              |             |
| Yuma       | L. indica 3/8       | MSUcp          | Intermediate | Light lavender |
|           | L. fauriei 3/8      |                |              |             |
|           | L. amabilis 1/4     |                |              |             |
| Zuni       | L. indica 3/4       | MSUcp          | Semidwarf    | Medium lavender |
|           | L. fauriei 1/4      |                |              |             |

*Names in parentheses indicate trademarked names when they differ from cultivar names.

*Proportion of species are provided if known.

*AUB = Auburn University cultivar trial plot, Gulf Coast Research and Extension Center, Fairhope, AL; BAN = Banting Nursery, Bridge City, LA; ECO = Ecolage Nursery, Lake Charles, LA; FLW = Flowerwood Nursery, Mobile, AL; MON = Monrovia Nursery, Cairo, GA; MSU = Mississippi State University Experiment Station, Poplarville, MS; MSUcp = Mississippi State University Experiment Station cultivar trial plot, Poplarville, MS; SHL = USDA Southern Horticulture Laboratory, Poplarville, MS; SHLcp = USDA Southern Horticulture Laboratory cultivar trial plot, McNeil, MS.

L. indica. Fluorescence-labeled PCR fragments were visualized by automated capillary gel electrophoresis on an ABI3130xl using ROX-500 size standard (Applied Biosystems, Foster City, CA). GeneMapper Version 4.0 was used to recognize and size peaks (Applied Biosystems).

**Data analysis.** Data from 96 SSR loci were compiled for 93 samples and analyzed. Allele frequencies, mean number of alleles per locus (A), and allelic richness (Rs) (El Mousadik and Petit, 1996) were computed using FSTAT 2.9.3.2 software (Goudet, 2001). Gene diversity (Nei, 1973) estimates were produced using Nei’s (1987) estimator for shared allele frequencies using Genepop 4.0.10 (Raymond and Rousset, 1995). Estimates of heterozygote deficit [Wright’s fixation index (Fis)] (Wright, 1978) overall loci was obtained using FSTAT software. Significance of Fis was determined using the randomization test implemented in FSTAT (Weir and Cockerham, 1984).

**Cluster analysis.** Populations 1.2.30 was used for phenetic analyses (Langella, 2002). Genetic distances between individual samples were calculated using shared allele distance to create a matrix (Jin and Chakraborty, 1993). Unweighted pair group method with arithmetic mean (UPGMA) with 100 bootstrap replicates was used to generate a dendrogram showing clustering of genetically similar samples (Saitou and Nei, 1987; Takezaki and Nei, 1996). Two species, L. limii and L. subcostata, were included as outgroups for rooting the dendrogram. Dendrograms were visualized with TreeView 1.6.6 (Page, 1996).

Results

All SSR loci used were trinucleotide repeats and amplified alleles (amplicons) were all in the expected size range. Of the 96 SSR loci examined, 18 loci were excluded from analyses to avoid possible distortion of genetic diversity estimates. Of these, four loci exhibited only one allele size (monomorphic) across all samples but were not known to be polymorphic in other species. Four loci had a high (greater than 10%) frequency of missing data within the 93 samples even after repeated amplification. The remaining 78 SSR loci were polymorphic, showed high reproducibility, had a low frequency of missing data (less than 5%), and were used for diversity study of the selected Lagerstroemia cultivars.

Gene diversity within and between groups (L. indica, L. fauriei, and interspecific hybrids), number of alleles, genotypes detected by per pocus, allelic richness per locus, and heterozygosity deficit were calculated overall loci for the 93 cultivars examined (Table 2). Allelic richness (Rs) for all samples ranged from 3.6 (locus 517_518) to 11.8 (locus 593_594) with an average of 6.4 alleles detected per locus. SSR loci detected a total of 1061 genotypes (ranging from four to 36 per locus) with an average of 13.6 genotypes detected per locus. Measures of genetic diversity overall samples varied considerably between loci. Observed genetic diversity (Ho) was calculated for each locus and ranged from 0.043 (locus 275_276) to 0.993
Table 2. Description of the simple sequence repeat loci used in the analysis of 93 Lagerstroemia cultivars and interspecific hybrids, including repeat motifs, primer sequences, and statistical analyses of results.

