# Development of Microsatellite Markers for Lagerstroemia indica (Lythraceae) and Related Species

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PRIMER NOTE

# DEVELOPMENT OF MICROSATELLITE MARKERS FOR *LAGERSTROEMIA INDICA* (LYTHRACEAE) AND RELATED SPECIES[1]

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- *Premise of the study:* Microsatellite markers were developed and characterized to analyze genetic diversity within *Lagerstroemia* cultivars and related species.
- *Methods and Results:* Using simple sequence repeat (SSR)–enriched libraries, 11 species-specific polymorphic genomic SSRs were developed from *L. indica* 'Hong Die Fei Wu'. All primers were tested on 48 *L. indica* individuals from China, the United States, and France. The primers amplified four to 12 alleles per locus, including di-, tri-, and tetranucleotide repeats. Observed and expected heterozygosities ranged from 0.1875 to 0.7609 and 0.2836 to 0.8385, respectively. The primers were also highly cross-transferrable to *L. subcostata*, *L. limii*, *L. fauriei*, *L. caudata*, and *L. speciosa.*
- *Conclusions:* The new primers will enlarge the bank of SSRs available to genetic research of *Lagerstroemia*. These SSR markers will facilitate population genetics and molecular marker–assisted selection of *L. indica*.

**Key words:** cross-transferrable; *Lagerstroemia indica*; Lythraceae; microsatellite; SSR-enriched library.

The Lythraceae family includes approximately 50 species of *Lagerstroemia* (Byers, 1997); species of this genus have become a mainstay in mild-climate habitats because of their ease of production and cultivation, long-lasting midsummer bloom, range of plant habits from miniature potted plant to large tree, and diversity of landscape uses (Pooler, 2006). *Lagerstroemia indica* L., native to China, has been widely cultivated in gardens for about 1800 years (Zhang, 1991). Since the 1960s, *L. fauriei* Koehne has played an important role in crape myrtle breeding programs because of its strong resistance to mildew diseases and to cold temperatures. *Lagerstroemia speciosa* (L.) Pers., *L. limii* Merr., and *L. subcostata* Koehne have also been introduced into crape myrtle breeding programs recently (Pooler, 2003; Pounders et al., 2007).

Undoubtedly, many *Lagerstroemia* cultivars and species with excellent ornamental traits will bring great changes to crape myrtle breeding. Therefore, to improve the usefulness of molecular marker–assisted selection programs in *Lagerstroemia*, suitable molecular markers are needed to identify, assess, conserve, and use these germplasms of *Lagerstroemia*. For such an objective, simple sequence repeats (SSRs) have proven to be effective and useful for the evaluation of genetic diversity among *Lagerstroemia* species and cultivars (Rinehart and Pounders, 2010; Wang et al., 2011; Cai et al., 2011) because of their codominance and hypervariablity. However, available SSR markers are relatively limited in crape myrtle (Rinehart and Pounders, 2010; Wang et al., 2011; Cai et al., 2011). Here we report the rapid development of 11 SSR markers and their cross-species transferability.

