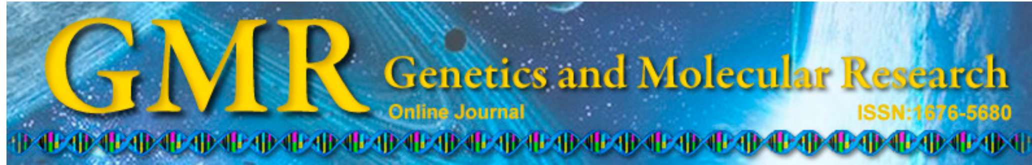

Screening of molecular markers linked to dwarf trait in crape myrtle by bulked segregant analysis

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ABSTRACT. Plant height is one of the most important traits of plant architecture as it modulates both economic and ornamental values. Crape myrtle (*Lagerstroemia indica* L.) is a popular ornamental woody plant because of its long-lasting mid-summer bloom, rich colors, and diversified plant architecture. These traits also make it an ideal model of woody species for genetic analysis of many ornamental traits. To understand the inheritance of plant height and screen for genes modulating plant height in *Lagerstroemia*, segregation of the plant height trait was analyzed using the F_1 population of *L. fauriei* (standard) x *L. indica* 'Pocomoke' (dwarf) with 96 seedlings, while dwarf genes were screened using the bulked segregant analysis method, combined with 28 amplified fragment length polymorphism primers and 41 simple sequence repeat primers. The results showed that the dwarf trait of crape myrtle was controlled by a major gene and modified by minor genes. An

amplified fragment length polymorphism marker, M53E39-92, which was 23.33 cM from the loci controlling the dwarf trait, was screened. These results provide basic information for marker-assisted selection in *Lagerstromia* and cloning of dwarf genes in future studies.

Key words: Amplified fragment length polymorphism; Bulked segregant analysis; *Lagerstroemia*; Plant architecture; Simple sequence repeat

INTRODUCTION

Plant height is one of the most important plant architecture traits and is closely related to production, quality, and ornamental value (Petersen and Krost, 2013). Studies of plant architecture have focused on the biology of this trait, beginning with the introduction of lodging-resistant semi-dwarf wheat and rice mutants that led to the Green Revolution in the 1960s (Peng et al., 1999). The genetic mechanism of plant architecture in crops has been improved (Gallavotti et al., 2010), and batch of genes modulating plant height have been successfully cloned (Dardick et al., 2013).

For woody plants, studies of plant architecture mainly focus on fruit trees. Different genes determining important traits such as columnar growth habit (Tian et al., 2005), and the rootstock dwarf gene *Dw1* in apple have been mapped, some of which are currently being used effectively in breeding programs (Rusholme Pilcher et al., 2008). Furthermore, to identify genes involved in columnar architecture, high-throughput RNA-Seq technology was used in a segregating population of ‘Fuji’ x ‘Telamon’ (Zhang et al., 2012). However, few studies on plant architecture of ornamental plants have been reported. Several transgenic chrysanthemum lines were produced and used to express the *Arabidopsis thaliana gai* gene (gibberellic acid insensitive) under its own promoter, which exhibited a range of dwarf phenotypes (Petty et al., 2003). Studies examining the inheritance of creeping habit and molecular markers linked to this habit were carried out to provide a basis for molecular marker-assisted selection breeding programs, and to clone creeping habit-related genes from the ground-cover *Chrysanthemum* (Zhao et al., 2009).