| Locus     | GenBank accession no. | Repeat | Primer sequence 5’-3’ | Expected allele size and (actual range) (bp) | A¢  | G¢  | Rs¢  | Ho¢  | Hs¢  | Ht¢  | Dst¢ | Gst¢ | Fis¢ |
|-----------|-----------------------|--------|------------------------|---------------------------------------------|------|-----|------|------|------|------|------|------|------|------|
| 217_218   | HQ677236              | (AAG)4 | F:ATTCTCTGACATGAACATGGCT, R:GTGGGAGTATAGAGGAGAGTAGAGG | 158 (150–253) | 5   | 5   | 4.767 | 0.261 | 0.243 | 0.242 | 0.000 | 0.000 | -0.105 |
| 223_224   | HQ677237              | (AAT)7 | F:GGGGATTTGCTAGTGAGTGA, R:AGGAAATTTGAGCCCCATCATAG | 98 (87–136) | 6   | 10  | 5.860 | 0.547 | 0.595 | 0.704 | 0.110 | 0.156 | 0.335*  |
| 225_226   | HQ677238              | (TTC)8 | F:AAAGGATCAAACCTGCAGCAT, R:AGGTTGAAGCCTTCATATGTCCT | 158 (140–158) | 8   | 13  | 7.386 | 0.683 | 0.638 | 0.738 | 0.100 | 0.135 | 0.080  |
| 227_228   | HQ677239              | (CTT)10| F:ACGAAAACAAATCTCCAGAAG, R:AGCTCAGAGAATGAGAGACCG | 120 (102–125) | 9   | 24  | 8.755 | 0.735 | 0.755 | 0.827 | 0.071 | 0.086 | 0.151  |
| 229_230   | HQ677240              | (TTC)10| F:AGGAAGGACACTTGATCTATCCA, R:TTCAAGGTAATTTGCCTTCATCC | 149 (135–150) | 6   | 15  | 6.000 | 0.236 | 0.693 | 0.730 | 0.038 | 0.051 | 0.550*  |
| 235_236   | HQ677242              | (AGA)7 | F:TACTGCCTCCTACTGAGTGTG, R:GTGCCTGAATCAGTGAAGAAGTG | 97 (90–105) | 6   | 19  | 6.000 | 0.520 | 0.528 | 0.700 | 0.100 | 0.135 | 0.090  |
| 243_244   | HQ677244              | (TTC)7 | F:TATCTCTGATCTGCATCAC, R:ACTTCTGAGATGGAGAGACAG | 144 (127–154) | 8   | 20  | 7.973 | 0.750 | 0.750 | 0.843 | 0.093 | 0.111 | 0.073  |
| 247_248   | HQ677246              | (TTC)5 | F:ACGCTAGCTCATCATCTCTCAT, R:AAACCTCCTCCACCTCCTCC | 143 (128–216) | 6  | 6   | 4.767 | 0.186 | 0.384 | 0.500 | 0.117 | 0.233 | 0.578*  |
| 253_254   | HQ677248              | (AGA)5 | F:GTAGAGGTACACTCGATGCAT, R:AGCTTCTGTGAGAGGAGGTG | 102 (87–98) | 4   | 10  | 3.975 | 0.283 | 0.307 | 0.366 | 0.059 | 0.161 | 0.224  |
| 255_256   | HQ677249              | (TCT)8 | F:AGAGAATTCGGCGTCGTAGGAAT, R:ATTGGCCAAAGCAATAAGAATC | 109 (98–108) | 4   | 10  | 4.000 | 0.499 | 0.583 | 0.685 | 0.102 | 0.149 | -0.005 |
| 257_258   | HQ677250              | (CTT)10| F:AGACTTCAAAGGATTCTTCCAT, R:ACCTTCTGAGAGGAGGAGAT | 132 (119–131) | 5   | 13  | 5.000 | 0.548 | 0.586 | 0.648 | 0.062 | 0.096 | 0.192  |
| 261_262   | HQ677251              | (CTT)8 | F:ACACTTTGAACCTACCTCCGCC, R:TTGCTACCGATAAACCTGTTGGTG | 141 (122–141) | 6   | 11  | 5.721 | 0.562 | 0.515 | 0.611 | 0.096 | 0.157 | -0.163 |
| 263_264   | HQ677252              | (GAA)12| F:CTGGTTAAGCTGTTGTCTGT, R:CAATTCCAGAGACCTGTG | 138 (94–145) | 10  | 28  | 9.729 | 0.462 | 0.681 | 0.815 | 0.134 | 0.165 | 0.185*  |
| 265_266   | HQ677253              | (CTT)10| F:AGACTTCAAAGGATTTTCTCTCG, R:ATGCACTGCTTTAAGAACTCC | 139 (106–152) | 9   | 26  | 8.591 | 0.771 | 0.726 | 0.822 | 0.096 | 0.117 | 0.093  |
| 267_268   | HQ677254              | (CTT)10| F:TTCTGATTTTACCCAACCCTCC, R:GGACGTTAGGAGGATGACAGAAA | 96 (81–94) | 5   | 11  | 5.000 | 0.235 | 0.502 | 0.712 | 0.210 | 0.295 | 0.431*  |
| 273_274   | HQ677255              | (TCT)8 | F:ACTTCACTTAAACCTGGGCAC, R:CTTCATGCTGCTG | 151 (139–150) | 4   | 7   | 3.796 | 0.581 | 0.498 | 0.604 | 0.106 | 0.175 | -0.141 |
| 275_276   | HQ677256              | (TCT)4 | F:TTTCTCACTTCTACGGTGCTCAT, R:AGGCTTACATGGGATG | 154 (143–154) | 5   | 5   | 4.635 | 0.043 | 0.264 | 0.280 | 0.016 | 0.056 | 0.839*  |
| 277_278   | HQ677257              | (AAG)10| F:GCAAGCTTCTGGATAAGTTCAGA, R:TTTGAGATCCTGCTTCTTCATG | 160 (152–161) | 5   | 9   | 4.860 | 0.440 | 0.460 | 0.564 | 0.104 | 0.184 | 0.112  |
| 279_280   | HQ677258              | (TTC)8 | F:AAATGTCTTCCACATGAAGTTCCTCCC, R:AAATCCCCAGCTCGTACACCTCTCT | 159 (137–169) | 9   | 21  | 8.794 | 0.310 | 0.555 | 0.775 | 0.220 | 0.284 | 0.420*  |