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## METHODS AND RESULTS

Samples of *L. indica* cultivars and related species were cultivated in the crape myrtle collection of the China National Engineering Research Center for Floriculture, Beijing (40°02′13.67″N, 115°50′5.58″E) (Appendix 1). Genomic DNA was extracted from silica-dried leaf tissue with the DNAsecure Plant Kit following the manufacturer's protocol (Tiangen Biotech, Beijing, China). A microsatellite-enriched library was constructed following a modified biotin-streptavidin capture method (Glenn and Schable, 2005). In brief, the genomic DNA of *L. indica* 'Hong Die Fei Wu' was digested into segments with the restriction enzymes *Rsa*I and *Xmn*I (New England Biolabs, Beijing, China), ligated to the double-stranded Super SNX-24 linker (F: 5′-GTTTAAGGCCTAGCTAGCAGAATC-3′, R: 5′-pGATTCTGCTAGCTAGGCCTTAAACAAA-3′; synthesized by Sangon Biotech, Shanghai, China), amplified using PCR, then hybridized with a mix of 3′ biotin-labeled oligonucleotide probes and captured for microsatellites using streptavidin-coated magnetic beads (Dynabeads M-280; Invitrogen, Carlsbad, California, USA). The captured DNA was amplified by PCR reaction using the Super SNX-24 forward linker as primer. The enriched DNA was inserted into pCR2.1-TOPO vectors (Invitrogen) following the manufacturer's instructions and transformed into One Shot Top10 Chemically Competent cells (Invitrogen). Recombinant clones were identified using blue/white screening on Luria-Bertani agar plates containing ampicillin and Xgal. A total of 175 bacterial colonies were picked out and analyzed using M13 primers to amplify the complete microsatellite-containing insert. SSR-containing clones were selected as positives when one well band was visible on a 2% agarose gel after PCR. Then, the positive clones were sequenced on an ABI 3730 DNA analyzer (Applied Biotech, San Diego, California, USA). Finally, sequence analysis was carried out using the EditSeq of the DNASTAR software package (DNASTAR, Madison, Wisconsin, USA). SSR loci were located using the program SSRHunter 1.3.0 (Qiang Li, Nanjing Agricultural University, Nanjing, China). Pairs of primers were designed to amplify the fragments with SSR loci using Primer Premier 5.0 (Premier Biosoft International, Palo Alto, California, USA). Designed primer pairs, labeled at the 5′ end using one of the conventional sequencing dyes 6-FAM or HEX (Applied Biosystems, Carlsbad, California, USA), were further accessed among 43 morphologically divergent

TABLE 1. Characteristics of 11 polymorphic microsatellite primers developed for *Lagerstroemia indica* 'Hong Die Fei Wu'.

| Locus | Primer sequences (5′–3′) | Repeat motif | Size (bp) | Fluorescent label | $T_a$ (°C) | GenBank accession no. |
|---|---|---|---|---|---|---|
| LAL1 | F: AGCTGGCTGGTTGGGAGT | $(ACTC)_3$ | 189 | 6-FAM | 54 | JQ740158 |
| | R: AAGGGTTTTACAAGAAATGGAC | | | | | |
| LAL3 | F: AAGCCCGACTCAGAAACT | $(TATG)_9$ | 129 | HEX | 50 | JQ740160 |
| | R: ACTATGACTCGGCCTTCC | | | | | |
| LAL4 | F: CGGGACCGACAAAATACT | $(TCTG)_5$ | 163 | HEX | 50 | JQ740161 |
| | R: AGGGAAGATGTTGGAAGG | | | | | |
| LAL5 | F: AATGAAGGTTCGGGTGC | $(AG)_5$ | 322 | 6-FAM | 54 | JQ740162 |
| | R: TCTGGCTTGAGGGTTTG | | | | | |
| LAL6 | F: GGAATCATCGACTGGGTAA | $(TGA)_3$ | 172 | 6-FAM | 54 | JQ740163 |
| | R: GCTCCTATGGCAGAACG | | | | | |
| LAL7 | F: GATGGGTTTGGCTCTGC | $(AG)_{17}$ | 348 | 6-FAM | 54 | JQ740164 |
| | R: GTCCTCCTCACTTGTTTCC | | | | | |
| LAL8 | F: TCAAGAGTGGCAGCATC | $(AGA)_4$ | 128 | 6-FAM | 52 | JQ740165 |
| | R: GGAACGGCTCTGATTGT | | | | | |
| LAL9 | F: TTCTTTCCCATTGTTATTCG | $(CATA)_4CATT(CATA)_4$ | 144 | 6-FAM | 50 | JQ740166 |
| | R: CGTCGTCGGTGAACCTCT | | | | | |
| LAL10 | F: CCCAAGTTCAACAAATCTCC | $(GAA)_6GAG(GAA)_3$ | 279 | 6-FAM | 56 | JQ740167 |
| | R: ATCGTTTCCTGGCGTCT | | | | | |
| LAL11 | F: GGGCAGAACCTGACTTA | $(ATG)_6$ | 164 | HEX | 48 | JQ740168 |
| | R: CTCCAACGGCTCAACTA | | | | | |
| LAL12 | F: GCGTCAGTCAACCCTAA | $(AG)_{21}$ | 475 | HEX | 50 | JQ740169 |
| | R: CGGACAGCCAGATACAG | | | | | |