Lagerstroemia (crape myrtle) belongs to the Lythraceae family, including at least 50 species native to southeastern Asia (Byers, 1997). China was the first country to cultivate crape myrtle, which took place approximately 1800 years ago (Zhang, 1991). Some species in this genus are of enormous significance in mild-climate habitats because of their long-lasting summer bloom, rich colors, and diverse patterns, and thus are widely used in gardens (Pooler, 2003). Additionally, crape myrtle is regarded as an indispensable source of income for companies and retail nursery growers. Recently, increased attention has been given to select dwarf crape myrtle mutants, and breeding programs to develop dwarf cultivars have been pursued in America, Italy, and Japan (Pooler, 2007). Because of the limited knowledge of inheritance of plant habit, a long period of time is required to breed dwarf cultivars. Significant progress has been made in the breeding of cultivars with diverse flower size and color (Whitcomb, 1985; Pounders et al., 2007), high resistance to powdery mildew (Pettis et al., 2004), and polyploidy cultivars (Ye et al., 2010; Wang et al., 2012) in crape myrtle. However, few studies have examined the inheritance of plant architecture in crape myrtle.

Compared with other woody plants, there are some advantages to investigate plant architecture using crape myrtle: 1) There are diverse patterns of plant types in *Lagerstroemia*, including arbors (>3 m), bushes, and mini shrubs (0.3-0.6 m); 2) large hybrid populations can be obtained easily by crossing for its abundant blooms in summer; and 3) crape myrtle seedlings

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grow rapidly and bloom in a short period of time. Thus, crape myrtle is an ideal model of woody plants for genetic analysis of many ornamental traits, including plant architecture characteristics.

Various agronomic traits, such as fertility, plant height, and resistance, are controlled by major genes and are considered qualitative traits. Traditionally, gene mapping is based on genetic linkage maps, which are restricted by mapping population, marker density, and resource consumption. Compared to genetic linkage maps, bulked segregant analysis (BSA) is an effective and efficient method of mapping target genes (Michelmore et al., 1991). With the rapid development of molecular biology techniques, DNA markers combined with BSA have been extensively used for qualitative traits analysis of many crops, including *Actinidia chinensis* (Xu et al., 2010), *Malus domestica* (Tian et al., 2004), and *Pyrus* (Wang et al., 2011). Among all DNA markers, amplified fragment length polymorphism (AFLP) is effective when little knowledge is available regarding the genome sequence (Waugh et al., 1997). Additionally, simple sequence repeats (SSRs) are abundant, co-dominant, and highly polymorphic in *Lagerstroemia* species and cultivars (Cai et al., 2011; Wang et al., 2011; He et al., 2012), and can be used as molecular markers for gene mapping in *Lagerstroemia*.

To determine the genetic mechanism behind the dwarf trait and screen for genes modulating plant height of *Lagerstroemia*, the segregation of plant height trait was analyzed using an F_1 population of *Lagerstroemia fauriei* (standard) x *Lagerstroemia indica* 'Pocomoke' (dwarf). Dwarf genes were screened using the BSA method combined with AFLP and SSRs. Our results provide a foundation for breeding of new crape myrtle cultivars using marker-assisted selection and for studying the inheritance of the plant height trait of *Lagerstroemia*.

MATERIAL AND METHODS

Plant materials

The F_1 population in our study was obtained from a cross of *Lagerstroemia fauriei* (female) x *L. indica* 'Pocomoke' (male) (Figure 1). Parents were selected for their contrasting plant architecture traits. The female parent was an arbor (>3 m) with wide leaves and long internodes, and the male parent was a dwarf shrub (0.3-0.6 m) with small leaves and short internodes (Pooler and Dix, 1999; Liu et al., 2013).

Figure 1. Standard type (3) and dwarf type (4) of 1-year-old seedlings in F_1 population of *Lagerstroemia fauriei* (female) (1) x *L. indica* 'Pocomoke' (male) (2).

In November 2011, mature hybrid seeds were harvested. At the beginning of 2012, seeds were sowed in a greenhouse, and then 142 seedlings were transplanted in June 2012 at the experimental field in ornamental plant germplasm and breeding nursery of China National Engineering Research Center for Floriculture in Beijing. To study the height trait, no training was used to maintain the original plant shapes. Additionally, regular irrigation and pest control were used throughout this study.