continued next page
| Locus  | GenBank accession no. | Repeat | Primer sequence 5’-3’ | Expected allele size and (actual range) (bp) | $A^e$ | $G^e$ | $R^e$ | $H^e$ | $H^s$ | $D_{st}^e$ | $G_{st}^e$ | $F_{is}^e$ |
|--------|-----------------------|--------|-----------------------|---------------------------------------------|-------|-------|-------|-------|-------|----------|----------|--------|
| 281_282 | HQ677259 (TCT)8       |        | F:TATGGCAGCTCGAGTCTCTACTC, R:TGTGCAATGGAATTAAATGCT | 99 (81–97) | 6 | 10 | 6.000 | 0.214 | 0.639 | 0.777 | 0.138 | 0.177 | 0.495*   |
| 285_286 | HQ677261 (GAA)8       |        | F:GCTGTCACCTGTGTTGATGTC, R:CTTCTGGGATCTCAATGTC | 123 (106–128) | 7 | 13 | 6.780 | 0.704 | 0.721 | 0.767 | 0.045 | 0.059 | 0.026   |
| 287_288 | HQ677262 (TTG)8       |        | F:CCATCACCAGTATGTCGAATTAG, R:CTAATGGAAGGTCGTAATTAGA | 112 (103–117) | 6 | 12 | 5.990 | 0.333 | 0.362 | 0.370 | 0.008 | 0.020 | 0.080   |
| 291_292 | HQ677263 (TTT)12      |        | F:GCTTCTAGCTGATAGATCCCGCC, R:CGGCTCAAAACCTCTCCCTTGA | 141 (119–156) | 8 | 12 | 7.534 | 0.670 | 0.668 | 0.761 | 0.154 | 0.202 | 0.075   |
| 295_296 | HQ677264 (GAA)8       |        | F:CGACAATTTCAACACTCAACAGA, R:AGTCTCAGTCACTCACCTC | 139 (128–143) | 8 | 21 | 7.796 | 0.429 | 0.534 | 0.747 | 0.214 | 0.286 | 0.218*  |
| 297_298 | HQ677265 (GAA)7       |        | F:AGATGAGAGAGTTGTTGGACAG, R:TGGACAAAGACTCTTCTCTC | 115 (95–118) | 8 | 15 | 7.741 | 0.317 | 0.663 | 0.777 | 0.114 | 0.146 | 0.513*  |
| 303_304 | HQ677266 (TTT)8       |        | F:CCAAAGGTGGTTAACAAATGGAA, R:AGGAGAGAGGAGGAGGAGG | 130 (123–197) | 4 | 7 | 4.000 | 0.872 | 0.604 | 0.707 | 0.102 | 0.145 | 0.237   |
| 309_310 | HQ677268 (GAA)12      |        | F:ACATGACCCAGTGCAAGATAAT, R:AGCCCGAGTCCTCTCTCTCT | 160 (136–156) | 6 | 10 | 5.608 | 0.383 | 0.439 | 0.649 | 0.210 | 0.324 | 0.201   |
| 311_312 | HQ677269 (AAG)7       |        | F:AGCAAGAAATGATTTGGAAGGAA, R:CTGGCAAAATCAAGATTTTCAA | 101 (94–112) | 8 | 15 | 7.867 | 0.658 | 0.584 | 0.736 | 0.151 | 0.206 | 0.136   |
| 319_320 | HQ677272 (GAA)4       |        | F:AGAGTTAAAGGAGTGACCCAG, R:CCTATATATTTCTCTCTCTGTGG | 110 (91–110) | 7 | 11 | 6.965 | 0.272 | 0.596 | 0.629 | 0.033 | 0.053 | 0.554*  |
| 323_324 | HQ677273 (AAG)8       |        | F:CAGAGCAGCTTTGTTGACCTG, R:TTAGCTACACTCCGACAGCTG | 136 (119–275) | 6 | 16 | 5.796 | 0.552 | 0.530 | 0.743 | 0.212 | 0.286 | 0.022   |
| 329_330 | HQ677274 (GA)10       |        | F:CCAGGCTCTTAAATCACTCATCT, R:CTGATATTTTCTGTGGGCTTCC | 131 (94–107) | 4 | 8 | 4.000 | 0.560 | 0.514 | 0.701 | 0.187 | 0.267 | 0.102   |
| 333_334 | HQ677276 (GAA)8       |        | F:CATGACGCTCCAAAACATGACG, R:TGGCTCTGCTGTTAAATTGAGGTC | 104 (92–100) | 6 | 9 | 5.842 | 0.158 | 0.574 | 0.591 | 0.017 | 0.029 | 0.656*  |
| 335_336 | HQ677277 (TCT)7       |        | F:GACACTAAAGTGCTCGGGAGGTG, R:TGGACAGTTGGAATCCAAATCC | 118 (110–146) | 8 | 12 | 7.369 | 0.493 | 0.669 | 0.687 | 0.018 | 0.026 | 0.270*  |
| 339_340 | HQ677278 (AAG)8       |        | F:GCTCAATCTTTGAGATAGCAAGC, R:GAGGGGAGGAATAGTTCAATAT | 145 (130–144) | 4 | 10 | 4.000 | 0.472 | 0.538 | 0.718 | 0.180 | 0.251 | 0.170   |
| 341_342 | HQ677279 (AAG)7       |        | F:GCGAGTGGTAAATATGAACTGGA, R:TGGAAATGAGTGGGAGGGGAAC | 158 (139–157) | 5 | 11 | 4.804 | 0.431 | 0.460 | 0.696 | 0.237 | 0.340 | 0.121   |
| 343_344 | HQ677280 (CTT)8       |        | F:GGATTTGCAAGACGTCCTCTGT, R:TCTCTCTTCTTGACACGAGC | 125 (112–138) | 9 | 22 | 8.602 | 0.749 | 0.776 | 0.834 | 0.058 | 0.070 | 0.088   |
| 345_346 | HQ677281 (AGA)5       |        | F:ACGATTTGCAAAGGAGACACACC, R:TGCTACTGATGCTGTGAGTGC | 157 (82–154) | 6 | 4 | 4.000 | 0.384 | 0.490 | 0.670 | 0.180 | 0.268 | 0.644*  |
| 347_348 | HQ677282 (TTG)10      |        | F:ACGTATCAACCAAGATGACACTT, R:GAAATCCAAAGCTCAAGTGAGG | 137 (123–137) | 8 | 19 | 7.948 | 0.528 | 0.552 | 0.723 | 0.171 | 0.236 | 0.099   |
| 349_350 | HQ677283 (AAGAAC)4    |        | F:ATGCGGTAGTGGAGAATACTAGA, R:AATGCAATTCAATGCTCTCTT | 124 (115–128) | 5 | 11 | 4.804 | 0.368 | 0.357 | 0.591 | 0.234 | 0.396 | 0.140   |