*Note*: $T_a$ = optimal annealing temperature.

cultivars of *L. indica* and five *Lagerstroemia* species with PCR amplification. The PCR amplification was performed in a 10 µL reaction volume containing 20 ng genomic DNA, 5 µL 2× *Taq* PCR Master Mix (Biomiga Inc., San Diego, California, USA), and 50 ng each of forward and reverse primer. The PCR profile consisted of an initial denaturing at 94°C for 3 min; followed by 30 cycles consisting of 30 s at 94°C for denaturation, 30 s at the specific annealing temperature (Table 1), 30 s at 72°C for extension; with a final extension of 72°C for 5 min. Amplified fragments were mixed using GeneScan 500 LIZ Size Standard (Applied Biosystems), separated on an Applied Biosystems 3730xl sequencer, and analyzed using GeneMapper version 3.0 software (Applied Biosystems). Number of alleles per locus ($A$), observed heterozygosity ($H_o$), and expected heterozygosity ($H_e$) were calculated using the software POPGENE version 1.31 (Yeh et al.,1999).

One hundred and fifty-five positive clones were successfully sequenced, among which 141 (90.9%) clones contained microsatellites (SSRs). When the duplicate loci and the SSR loci that had been developed (Rinehart and Pounders, 2010; Wang et al., 2011; Cai et al., 2011) were removed, 64 (41.29%) unique sequences remained. These fragments contain 74 SSRs, among which 33 (44.59%) were tetranucleotide repeats, 27 (36.49%) were binucleotide repeats, and 14 (18.92%) were trinucleotide repeats. Fifty-four sequences with long enough sequences (>20 bp) on the upper and lower side of the repeats were suitable for primer design.

TABLE 2. Results of initial primer screening using 48 accessions of *Lagerstroemia* germplasm including 43 cultivars and five *Lagerstroemia* species.

| Locus | $A$ | $H_o$ | $H_e$ |
|---|---|---|---|
| LAL1 | 6.0000 | 0.5106 | 0.4425 |
| LAL3 | 6.0000 | 0.6809 | 0.6976 |
| LAL4 | 5.0000 | 0.5745 | 0.7149 |
| LAL5 | 5.0000 | 0.3404 | 0.5145 |
| LAL6 | 4.0000 | 0.6458 | 0.5090 |
| LAL7 | 13.0000 | 0.7609 | 0.8385 |
| LAL8 | 5.0000 | 0.1875 | 0.2836 |
| LAL9 | 7.0000 | 0.6596 | 0.6511 |
| LAL10 | 6.0000 | 0.3191 | 0.4322 |
| LAL11 | 6.0000 | 0.3333 | 0.3296 |
| LAL12 | 12.0000 | 0.5000 | 0.6277 |
| Mean | 6.8182 | 0.5011 | 0.5492 |

*Note*: $A$ = number of alleles; $H_e$ = expected heterozygosity; $H_o$ = observed heterozygosity.

Of these, 11 pairs of primers were successfully amplified and found to be polymorphic (Table 1). We detected 75 alleles in the 48 genotypes. The $A$ per locus ranged from four to 13 with an average of 6.8182, $H_o$ ranged from 0.1875 to 0.7609 with an average of 0.5011, and $H_e$ ranged from 0.2836 to 0.8385 with an average of 0.5492 (Table 2). All loci could cross-amplify in *L. subcostata* and *L. limii*; LAL4 and LAL12 failed to amplify in *L. fauriei*; and LAL11 did not amplify in *L. caudata*. Because the genetic relationship between *L. speciosa* and *L. indica* is distant (Pooler, 2006), only four pairs of primers (LAL4, LAL6, LAL8, and LAL9) showed the expected allele sizes in *L. speciosa*. The primers cross-amplified in the related species, but whether they are polymorphic still needs to be determined.