Phenotypic measurements and statistical analysis

Remarkable character separation of plant height was observed in the F_1 population after the first year of growth. At that time, we randomly selected 96 individuals to measure plant height and constructed the frequency distribution histogram using Spass17.0 and Excel (Zhao et al., 2009). Using the mean height of 1-year-old standard seedlings as a reference, dwarf seedlings were distinguished based on half height of the standard seedlings (Xu et al., 2010). Mendelian segregation was tested at a 1% signification level between standard type and dwarf type individuals by performing a chi-square goodness-of-fit test.

DNA extraction

Genomic DNA was extracted from fresh young leaves using the FastDNA kit (Tiangen Biotech, Beijing, China) following the manufacturer protocol. DNA quality and concentration were measured by 1% agarose gel electrophoresis containing Gel Red at 0.1 μg/mL 1X TAE buffer, with bands visualized under UV light (Zhu et al., 2011).

AFLP protocol

AFLP was performed according to the method described by He et al. (2014), including restriction enzyme digestion, a pre-amplification reaction, and selective amplification. In this study, a total of 384 AFLP primer combinations were used. Primers and adapters were synthesized by Beijing Ruibo Biotech Co., Ltd. (Beijing, China). We digested 100 ng DNA for 14 h at 37°C in a mixture of 5 pM *Eco*RI adapter, 50 pM *Mse*I adapter, 5 U *Eco*RI, 5 U *Mse*I, 1X T_4 ligase buffer, and 4 U T_4 DNA ligase (New England Biolabs, Ipswich, MA, USA). Pre-amplification reactions were performed in a 20-μL volume containing 3 μL enzyme-digested products, 10 μL 2X *Taq* PCR Master Mix (Biomiga, San Diego, CA, USA), 0.6 μL of each forward and reverse primer, and 5.8 μL ddH_2O. The polymerase chain reaction (PCR) procedure was as follows: 94°C for 5 min; followed by 30 cycles of 94°C for 35 s, 56°C for 35 s, and 72°C for 1 min; final extension step at 72°C for 10 min. The pre-amplification products were diluted 1:20 with ddH_2O, and 3 μL was used for selective amplification. The PCR procedure was performed in a 20-μL volume containing 3 μL pre-amplification products, 10 μL 2X *Taq* PCR Master Mix, 0.6 μL of each forward and reverse primer, and 5.8 μL ddH_2O. Among the primers, M19, M51, M52, M24, M25, and M53 were labeled with a fluorescent tag. The selective amplification procedure was as follows: 94°C for 5 min; followed by 13 cycles of 94°C for 35 s, 56°C for 30 s, and 72°C for 1 min; 30 cycles of 94°C for 35 s, 56°C for 35 s, and 72°C for 1 min; final extension step at 72°C for 10 min.

SSR protocol

A total of 41 SSR primers demonstrated to be highly polymorphic in crape myrtle were tested (Wang et al., 2011; He et al., 2012). All primers were synthesized by Sangon Biotech (Shanghai) Co., Ltd. (Shanghai, China) (Table 1). The forward primer labeled with a fluorescent tag (Sangon) matched the reverse primer. SSR reactions were performed in a 10-μL volume containing 100 ng DNA, 5 μL 2X *Taq* PCR Master Mix, 0.5 μL of each forward and reverse primer, and 3 μL ddH$_2$O. The PCR procedure was as follows: 94°C for 5 min; followed by 30 cycles of 94°C for 30 s, appropriate anneal temperature (45°-60°C) for 30 s, and 72°C for 1 min; final extension was performed at 72°C for 5 min.

Pool construction

Pool construction was conducted according to the method of Michelmore et al. (1991) with some modifications. Individuals in the F$_1$ population were divided into standard and dwarf types based on plant height when the plants stopped growing in winter. We randomly selected 8 plants from standard and dwarf type, and then mixed their DNA in the same volume separately. Different primer combinations were tested to screen for polymorphisms. If a polymorphic band was screened in 1 pool but not screened in another, a polymorphic marker was identified in the corresponding locus.

