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Research Article
Development and Application of EST-SSR Markers in *Koelreuteria paniculata* Laxm. Using a Transcriptomic Approach

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Abstract

Background and Objective: *Koelreuteria* as important native tree species, have multiple ornamental, ecological and medicinal values. However, research on the germplasm resources of *Koelreuteria* is relatively limited and relies primarily on the use of morphological differences to distinguish trees; thus the aim of this study was to develop effective EST-SSR markers to provide reference for the genetic diversity evaluation of the species. **Methodology:** In this study, 2871 microsatellite markers were assessed by mining a transcriptomic database of *Koelreuteria paniculata* Laxm. and 65,360 pairs of primers were designed. Based on the length of the products, the stability and permeability of primers and primer annealing temperatures, 96 pairs were screened and tested in a preliminary test. Thirteen pairs were selected for capillary electrophoresis analysis. The observed heterozygosity and expected heterozygosity were collected by GenAlEx software and the allelic polymorphism information content were calculated using CERVUS. **Results:** Ninety two pairs that gave clear bands in PCR amplification. For capillary electrophoresis analysis, in the microsatellite loci that showed polymorphisms, the number of alleles ranged from 2-5 per locus and the polymorphic information content ranged from 0.124-0.726, respectively. Additionally, based on capillary electrophoresis results, this study established unique fingerprints for four particular variants of *K. paniculata* Laxm. **Conclusion:** These polymorphic markers will be in a great favour of evaluating genetic biodiversity. The fingerprints would provide a foundation for variety protection.

Key words: *Koelreuteria paniculata* Laxm., molecular marker, transcriptome, EST-SSR, polymorphism

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Koelreuteria is an important ornamental tree that has various economically important uses[1]. Plants in the genus contain a variety of compounds with great medicinal value, such as *K. paniculata* Laxm., which contains flavonoids[2]. However, research on the germplasm resources of *Koelreuteria* is relatively limited and relies primarily on the use of morphological differences to distinguish trees; this means it lacks clear molecular markers. Thus, this study used the universal molecular marker, simple sequence repeats (SSRs), to assess *K. paniculata* with a view to developing primers for helpful SSR markers. The study also used the SSRs to develop unique fingerprints for four special variants of *K. paniculata* Laxm., samples A2, A3, A4 and A5, which differ from 'ordinary' individuals in terms of leaf color (Fig. 1).

In recent years, many genetic markers have been developed, such as random amplified polymorphic DNA (RAPD), simple sequence repeats (SSRs) and single-nucleotide polymorphisms (SNPs). The SSRs occupy an important position in the field of genetic markers because of their high polymorphism, especially in plants[3]. The SSRs, also known as microsatellites, consist of short repeated DNA motifs. Compared with other genetic markers, SSRs also have the advantages of high abundance, a random distribution in the genome and co-dominant inheritance. As a result, SSRs play important roles in linkage mapping and DNA fingerprinting, genetic diversity analysis, identification of genetic relationships and functional gene tagging[4].

However, the development of SSRs from the genome is relatively expensive and has limitations in terms of the research material, which should be genome-wide sequences. Alternatively, expressed sequence tag (EST)-SSRs, developed from expressed sequence regions, reduce the costs and are used widely in many fields.

Compared with genomic SSRs, EST-SSRs have a high level of transferability in both intraspecific and interspecific manners[5,6]. With these advantages, EST-SSRs have great value in genetic research and have been used in many plants, including angiosperms and gymnosperms, such as kenaf[7], black pepper[8], *Amentotaxus*[5], pigeon pea[9], grape[10], white poplar[11], eucalyptus[6] and wheat[12]. In this study, EST-SSR markers are first developed in *K. paniculata* Laxm. and the study provide a primary situation about the distribution of SSR markers in the species expressed sequence.

The aim of study was the development of primers for EST-SSRs in *K. paniculata* Laxm. and the assessment of the utility of 92 primers pairs. Furthermore, this study obtained fingerprints of four special varieties and provided a foundation for variety protection.

MATERIALS AND METHODS

Plant materials: In total, 7 genotypes were used: Five *K. paniculata* Laxm. from Changzhi, Shanxi province, China (adult tree, collected on May 10, 2016) and two from Beijing, China (adult tree, collected on March 1, 2016). The detailed information about materials is shown in Table S1. All genotypes were collected and stored in -76°C. Part of the material was used for transcriptome sequencing and the rest was used for the development of primers.