*continued next page*
| Locus | GenBank accession no. | Repeat | Primer sequence 5'-3' |
|-------|-----------------------|--------|----------------------|
| 355_356 | HQ677285 | (TCC)4 | F:CTCTCTGCTTTTTCTACACAG, R:AGAGGAAGGAGGAGGAAGAGAG |
| 359_360 | HQ677286 | (TCT)5 | F:CTTCCCTCAAAACAAAACACTTAC, R:TGTGAGTGGAGTCAAGTGAAGAGAC |
| 361_362 | HQ677287 | (CTT)7 | F:ATGTCTCTAGTGTGAGACATCCT, R:CTCTGCTGAGAGGAGAGAGAGAGG |
| 363_364 | HQ677288 | (TTC)8 | F:TCGGTTTGTGAGAATGGAATGAGA, R:TGGGATCAGTACGATGATGAG |
| 369_370 | HQ677290 | (AGA)7 | F:CGAGAAAGGAATATTGCAATGAGGAGG, R:GCTTCCTTAGAGAACGATGTCCT |
| 373_374 | HQ677292 | (CTT)8 | F:ATCAATGTTCCAGAACAACCTTGCC, R:GCTTGGTCAGTTGAGAGAGAGG |
| 377_378 | HQ677293 | (CTT)8 | F:TTGCTTCTTATTTAAACAGTGTCC, R:AGACACAGGTGAAAGAGGAGAGG |
| 379_380 | HQ677294 | (TCT)6 | F:GCTCTACTCTCTCAACTCAAGGAGG, R:TGAATAAGGAGGAGGAGAGGAGG |
| 381_382 | HQ677296 | (TCT)8 | F:AGATTGGAGGTGAGGTGAGGTGAGG, R:GGCTTCCTTAGAGAACGATGTCCT |
| 387_388 | HQ677299 | (ATG)7 | F:TGACTGTCTTTTTTTGTGACTGACTCA, R:TGCAAGCTTACTCATCCTCCTT |
| 399_400 | HQ677300 | (ATG)7 | F:TGGAAAGTATCCCATAATGACG, R:TGAATGGACTGAAGTGGAGTGAG |
| 401_402 | HQ677301 | (CAT)5 | F:GGAGGATGTTGAAGAGGAGAGG, R:TGCCTCTCAGCTTCCCTCCCTT |
| 403_404 | HQ677302 | (CAT)4 | F:TTCACATCAGCAGCAGAGGAGG, R:ATAGAAGGTTGAGGAGGAGGAGG |
| 409_410 | HQ677303 | (GAT)8 | F:GAAATGTTTTATGTGCTCCGCTTCA, R:AAATCATGTTGTTCTTTCCTCC |
| 415_416 | HQ677304 | (TCA)10 | F:CTGTCTCTTTCTCTCTCTCTACTCAA, R:GAGACAAGGCTGAAAGAAAAGCAT |
| 425_426 | HQ677305 | (TCA)5 | F:GCAGTCTCCCTCTCTCTCAATC, R:GAAACAGAGGCTGAAAGAAAAGCAT |
| 427_428 | HQ677306 | (AAG)4 | F:CCAGCAGAATCATTACCCTCCTC, R:TTCATACATGAAAGGAGGAGGAGG |
| 469_470 | HQ677308 | (AGC)7 | F:ATGTCACAGTGAAATGGAGACCAA, R:GACTACAGGCGGGAAGGAGG |
| 477_478 | HQ677309 | (CTG)8 | F:TAGATCCCTCTGTTAAATGGGAGGC, R:AGGTCACTTTTTGAGGGAGGACT |

| Locus | Expected allele size and (actual range) (bp) | A | G | Rs | Ho | Hs | Ht | Dst | Gst | Fis |
|-------|---------------------------------------------|---|---|---|---|---|---|---|---|---|
| 355_356 | 122 (114–120) | 4 | 8 | 4.000 | 0.371 | 0.345 | 0.587 | 0.241 | 0.411 | 0.074 |
| 359_360 | 87 (80–90) | 4 | 9 | 4.000 | 0.471 | 0.485 | 0.650 | 0.165 | 0.254 | 0.036 |
| 361_362 | 133 (107–141) | 6 | 5 | 5.387 | 0.993 | 0.532 | 0.627 | 0.095 | 0.152 | –0.754 |
| 363_364 | 152 (142–154) | 7 | 15 | 6.807 | 0.331 | 0.365 | 0.629 | 0.264 | 0.420 | 0.207 |
| 369_370 | 131 (112–148) | 7 | 10 | 6.912 | 0.105 | 0.346 | 0.391 | 0.045 | 0.115 | 0.703* |
| 373_374 | 158 (145–162) | 8 | 24 | 7.595 | 0.563 | 0.613 | 0.782 | 0.168 | 0.215 | 0.136 |
| 377_378 | 141 (119–159) | 9 | 14 | 8.258 | 0.577 | 0.582 | 0.694 | 0.112 | 0.162 | 0.028 |
| 379_380 | 137 (111–159) | 11 | 27 | 10.766 | 0.737 | 0.742 | 0.843 | 0.101 | 0.120 | –0.001 |
| 381_382 | 143 (117–152) | 8 | 11 | 7.431 | 0.216 | 0.474 | 0.690 | 0.217 | 0.314 | 0.443* |
| 387_388 | 154 (131–155) | 10 | 20 | 9.564 | 0.464 | 0.633 | 0.829 | 0.196 | 0.237 | 0.180 |
| 399_400 | 126 (113–133) | 5 | 3 | 3.644 | 0.102 | 0.310 | 0.415 | 0.106 | 0.254 | 0.463* |
| 401_402 | 152 (127–158) | 5 | 10 | 4.959 | 0.394 | 0.465 | 0.605 | 0.140 | 0.232 | 0.183 |
| 403_404 | 139 (124–141) | 9 | 7 | 5.387 | 0.527 | 0.503 | 0.647 | 0.144 | 0.223 | 0.244 |
| 405_406 | 140 (124–138) | 7 | 5 | 4.975 | 0.255 | 0.410 | 0.479 | 0.069 | 0.143 | 0.416* |
| 409_410 | 154 (138–161) | 8 | 18 | 7.620 | 0.742 | 0.715 | 0.828 | 0.112 | 0.135 | –0.007 |
| 415_416 | 150 (131–149) | 4 | 6 | 3.998 | 0.222 | 0.262 | 0.261 | 0.000 | 0.000 | 0.182 |
| 425_426 | 95 (84–98) | 6 | 11 | 5.626 | 0.253 | 0.290 | 0.316 | 0.026 | 0.084 | 0.162 |
| 427_428 | 110 (96–111) | 5 | 9 | 4.755 | 0.291 | 0.386 | 0.657 | 0.271 | 0.412 | 0.384* |
| 469_470 | 125 (103–120) | 7 | 13 | 6.755 | 0.556 | 0.536 | 0.706 | 0.171 | 0.242 | 0.033 |
| 477_478 | 156 (141–157) | 10 | 10 | 4.755 | 0.372 | 0.374 | 0.636 | 0.262 | 0.412 | 0.170 |

continued next page
Table 2. Continued.