Data analysis

PCR products of AFLP and SSR were verified using GeneMarker Version 1.71 (SoftGenetics, State College, PA, USA) according to the method described by Jakubowski et al. (2011). AFLP data were scored as '1' or '0' in the form of Excel, while SSR data were exported as fragment sizes. The primer combinations yielding candidate markers were subsequently applied to each individual from the F$_1$ population (Cervera et al., 1996). Genetic distance in centimorgans (cM) were calculated using the Kosambi mapping function (Kosambi, 1944). Furthermore, the validity of screened polymorphic marker was verified by replicate tests and testing individual plants in the F$_1$ population.

RESULTS

Segregation analysis of plant height in F$_1$ population

Ninety-six individuals from the F$_1$ population were selected randomly for measurement of plant height; the frequency distribution histogram was constructed using spass 17.0 and Excel. The distribution of plant height of 1-year-old F$_1$ seedlings showed continuous variation and was mainly concentrated on the ranges of 1015 and 3040 cm (Figure 2). As shown in Figure 2, the number of individuals taller than 30 cm and shorter than 15 cm was surveyed. Statistical analysis showed that there were 33 standard individuals and 36 dwarf individuals. Chi-square test showed that the Mendelian segregation was in the theoretical ratio of 1:1 (Table 2). Combined with the parent phenotypes, the dwarf trait in crape myrtle was likely controlled by a major gene and modified by minor genes.

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Table 1. Nucleotide sequences of 41 SSR primers.