## CONCLUSIONS

Genetic diversity parameters indicated that these polymorphic microsatellite loci will be a promising tool for investigations of current genetic diversity and genetic structure in *L. indica*. Our results showed better transferability of the tested markers to *L. subcostata* and *L. limii* than to other species. Given that most of the primers successfully amplified a band of the expected size in several *Lagerstroemia* species, these microsatellites have the potential to become an efficient molecular tool to address similar questions in other *Lagerstroemia* species. With the development of microsatellite markers from crape myrtle, there will be more SSRs available for use in studies such as the construction of linkage maps, mapping of useful genes, marker-assisted breeding, and evaluation of genetic diversity.

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Appendix 1. Information for taxa used in this study. All samples were cultivated in the crape myrtle collection of the China National Engineering Research Center for Floriculture, Beijing, China.

| Taxon | Collection no. | Locality |
|---|---|---|
| *L. indica* ‘Hong Die Fei Wu’* | LI2008-NZ1 | Beijing, China (40°02′13.67″N, 115°50′5.58″E) |
| *L. indica* ‘Ceng Yun Ji Xue’ | LI2008-NZ3 | Beijing, China (40°02′13.65″N, 115°51′5.51″E) |
| *L. indica* ‘Duo Hua Fen’ | LI2008-NZ4 | Beijing, China (40°02′13.57″N, 115°52′6.32″E) |
| *L. indica* ‘Huan Fen Liu Yun’ | LI2008-NZ5 | Beijing, China (40°02′13.46″N, 115°53′4.36″E) |
| *L. indica* ‘Bing Qing Yu Die’ | LI2008-NZ6 | Beijing, China (40°02′13.38″N, 115°54′4.30″E) |
| *L. indica* ‘Fu Jian Za Zhong Bai’ | LI2008-NZ8 | Beijing, China (40°02′13.27″N, 115°55′8.25″E) |
| *L. indica* ‘Zi Sha’ | LI2008-NZ10 | Beijing, China (40°02′13.23″N, 115°56′5.21″E) |
| *L. indica* ‘Fen Jing’ | LI2008-NZ11 | Beijing, China (40°02′13.18″N, 115°57′3.19″E) |
| *L. indica* ‘Zi Jin’ | LI2008-NZ12 | Beijing, China (40°02′13.10″N, 115°58′4.30″E) |
| *L. indica* ‘Muskogee’ | LI2010-02 | Beijing, China (40°02′13.06″N, 115°59′5.24″E) |
| *L. indica* ‘Tuskegee’ | LI2010-03 | Beijing, China (40°02′12.93″N, 115°60′6.70″E) |
| *L. indica* ‘Dallas Red’ | LI2010-05 | Beijing, China (40°02′12.89″N, 115°61′7.20″E) |
| *L. indica* ‘Miami’ | LI2010-08 | Beijing, China (40°02′12.84″N, 115°62′6.90″E) |
| *L. indica* ‘Osage’ | LI2010-12 | Beijing, China (40°02′12.81″N, 115°63′8.86″E) |
| *L. indica* ‘Yuma’ | LI2010-17 | Beijing, China (40°02′12.77″N, 115°64′4.25″E) |
| *L. indica* ‘Centennial Spirit’ | LI2010-19 | Beijing, China (40°02′12.70″N, 115°65′9.14″E) |
| *L. indica* ‘William Toovey’ | LI2010-20 | Beijing, China (40°02′12.65″N, 115°66′5.21″E) |
| *L. indica* ‘Okmulgee’ | LI2010-22 | Beijing, China (40°02′12.58″N, 115°67′5.67″E) |
| *L. indica* ‘Acoma’ | LI2010-23 | Beijing, China (40°02′12.53″N, 115°68′6.32″E) |
| *L. indica* ‘Zuni’ | LI2010-27 | Beijing, China (40°02′12.50″N, 115°69′3.53″E) |
| *L. indica* ‘Prairie Lace’ | LI2010-29 | Beijing, China (40°02′12.49″N, 115°70′1.26″E) |
| *L. indica* ‘Tonto’ | LI2010-30 | Beijing, China (40°02′12.43″N, 115°71′6.45″E) |
| *L. indica* ‘Velma’s Royal Delight’ | LI2010-31 | Beijing, China (40°02′12.40″N, 115°72′9.10″E) |
| *L. indica* ‘Powhatan NEW’ | LI2010-33 | Beijing, China (40°02′13.36″N, 115°73′4.39″E) |
| *L. indica* ‘Sacramento’ | LI2010-35 | Beijing, China (40°02′13.31″N, 115°74′5.08″E) |