| Locus | GenBank accession no. | Repeat | Primer sequence 5'-3' | Expected allele size and (actual range) (bp) | A | G | Rs | Ho | Hs | Ht | Dst | Gst | Fis* |
|-------|-----------------------|--------|-----------------------|---------------------------------------------|----|---|----|----|----|----|-----|-----|-----|
| 481_482 | HQ677310 (CAG)7 | F:GACCTGAAGCCTGGATCAAC, R:CACTTCCCCGAACCTCTCTTCATGC | 129 (117–130) | 6 | 11 | 5.591 | 0.470 | 0.555 | 0.721 | 0.165 | 0.229 | 0.254* |
| 497_498 | HQ677311 (GCT)7 | F:GGTTGATGTTGTATCTGCTGCTGTC, R:GGCAACGCTTTAATCTGAACACT | 122 (116–127) | 6 | 11 | 5.796 | 0.605 | 0.466 | 0.667 | 0.201 | 0.302 | 0.038 |
| 501_502 | HQ677312 (AGA)10 | F:TTGTCAATGATGGTGATGTTTT, R:CAAGACAGGATAGGATAGGAT | 148 (128–1470 | 7 | 23 | 6.995 | 0.683 | 0.650 | 0.817 | 0.167 | 0.205 | 0.019 |
| 507_508 | HQ677315 (GAA)6 | F:ACAGCAGGGAGGAGAGAATGGA, R:TATGCAGTTACCCATCTTCAT | 150 (139–158) | 4 | 4 | 3.591 | 0.500 | 0.429 | 0.475 | 0.046 | 0.097 | 0.100 |
| 517_518 | HQ677316 (AGA)7 | F:CAATATGTTGGATGTAATGGA, R:TATGCAGTTACCCATCTTCAT | 154 (110–155) | 9 | 13 | 8.326 | 0.673 | 0.612 | 0.772 | 0.160 | 0.207 | 0.227* |
| 521_522 | HQ677317 (AGA)10 | F:CAGCAGGTATGATGGTAATGGA, R:TATGCAGTTACCCATCTTCAT | 150 (139–158) | 4 | 4 | 3.591 | 0.500 | 0.429 | 0.475 | 0.046 | 0.097 | 0.100 |
| 525_526 | HQ677318 (TCT)7 | F:TATCGTAACAAATCCATTTGCG, R:TCATCATCATCATCATCATCATCATCAT | 110 (95–111) | 5 | 12 | 5.000 | 0.656 | 0.656 | 0.727 | 0.071 | 0.098 | 0.259* |
| 527_528 | HQ677319 (GAA)6 | F:CACGCGAGGAGGAGGAGAATGGA, R:TGTGACTAAGATTCTCCTGAC | 151 (127–166) | 6 | 10 | 5.680 | 0.521 | 0.505 | 0.529 | 0.024 | 0.046 | 0.087 |
| 529_530 | HQ677320 (TCT)7 | F:ATGTACACCCGAAACCCTTTATG, R:TCTGAGTTGCAGTCTGACATTAC | 131 (124–139) | 6 | 10 | 5.680 | 0.521 | 0.505 | 0.529 | 0.024 | 0.046 | 0.087 |
| 541_542 | HQ677324 (CAT)4 | F:CAATATGTTGGATGTAATGGA, R:TATGCAGTTACCCATCTTCAT | 158 (127–159) | 4 | 7 | 3.963 | 0.614 | 0.503 | 0.538 | 0.035 | 0.065 | –0.013 |
| 543_544 | HQ677325 (TCA)8 | F:CAATATGTTGGATGTAATGGA, R:TATGCAGTTACCCATCTTCAT | 160 (147–167) | 7 | 16 | 6.755 | 0.731 | 0.707 | 0.747 | 0.041 | 0.055 | 0.114 |
| 563_564 | HQ677326 (GCT)5 | F:GACCTGAAGCCTGGATCAAC, R:CACTTCCCCGAACCTCTCTTCATGC | 146 (138–242) | 9 | 20 | 8.752 | 0.890 | 0.723 | 0.849 | 0.126 | 0.148 | –0.024 |
| 581_582 | HQ677327 (AGA)10 | F:CACGCGAGGAGGAGGAGAATGGA, R:TGTGACTAAGATTCTCCTGAC | 159 (96–151) | 5 | 9 | 5.000 | 0.665 | 0.532 | 0.566 | 0.034 | 0.060 | –0.079 |
| 583_584 | HQ677328 (AGA)10 | F:CACGCGAGGAGGAGGAGAATGGA, R:TGTGACTAAGATTCTCCTGAC | 145 (111–151) | 10 | 28 | 9.951 | 0.715 | 0.665 | 0.815 | 0.150 | 0.184 | 0.030 |
| 593_594 | HQ677329 (GAA)6 | F:CAATATGTTGGATGTAATGGA, R:TATGCAGTTACCCATCTTCAT | 140 (120–161) | 12 | 36 | 11.791 | 0.733 | 0.756 | 0.861 | 0.105 | 0.122 | 0.089 |
| 597_598 | HQ677331 (CAT)5 | F:CAATATGTTGGATGTAATGGA, R:TATGCAGTTACCCATCTTCAT | 114 (97–125) | 7 | 13 | 6.635 | 0.516 | 0.552 | 0.730 | 0.178 | 0.244 | 0.208* |

Mean 6.628 13.60 6.409 0.491 0.541 0.665 0.124 0.181 0.174

A = number of allele sizes detected per locus; G = genotypes detected per locus; Rs = allelic richness; Ho = observed heterozygosity overall samples; Hs = average gene diversity within groups; Ht = total gene diversity; Dst = average gene diversity between groups; Gst = genetic differentiation between groups; Fis = heterozygote deficit.

*Significant at P ≤ 0.05 via randomization test (Weir and Cockerham, 1984).
(locus 361_362) with a mean of 0.491. The total genetic diversity ($H_t = 0.665$) was slightly higher than the value observed ($H_o = 0.491$), indicating a higher heterozygote deficit ($F_{is} = 0.174$) (Table 2).

Total gene diversity ($H_t$) was calculated for each locus; the mean for all loci was 0.665, whereas the average gene diversity for each locus was 0.541 (Table 2). If samples are randomly chosen from within a group ($L. indica$, $L. fauriei$, or interspecific hybrids), they should differ on average at 54.1% of their loci. If they are chosen from the whole sample, the differences increase to 66.5%. Genetic differentiation ($Gst$) and average gene diversity ($Dst$) values between species were 0.181 and 0.126, respectively. Thus, only 18.1% of the overall variation was the result of differences between groups ($L. indica$, $L. fauriei$, and interspecific hybrids), whereas diversity among groups was 12.6%. Locus 593_594 was the most informative with the highest gene diversity ($H_t = 0.861$) and detected the most unique genotypes ($G = 36$) and number of alleles ($A = 12$). Locus 361_362 showed the highest observed heterozygosity overall samples ($H_o = 0.993$), whereas locus 275_276 showed the least heterozygosity ($H_o = 0.043$) (Table 2).