Locus	Primer sequence (5'-3')	Repeat	Tm (°C)	Allele size (bp)
SSR1	F: <FAM>AGAGAAAGAGAGGAGCGGGAGT R: ACCTTCTTCCCCAATTCAATCC	$(GT)_6GC(GT)_6$	55	213
SSR2	F: <FAM>ACGTTTAGCACACCGGTACTGT R: GGAAGCACATCACTATGGCAAG	$(GT)_7$	55	181
SSR3	F: <FAM>GGTGGAGATGCTAACAAGCAAG R: GGATTTTTGCTGTAGGGTGATT	$(TG)_{16}$	55	161
SSR4	F: <FAM>CCCTCATACCTTCTTTATCAAGTCA R: ATCCCCACAAAATCTCTCCTTC	$(AC)_{13}$	55	215
SSR5	F: <FAM>TGGGATCGATGCTATTAATGTTG R: TACACCAATTCACACCTCCACTC	$(GT)_8$	55	207
SSR6	F: <FAM>ACATGGCTCCCATCACACAG R: CAGGGGATTCTTGTTTTGCTTT	$(AC)_6$	55	160
SSR7	F: <ROX>CTCTCAAATGACCCTCTT R: TTGAGTAATAACAAGTCCC	$(AAAG)_5$	48	255
SSR8	F: <HEX>GAGTTCATGCAGTTAGGT R: ATATCGGATTTATCTTCC	$(AAG)_6$	48	130
SSR9	F: <ROX>GGAAGAGGGATTGGAACC R: TCTCACTGAAAGAAACTA	$(GA)_5G(GGA)_4$	48	134
SSR10	F: <HEX>ACGGTAGATAAGGTGAGC R: GGTTTCGTATCGTCGTAG	$(CT)_9T(CTGT)GT(CTGT)_4$	52	166
SSR11	F: <HEX>TACTGGGTATCCGTTTCT R: ACAGGTGCATTTACTTCC	$(CT)_6CC(CT)_{12}$	50	317
SSR12	F: <FAM>TTCTGACCCAGCAGTAAA R: CGTATCTCATCTGTAGCGTA	$(AGGT)_4$	50	138
SSR13	F: <HEX>GGGAATTTGGGATATGGA R: TAAAGAAACGACCGAGCC	$(AAAG)_5$	52	179
SSR14	F: <HEX>GTCACAGGTTACCGAATC R: ATGTAAATGGTGAGGAGG	$(AATC)_5$	50	253
SSR15	F: <ROX>TTCTTGTCTTGGGTATCGC R: GAGCCAGTATTGTCTTCACG	$(CCTT)_7$	50	228
SSR16	F: <HEX>TTCTTCCACTTCCTCCTT R: CAGCCCACATTAACTTTT	$(AG)_{12}$	50	204
SSR17	F: <FAM>AAAGACGCAGAAGGATGG R: CGATTAGTTTCAGCTCGT	$(AG)_{20}$	50	420
SSR18	F: <FAM>GGACCAGATTGTAAATGC R: CTGCTCCTAATATCAGTGTC	$(CT)_{15}$	50	289
SSR19	F: <HEX>TAGTCCATTCATGTCAAG R: GGATTCACCAAACTACTT	$(AG)_{14}$	52	246
SSR20	F: <FAM>TTTGGTGGTAGTGGGAGT R: GTGTCTGCATGGCTGTAA	$(CTGT)_6$	54	305
SSR21	F: <ROX>CCTAACAAGAAAGGAACAG R: TTTCAGGACATCAGCACC	$(AG)_{11}$	60	144
SSR22	F: <HEX>CCTCCTCCTGCCACTCCTCT R: CCCGTCGTCTCCTCAGTTCTC	$(AAG)_4AG(AAG)_2$	54	194
SSR23	F: <FAM>CAACAGTAAAATTGGAGC R: AGTAGTGATTCGGGTGGA	$(CTTT)_7$	55	144
SSR24	F: <HEX>ATCAATGTCCACAACAACTTGCC R: GGTTTGGTCGATTTGGTTCAGTTA	$(AAG)_7$	55	158
SSR25	F: <FAM>TACACTCCCTCCCATTCAGATTGT R: GCTGCCTGAATCAGTGAAGAGAGT	$(AGA)_7$	55	97
SSR26	F: <HEX>ACGTATCAACCGAATGACCACTTT R: GAATTCAAAGCTCAAGTGGGGAC	$(TTC)_{10}$	55	137
SSR27	F: <HEX>GTCTCACTCTCTCAACTCAAGGGC R: TGAGAAAGAATTTTTCCTGAACCG	$(TCT)_6$	55	137
SSR28	F: <HEX>ATGTACACCCGAAACCCTTTAGGT R: TCCATGTCTTGTCACAGCCTCTAC	$(TCT)_7$	55	131
SSR29	F: <FAM>TGTCACTTCTGCAAATAT R: AACTACTGCCATCATACT	$(TC)_7$	50	229
SSR30	F: <FAM>GTGTTGGGAGTCAGATGG R: ACAGCCGTTCGACATTAA	$(AC)_6$	48	191
SSR31	F: <FAM>AGCTGGCTGGTTGGGAGT R: AAGGGTTTTACAAGAAATGGAC	$(ACTC)_3$	54	189

Continued on next page

Table 1. Continued.

Locus	Primer sequence (5'-3')	Repeat	Tm (°C)	Allele size (bp)
SSR32	F: <HEX>AAGCCCGACTCAGAAACT R: ACTATGACTCGGCCTTCC	$(TATG)_9$	50	129
SSR33	F: <HEX>CGGGACCGACAAAATACT R: AGGGAAGATGTTGGAAGG	$(TCTG)_5$	50	163
SSR34	F: <FAM>AATGAAGGTTCGGGTGC, R: TCTGGCTTGAGGGTTTG	$(AG)_5$	54	322
SSR35	F: <FAM>GGAATCATCGACTGGGTAA R: GCTCCTATGGCAGAACG	$(TGA)_3$	54	172
SSR36	F: <FAM>GATGGGTTTGGCTCTGC R: GTCCTCCTCACTTGTTTCC	$(AG)_{17}$	54	348
SSR37	F: <HEX>TCAAGAGTGGCAGCATC R: GGAACGGCTCTGATTGT	$(AGA)_4$	52	128
SSR38	F: <FAM>TTCTTTCCCATTGTTATTCG R: CGTCGTCGGTGAACCTCT	$(CATA)_4CATT(CATA)_4$	50	144
SSR39	F: <FAM>CCCAAGTTCAACAAATCTCC R: ATCGTTTCCTGGCGTCT	$(GAA)_6GAG(GAA)_3$	56	279
SSR40	F: <HEX>GGGCAGAACCTGACTTA R: CTCCAACGGCTCAACTA	$(ATG)_6$	48	164
SSR41	F: <FAM>GCGTCAGTCAACCCTAA R: ACAGCCGTTCGACATTAA	$(AG)_{21}$	50	475