Appendix 1. Continued.

| Taxon | Collection no. | Locality |
|---|---|---|
| *L. indica* 'Pink Blush' | LI2010-37 | Beijing, China (40°02′13.28″N, 115°75′7.15″E) |
| *L. indica* 'World's Fair' | LI2010-39 | Beijing, China (40°02′13.23″N, 115°76′10.25″E) |
| *L. indica* 'Houston NEW' | LI2010-40 | Beijing, China (40°02′13.20″N, 115°77′8.94″E) |
| *L. indica* 'Pixie White' | LI2010-41 | Beijing, China (40°02′13.17″N, 115°78′12.30″E) |
| *L. indica* 'Mardi Gras' | LI2010-42 | Beijing, China (40°02′13.11″N, 115°79′4.57″E) |
| *L. indica* 'Baton Rouge' | LI2010-43 | Beijing, China (40°02′13.07″N, 115°80′3.96″E) |
| *L. indica* 'Creole' | LI2010-46 | Beijing, China (40°02′13.05″N, 115°81′10.54″E) |
| *L. indica* 'Cordon Bleu' | LI2010-47 | Beijing, China (40°02′12.98″N, 115°82′4.78″E) |
| *L. indica* 'Bicolor' | LI2010-48 | Beijing, China (40°02′12.92″N, 115°83′8.59″E) |
| *L. indica* 'Chickasaw' | LI2010-51 | Beijing, China (40°02′12.90″N, 115°84′9.10″E) |
| *L. indica* 'Pocomoke' | LI2010-52 | Beijing, China (40°02′12.87″N, 115°85′6.22″E) |
| *L. indica* 'Arapahoe' | LI2010-54 | Beijing, China (40°02′12.84″N, 115°86′1.48″E) |
| *L. indica* 'Apalachee' | LI2010-55 | Beijing, China (40°02′12.80″N, 115°87′5.32″E) |
| *L. indica* 'Dynamite' | LI2010-56 | Beijing, China (40°02′12.77″N, 115°88′4.26″E) |
| *L. indica* 'Berlingot Menthe' | LI2010-57 | Beijing, China (40°02′12.75″N, 115°89′6.74″E) |
| *L. indica* 'Niver' | LI2010-58 | Beijing, China (40°02′12.70″N, 115°90′11.24″E) |
| *L. indica* 'Petite Pink' | LI2010-59 | Beijing, China (40°02′12.65″N, 115°91′2.32″E) |
| *L. indica* 'Rosea Nova' | LI2010-60 | Beijing, China (40°02′12.63″N, 115°92′5.46″E) |
| *L. indica* 'Violet' | LI2010-61 | Beijing, China (40°02′12.60″N, 115°93′5.33″E) |
| *L. speciosa* (L.) Pers. | LI2009-01 | Beijing, China (40°02′12.55″N, 115°94′4.98″E) |
| *L. subcostata* Koehne | LI2009-02 | Beijing, China (40°02′12.51″N, 115°95′3.26″E) |
| *L. limii* Merr. | LI2009-03 | Beijing, China (40°02′12.44″N, 115°96′4.37″E) |
| *L. fauriei* Koehne | LI2009-04 | Beijing, China (40°02′12.40″N, 115°97′12.67″E) |
| *L. caudata* Chun & F. C. How ex S. K. Lee & L. F. Lau | LI2009-05 | Beijing, China (40°02′12.35″N, 115°98′5.19″E) |

*Indicates that the individual was used to construct the microsatellite-enriched library.