Genetic similarities among individuals sampled are shown in the dendrogram in Figure 1, which was rooted with $L. limii$ and $L. subsicotata$. Clustering among cultivars and hybrids is in general agreement with their genetic background and pedigrees (Fig. 1; Table 1). As expected, three main clusters can be inferred

---

**Fig. 1.** Dendrogram of 93 *Lagerstroemia* cultivars generated by unweighted pair group method with arithmetic mean (UPGMA) cluster analysis using shared allele distance (DAS). The tree is rooted with *L. limii* and *L. subscotata*. Three main clusters represent cultivars derived from *L. indica*, *L. fauriei*, and interspecific hybrids between the two species. Asterisks indicate nine cultivars that cluster with *L. indica* but supposedly contain other *Lagerstroemia* species in their genetic background (see Table 1). Bootstrap values greater than 50 were limited to relationships between two and three samples to the right of the dashed line and are not shown.
from this dendrogram. The \textit{L. indica} cluster includes all \textit{L. indica} cultivars except Red River, which is labeled as \textit{L. indica} but located in the interspecific hybrid cluster. The interspecific hybrid cluster includes 28 of 37 selected interspecific hybrids between \textit{L. indica} and \textit{L. fauriei}. The other nine interspecific hybrids clustered with \textit{L. indica}. All five \textit{L. fauriei} cultivars examined were tightly grouped in a single cluster (Fig. 1).

**Discussion**

This is the first large-scale evaluation of genetic relationships among crapemyrtle cultivars and is expected to be useful in efficiently combining existing genetic diversity in novel ways. Significant deviations from HWE were detected for all loci (data not shown) and are associated with positive Fis values, revealing deviation in the direction of heterozygote deficiency, but this is expected for samples derived exclusively from vegetatively propagated cultivated materials. Gene diversity measures were also calculated for pure species \textit{L. indica} and \textit{L. fauriei} overall loci and were 0.578, 0.367, and 0.656 for \textit{L. indica}, \textit{L. fauriei}, and interspecific hybrids, respectively. When analyzing pure \textit{L. indica} and \textit{L. fauriei} samples only, Dst and Gst values are 0.169 and 0.255, respectively, slightly higher than those when including interspecific hybrids. Thus, 25.5% of the overall variation is the result of differences between these species, whereas diversity among species was 16.9%, indicating that a large number of the allele sizes is shared between these species. These results support the observed fertility after interspecific hybridization between \textit{L. fauriei} and \textit{L. indica} and the large number of cultivars derived from hybrid parents. Further estimates of species diversity are needed to build a roadmap for efficient and successful interspecific hybridizations to capitalize on untapped genetic potential.

In general, clustering based on UPGMA grouped cultivars and hybrids in agreement with their pedigree. As expected, the \textit{L. fauriei} cluster contains all five \textit{L. fauriei} samples. Pooler (2003) produced three distinct groups among 12 clones of \textit{L. fauriei} using AFLP and RAPD markers. Although only five \textit{L. fauriei} cultivars were included in this analysis, clustering agrees with the groups reported in Pooler (2003). The \textit{L. indica} group contains 50 of the 51 \textit{L. indica} cultivars. The missing cultivar, Red River, was purchased from a commercial nursery and may have been mislabeled. Mislabeling can occur in crapemyrtle production when plants that are similar in flower color and growth habit are propagated, transported, and sold. Alternatively, ‘Red River’ lacks a robust description and may be a hybrid although it is labeled as \textit{L. indica}.

The hybrid cluster contains 28 of the 37 interspecific hybrids between \textit{L. fauriei} and \textit{L. indica} as well as hybrids containing \textit{L. limii} such as ‘Arapaho’ and ‘Cheyenne’. As noted in Table 1 and depicted in Figure 2, most hybrid crapemyrtles are derived from complex crosses containing varying percentages of \textit{L. indica} and \textit{L. fauriei}. For example, ‘Arapaho’ and ‘Cheyenne’ contain \textit{L. limii} but are primarily composed of \textit{L. indica} and \textit{L. fauriei} so their inclusion in the hybrid group is not unexpected.

![Fig. 2. Familial relationships between Lagerstroemia cultivars released by the U.S. National Arboretum are depicted by pedigree with cultivar names listed below symbols. Circles indicate plants used as maternal sources and squares for paternal contributions. Filled symbols indicate \textit{L. indica}, and open symbols indicate \textit{L. fauriei}. Gray symbols indicate hybrid plants between \textit{L. indica} and \textit{L. fauriei}, whereas gradients were used to mark other \textit{Lagerstroemia} species contributions. ‘Apalachee’ could not be connected to other pedigrees and is shown separately in B. ‘Low Flame’, ‘Red’, and ‘Basham’s Party Pink’ are shown more than once because they were used as maternal and paternal parents of several cultivars. Cultivar names marked with asterisks were not included in this study; descriptions of all other cultivars can be found in Table 1.](image-url)
Several known hybrid cultivars cluster with *L. indica*. For example, ‘GAMAD I’, ‘GAMAD II’, and ‘GAMAD IV’ originated as open-pollinated seed from ‘Pocomoke’, which is a confirmed interspecific hybrid developed by the U.S. National Arboretum. Although the paternal contributions are not known, all of these cultivars should be included in the hybrid cluster. However, ‘GAMAD I’, ‘GAMAD II’, ‘GAMAD IV’, and ‘Pocomoke’ cluster within the *L. indica* group with ‘Chickasaw’, which is also an interspecific hybrid between *L. indica* and *L. fauriei*. ‘McFadden’s Pinkie’ contains half *L. subcostata* genetic material and ‘Monia’ contains half *L. speciosa* genetic material in their background (Table 1). These two cultivars also cluster with *L. indica* group (Fig. 1).