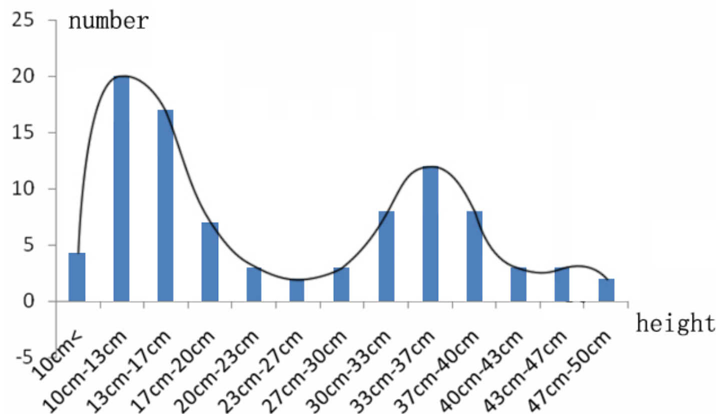

Figure 2. Distribution of plant height trait of 1-year-old seedlings in F_1 population of *Lagerstroemia fauriei* (female) (1) x *L. indica* 'Pocomoke' (male).

Table 2. Segregation of different plant height in F_1 population of *Lagerstroemia fauriei* x *L. indica* 'Pocomoke'.

Progeny	No. of plants	No. of individuals of different plant type		Theoretical ratio	χ^2	P
		Standard type	Dwarf type			
F_1	96	33	36	1:1	0.083	0.01

Polymorphisms revealed by molecular markers

Among the 384 AFLP primer combinations, 28 pairs showing polymorphisms were selected, including 12 sequences for the E_{+3} primer and 6 sequences for the M_{+3} primer (Table 3). In AFLP analysis, the fragment size of PCR products ranged from 50-467 bp. A total of 192 polymorphic loci were screened, of which 25 were amplified by M24E36 (Table 4). The results revealed a

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specific fragment at 92 bp amplified by M53E39-92, which was screened in both the male parent and dwarf type pool, but not in the female parent and standard type pool (Figure 3). Additionally, 27 SSR primers were polymorphic and 72 polymorphic loci were amplified in the subject materials. However, we did not screen any specific fragment that was different in relative pools.

Table 3. Primer sequences of AFLP primer.

Primer No.	Primer sequences (5'-3')	Primer No.	Primer sequences (5'-3')
E31	GACTGCGTACCAATTCAAA	E41	GACTGCGTACCAATTCAGG
E32	GACTGCGTACCAATTCAAC	E42	GACTGCGTACCAATTCAGT
E33	GACTGCGTACCAATTCAAG	E43	GACTGCGTACCAATTCATA
E34	GACTGCGTACCAATTCAAT	M19	GATGAGTCCTGAGTAAGA
E36	GACTGCGTACCAATTCACC	M24	GATGAGTCCTGAGTAATC
E37	GACTGCGTACCAATTCACG	M25	GATGAGTCCTGAGTAATG
E38	GACTGCGTACCAATTCACT	M51	GATGAGTCCTGAGTAACCA
E39	GACTGCGTACCAATTCAGA	M52	GATGAGTCCTGAGTAACCC
E40	GACTGCGTACCAATTCAGC	M53	GATGAGTCCTGAGTAACCG

Table 4. Number of AFLP amplification loci amplified by 28 different primer combinations.

Primer combination	Polymorphic fragment	Size range (bp)	Primer combination	Polymorphic fragment	Size range (bp)
M19E33	5	55-289	M51E33	8	56-416
M19E32	3	57-153	M51E36	2	56-86
M19E37	2	57-87	M51E37	3	56-188
M24E33	22	51-416	M51E39	2	56-85
M24E36	25	53-228	M51E40	2	55-85
M24E37	12	52-215	M51E41	4	55-489
M24E38	22	56-300	M52E31	2	57-86
M24E39	14	50-169	M52E32	2	57-87
M24E42	7	51-339	M52E33	2	56-85
M24E43	13	54-175	M52E34	3	56-174
M25E37	1	152	M52E36	7	56-316
M25E40	3	63-85	M53E33	1	416
M25E42	1	90	M53E39	3	54-182
M25E43	2	90-239	M53E41	19	50-489

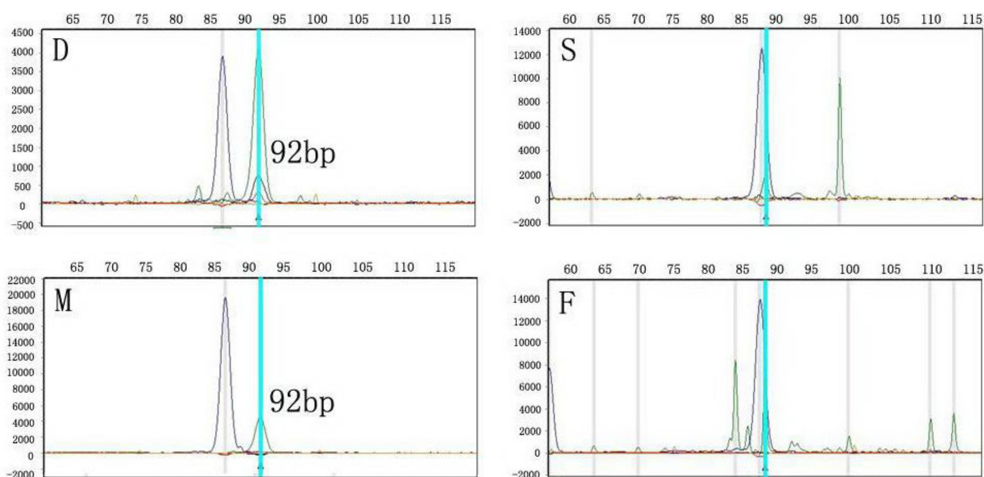

Figure 3. Amplification in pools and parents for primer M53E39 on the automated fluorescent-labeled system. **D.** Dwarf pool; **M.** male parent; **S.** standard pool; **F.** female parent.

Linkage analysis

The specific AFLP marker M53E39-92 was found to be linked to the dwarf gene in crape myrtle. According to phenotypic measurements and statistical analysis, 33 standard individuals and 36 dwarf individuals in the F_1 population were selected randomly to verify the linkage of the AFLP marker. Sixteen recombination individuals were detected, occurring 23.33 cM from dwarf genes.

DISCUSSION

Dwarf crape myrtles are becoming increasingly popular and widely used worldwide for various reasons, including being used as potted plants (Guidry and Einert, 1975) and bedding plants. However, the inheritance of this anomalous habit remains unclear (Pooler, 2007). We found that the dwarf trait of crape myrtle is likely controlled by a major gene and modified by minor genes. The F_1 population, containing 96 individuals obtained from a cross of *L. fauriei* (female) x *L. indica* 'Pocomoke' (male), were observed in our study. Approximate discrete characters with respect to plant height was isolated in the F_1 population. The distribution of plant height in the F_1 population showed continuous variation. The chi-square test showed that both standard and dwarf plants were in the Mendelian ratio of 1:1. Combined with the results of other woody plants, the dwarf trait of crape myrtle was most likely controlled by a major gene (Xu et al., 2010). As an important ornamental character in crape myrtle, whether the dwarf trait is controlled by regulators or structure genes must be confirmed in future studies.