Within the interspecific hybrid group, there are several subclusters that corresponded with growth habit, particularly among most dwarf and semidwarf cultivars. As mentioned, ‘GAMAD I’, ‘GAMAD II’, ‘GAMAD IV’, ‘Pocomoke’, and ‘Chickasaw’ cluster together (Fig. 1). All of these cultivars are dwarf crapemyrtles (Fig. 1). Dwarf cultivars GAMAD V and GAMAD III cluster with ‘White Chocolate’, which is a semidwarf (Fig. 1). Likewise, dwarf and semidwarf cultivars Okmulgee, Rubra Compacta, and Dwarf Red cluster with each other near additional dwarf and semidwarf cultivars Velma’s Royal Delight, Low Flame, and McFadden’s Pinkie (Fig. 1). Clustering resulting from growth habit is likely the result of shared pedigrees, especially for cultivars named GAMAD, which are all siblings (Dirr et al., 2005).

White flower color is associated with *L. fauriei*. Generally, the more *L. fauriei* in a cultivar’s genetic background, the lighter the flower color might be and this trend is evident in Table 1 in which most interspecific hybrids produce lavender or pink flowers. Presumably, red and purple flower colors are the result of specific anthocyanin accumulations that are inherited from *L. indica* (Zhang et al., 2008). Although not genetically verified, it is generally accepted that hybrids observed to have increased resistance to powdery mildew acquired this trait from *L. fauriei* (Hagan et al., 1998).

Within the last decade, almost all interspecific hybridizations were the result of crossing the best selections (i.e., named cultivars) with other cultivars. This can be seen in the U.S. National Arboretum releases in which detailed breeding notes are available (Fig. 2). Although this approach is easy and cost-effective, gains from selection can be hindered by a lack of genetic diversity in the breeding pool, especially because crapemyrtle breeding programs are restricted to only a few species. A low proportion of the genetic diversity (25.5%) is the result of differences between species and the moderate observed genetic diversity within the hybrids (average 66.5%) indicates that there is genetic diversity available for breeding. Understanding the extent and distribution of this variation within endemic and breeding populations is important for efficient germplasm preservation and accelerated breeding.

Complex crosses and high heterozygosity suggest that pedigree-based analysis may uncover markers associated with important traits in previously selected cultivars. Because SSRs are codominant and highly heterozygous, allele sizes documented here suggest that there may be an increased frequency of different alleles on homologous chromosome. Linking SSR markers with important traits may be possible because the same parents are used through multiple generations and they are often commercial cultivars that are easily accessible and have already been evaluated for important horticultural traits.

Pedigree-based analysis of the U.S. National Arboretum releases shown in Figure 2 has already identified markers potentially associated with ‘Dwarf Red’, ‘Pink Lace’, and ‘Basham’s Party Pink’. Cultivars derived from ‘Dwarf Red’ seedlings include Biloxi, Zuni, Pecos, and Osage (Fig. 2). Seventeen SSR loci produced the same allele sizes found in the ‘Dwarf Red’ parent and all four derived cultivars. Two of these loci show a dramatic increase in heterozygosity when compared with the remaining 91 genotypes. For example, locus 253_254 has observed heterozygosities of 0.600 in ‘Dwarf Red’ and derived cultivars and 0.093 in the other 88 samples. Cultivars containing ‘Pink Lace’ as the parent or grandparent include Acoma, Natchez and Muskogee. ‘Osage’, ‘Miami’, ‘Wichita’, ‘Choctaw’, ‘Hopi’, ‘Sioux’, ‘Yuma’, and ‘Lipan’ were derived from ‘Pink Lace’ after additional generations (Fig. 2). Nine loci produced the same allele sizes found in ‘Pink Lace’. Likewise, 12 SSR loci produced the same allele sizes found in ‘Basham’s Party Pink’ when comparing derived cultivars such as Tuskegee and Tuscarora and more distantly cultivars, Caddo, Lipan, Tonto, Arapaho, and Cheyenne (Fig. 2). However, the statistical significance of allele frequency differences was limited by the small sample size in our pedigreed samples. At this time, SSR loci producing the same allele sizes, increased heterozygosity, and/or increased allele frequency within related groups do not necessarily confirm selection pressure for those alleles.

A larger number of genotypes needs to be evaluated to compensate for the increased number of alleles (Nei et al., 1983), but once a sufficient number of genotypes and phenotypes has been evaluated, linkage results might warrant MAS. Substantial crapemyrtle populations already exist in the form of diallel populations that have been analyzed for quantitative traits (Pounders et al., 2007b). These same populations could also be sampled to look for qualitative traits associated with the SSR markers described here, especially because the parents of the diallel crosses overlap with the cultivars used in this study and the progeny were already evaluated for a range of traits (Pounders et al., 2007b). It is likely that additional associations might be uncovered as more SSR markers are screened (Wang et al., 2010).

**Literature Cited**

Ali, R. 1977. Chromosome numbers in some species of Lagerstroemia. Curr. Sci. 46:579–580.

Bowden, W.M. 1945. A list of chromosome numbers in higher plants. I. Acanthaceae to Myrtaceae. Amer. J. Bot. 32:81–92.

Cabrera, R.I. 2004. Evaluating and promoting the cosmopolitan and multipurpose Lagerstroemia. Acta Hort. 630:177–184.

Cabrera, R.I., J.A. Reinert, and C.B. McKenney. 2008. Differential resistance among crapemyrtle (Lagerstroemia) species, hybrids, and cultivars to foliar feeding by adult flea beetles (Altica litigata). HortScience 43:403–407.

Caetano-Anollés, G., S.E. Schlärbaum, and R.N. Trigiano. 1999. DNA amplification fingerprinting and marker screening for pseudo-testcross mapping of flowering dogwood (Cornus florida L.). Euphytica 106:209–222.

Creech, J.L. 1985. Asian natives for American landscapes—The National Arboretum does more than gather seeds. Amer. Nurseryman 161:81–82.

Dirr, M., V. Waters, and J. Kardos. 2005. New protected woody plant selections. Acta Hort. 689:209–222.

Dix, R.L. 1999. Cultivars and names of Lagerstroemia. 1 Dec. 2010. <http://www.usda.gov/Research/Herbarium/Lagerstroemia/index.html>.
Egolf, D. and A.O. Andrick. 1978. The Lagerstroemia handbook/checklist. American Association of Botanical Gardens and Arboreta, Wilmington, DE.