Based on our genetic analysis, BSA is a rapid and effective method for identifying markers linked to dwarf genes (Tian et al., 2004). BSA was first applied in linkage analysis of discrete traits in the F_2 population (Michelmore et al., 1991), and also widely used to screen target traits in F_1 populations (Zhao et al., 2009; Wang et al., 2011). The underlying principle of BSA is the grouping of informative individuals together so that a particular genomic region can be studied against a randomized genetic background of unlinked loci. In the present study, 8 individuals were selected randomly each from standard and dwarf type so that the insignificant signals derived from background discrepancies could be eliminated at the extreme, because the most appropriate number of individuals applied to construct gene bulks was 510 to avoid false-positives (Goivannoni et al., 1991). Moreover, *L. fauriei* (standard) and *L. indica* 'Pocomoke' (dwarf) showed large differences in plant height, indicating that the parents tested are highly polymorphic in the target region. The F_1 individuals showed significant differences in plant height, internode length, and leaf size.

Twenty-eight AFLP primers from 384 primer combinations were examined to identify polymorphisms in this study. A total of 192 polymorphic loci were screened, of which 25 were amplified by M24E36. Of all primer combinations, the mean length of the amplified polymorphic site was 6.86. AFLP is an effective marker-based PCR technique characterized by dominant, is highly polymorphic, and requires little sequence information for the target species and template DNA. The prominent advantages of the AFLP technique compared to other PCR-based marker technologies are the reproducibility and number of screened markers (Powell et al., 1996). Until now, the AFLP technique combined with BSA have been widely employed for gene mapping studies (Aranda et al., 2014). For a dominant AFLP marker, the probability of an unlinked locus being polymorphic between 2 pools of 10 individuals was calculated to be 2 x 10^{-6} (Michelmore et al., 1991). In addition, 41 SSR primers known to be high polymorphic

in crape myrtle were analyzed. However, specific markers linked to dwarf characters were not screened. SSR markers are preferred to other markers because SSR sequences generally show a high level of similarity across different species, genera, and families (Yang et al., 2014). Thus far, the number of SSR markers used in *Lagerstroemia* studies was less than 200, indicating that a very large gap remains compared with other species. Although SSR markers are generally regarded to be valuable for gene mapping, the lack of sufficient SSR markers still cannot evenly cover the entire genome, including the target region in crape myrtle.

Data analysis showed that a 92-bp fragment was screened in both the male parent and dwarf pool, but not in the female parent and standard pool. Finally, a molecular marker was identified and linked to the dwarf gene in crape myrtle. Replicating and testing the validity of this marker in the F_1 population, the distance between the molecular marker and dwarf gene was found to be 23.33 cM. According to Michelmore et al. (1991), all polymorphic loci assayed within 15 cM of the target locus can be identified. Loci are detected with decreasing frequency as genetic distance increases. Therefore, with a genetic distance of 23.33 cM from dwarf genes, uncertainties regarding this *Lagerstromia* species may not be revealed by the specific marker. In the future, the inheritance of dwarf trait must be verified in the F_2 or BC_1 populations. Additionally, the number of molecular markers is a key factor that influenced the results in this study. To achieve an ideal effect in marker-assisted selection of the dwarf character, we will develop additional molecular markers to enhance the coverage area of *Lagerstromia* species.

In conclusion, 96 seedlings in an F_1 population were employed to screen molecular markers linked to the dwarf trait of crape myrtle using BSA combined with the AFLP and SSR technologies. An AFLP marker M53E39-92 linked to the dwarf gene was detected which was 23.33 cM from the loci controlling the dwarf trait. This study revealed that BSA is a valuable method for identifying important traits in crape myrtle and the first to map dwarf genes in this species. Our results provide basic information for marker-assisted selection and cloning of the dwarf gene to improve crape myrtle breeding programs.

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