El Mousadik, A. and R.J. Petit. 1996. High level of genetic differentiation for allelic richness among populations of the argan tree [Argania spinosa (L.) Skeels] endemic to Morocco. Theor. Appl. Genet. 92:832–839.

Furtado, C.X. and S. Montien. 1969. A revision of Lagerstroemia L. (Lythraceae). Garden Bul. Singapore 24:185–335.

Goldstein, D.B. and D.D. Pollock. 1997. Launching microsatellites: A review of mutation processes and methods of phylogenetic inference. J. Hered. 88:335–342.

Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (Version 2.9.3). 1 Dec. 2010. <http://www2.unil.ch/popgen/softwares/fstat.htm>.

Guha, S. 1972. Cytotaxonomic studies on the family Lythraceae. Proc. Indian Acad. Sci. Congr. Assn. 59:344–345.

Herbert, J.J., R.F. Mizell, III, and H.J. McAuslane. 2009. Host suitability of crapemyrtle cultivars to powdery mildew and cercospora leaf spot in Alabama. J. Environ. Hort. 16:143–147.

Hagan, A.K., G.J. Keever, C.H. Gilliam, J.D. Williams, and G. Creech. 1998. Susceptibility of crapemyrtle cultivars to powdery mildew and cercospora leaf spot in Alabama. J. Environ. Hort. 16:143–147.

Herbert, J.J., R.F. Mizell, III, and H.J. McAuslane. 2009. Host preference of the crapemyrtle aphid (Hemiptera: Aphididae) and host suitability of crapemyrtle cultivars. Environ. Entomol. 38:1155–1160.

Jin, L. and R. Chakraborty. 1993. Estimation of genetic distance and coefficient of gene diversity from single-probe multilocus DNA fingerprinting data. Mol. Biol. Evol. 11:120–127.

Langella, O. 2002. Populations, a free population genetics software (Version 1.2.30). 1 Dec. 2010. <http://bioinformatics.org/~tryphon/populations/>.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. USA 7012:3321–3323.

Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York, NY.

Nei, M., F. Tajima, and Y. Tateno. 1983. Accuracy of estimated phylogenetic trees from molecular data. J. Mol. Evol. 19:153–170.

Page, R.D. 1996. TreeView: An application to display phylogenetic trees on personal computers. Comput. Appl. Biosci. 124:357–358.

Petit, G.V., D. Boyd, S. Braman, and C. Pounders. 2004. Potential resistance of crapemyrtle cultivars to flea beetle (Coleoptera: Chrysomelidae) and japanese beetle (Coleoptera: Scarabaeidae) damage. J. Econ. Entomol. 97:981–992.

Pollock, D.D., A. Bergman, M.W. Feldman, and D.B. Goldstein. 1998. Microsatellite behavior with range constraints: Parameter estimation and improved distances for use in phylogenetic reconstruction. Theor. Popul. Biol. 533:256–271.

Pooler, M.R. 2003. Molecular genetic diversity among 12 clones of Lagerstroemia fauriei revealed by AFLP and RAPD markers. HortScience 38:256–259.

Pooler, M.R. 2006a. ‘Arapaho’ and ‘Cheyenne’ Lagerstroemia. HortScience 41:855–856.

Pooler, M.R. 2006b. Crapemyrtle, p. 439–457. In: Anderson, N. (ed.). Flower breeding and genetics. Springer, Dordrecht, The Netherlands.

Pounders, C., S.M. Reed, and M.R. Pooler. 2006. Pollination biology of Lagerstroemia indica and several interspecific hybrids. HortScience 413:575–578.

Pounders, C., T. Rinehart, and H. Sakhanokho. 2007a. Evaluation of interspecific hybrids between Lagerstroemia indica and L. speciosa. HortScience 42:1317–1322.

Pounders, C., T. Rinehart, N. Edwards, and P. Knight. 2007b. An analysis of combining ability for height, leaf out, bloom date and flower color for crapemyrtle. HortScience 42:1317–1322.

Raven, P.H. 1975. The bases of angiosperm phylogeny: Cytology. Ann. Missouri Bot. Garden 62:724–764.

Raymond, M. and F. Rousset. 1995. GENEPOP Version 1.2: Population genetics software for exact tests and ecumenicism. J. Hered. 86:248–249.

Rinehart, T.A., B.E. Scheffler, and S.M. Reed. 2006. Genetic diversity estimates for the genus Hydrangea and development of a molecular key based on SSR. J. Amer. Soc. Hort. Sci. 131:787–797.

Rossetto, M. 2001. Sourcing of SSR markers from related plant species, p. 211–224. In: Henry, R.J. (ed.). Plant genotyping: The DNA fingerprinting of plants. CAB International, Wallingford, UK.

Saitou, N. and M. Nei. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4:406–425.

Takezaki, N. and M. Nei. 1995. Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. Genetics 144:389–399.

Tobe, H., P.H. Raven, and S.A. Graham. 1986. Chromosome counts for some Lythraceae sens. str. (Myrtales), and the base number for the family. Taxon 35:13–20.

Waldbiers, G.C., S.M.A. Quiniou, and A. Karsi. 2003. Rapid development of gene-tagged microsatellite markers from bacterial artificial chromosome clones using anchored TAA repeat primers. Biotechniques 35:976–979.

Wang, X.W., D. Dean, P. Wadl, D. Hadziabdic, B. Scheffler, T. Rinehart, R. Cabrera, and R. Trigiano. 2010. Development of microsatellite markers from crapemyrtle (Lagerstroemia L.). HortScience 45:842–844.

Wang, Z., J.L. Weber, G. Zhong, and S.D. Tanksley. 1994. Survey of plant short tandem DNA repeats. Theor. Appl. Genet. 88:1–6.

Weir, B. and C.C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38:1358–1370.

Williams, D., K. Tilt, and S. Valenti-Windson. 1998. Common crapemyrtle. Alabama Coop. Ext. Serv. Publ. ANR-1063.

Wright, S. 1978. Variability within and among natural populations in evaluation and the genetics of populations. University of Chicago Press, Chicago, IL.

Zhang, J., L.S. Wang, J.M. Gao, Q.Y. Shu, C.H. Li, J. Yao, Q. Hao, and J.J. Zhang. 2008. Determination of anthocyanins and exploration of relationship between their composition and petal coloration in crapemyrtle (Lagerstroemia hybrid). J. Integr. Plant Biol. 50:581–588.