Development of EST-SSR primers: The study screened for potential SSRs having at least five repeated SSR motifs

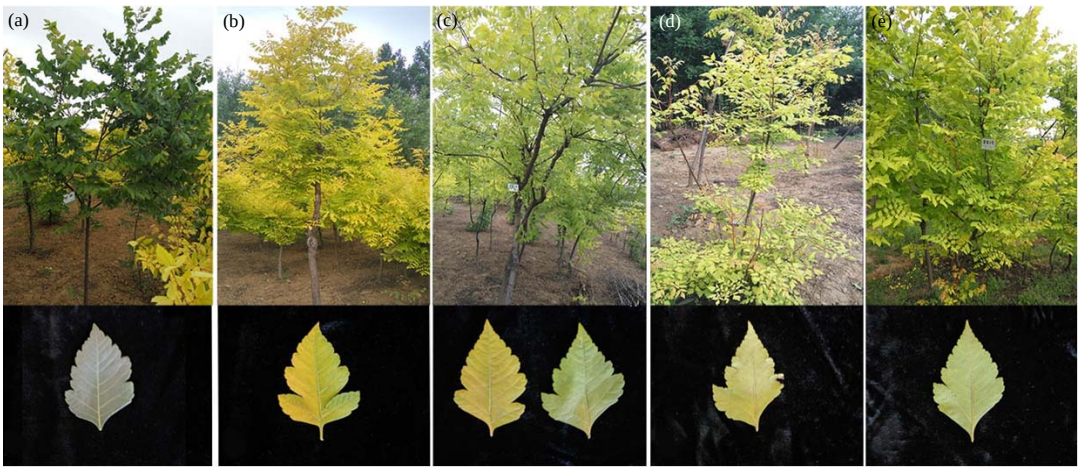

Fig. 1(a-e): (a) Photo of sample A1, the 'ordinary' golden rain tree and (b-e) Four specific varieties of *K. paniculata* Laxm. (A2-A5)

from the transcriptome of *K. paniculata* Laxm. Based on the data, 65,360 pairs of primers were designed for 2871 SSR loci using QDD (ver. 3.1)[13]. Based on the length of the products, stability and permeability of the primers and primer annealing temperature (which affects testing methods and results), 96 primer pairs were screened and tested. The information about the primers is shown in Table S2.

DNA extraction and PCR amplification: Genomic DNA samples were extracted from young leaves with a modified

Table S1: Material sources

No.	Name	District of collection	Time of collection
A1	*K. paniculata* Laxm.	Changzhi, Shanxi province, China	2016.5.10
A2*	*K. paniculata* Laxm.	Changzhi, Shanxi province, China	2016.5.10
A3*	*K. paniculata* Laxm.	Changzhi, Shanxi province, China	2016.5.10
A4*	*K. paniculata* Laxm.	Changzhi, Shanxi province, China	2016.5.10
A5*	*K. paniculata* Laxm.	Changzhi, Shanxi province, China	2016.5.10
B1	*K. paniculata* Laxm.	Beijing, China	2016.3.10
B2	*K. paniculata* Laxm.	Beijing, China	2016.3.10

Table S2: Primers used for SSR analysis of *Koelreuteria*

No.	PRIMER_LEFT_SEQUENCE	PRIMER_RIGHT_SEQUENCE	TM (°C)	MOT_TRANS
K-SSR-1	AAGAACCTCCTGTACGCCAA	AGCCATCAAATCTTCACGAAA	52	AGG
K-SSR-2	ATCTATCCTTCCACAGGGCA	GAATTTGCGAAGATTCAGGC	54	AT
K-SSR-3	TGTCAACTTTCATGATCGCC	GCCTCTGGATTTCCTCCC	53	AG
K-SSR-4	AAACCAATGATTACGACCCAA	TCTCGTCTCTGTGTGTCGGT	53	AG
K-SSR-5	GCTTTCTTCACTGTATTGCCG	GACACAATCACAGACTCGCCT	55	AG
K-SSR-6	GGAAGACACGATTTCTTGGG	CCCACAACACCATAACGAAA	53	AG
K-SSR-7	CATTGAGAACATTCAGGCCC	CGTGTATTGTAATTTCCCACCC	54	AG
K-SSR-8	CAAGCCAAATCATACTTGCG	CTGAACAGACTGATGGCAGC	54	AG
K-SSR-9	ACTAGTGGTTCCAAGGCGG	GTCAGCACTTGAATCTGGCA	54	ATC
K-SSR-10	CATAGAGAGTATCCGGCGGT	CGCACAAATCTTATAGAGCCC	55	AG
K-SSR-11	ACGTTACTAAGGAGCGCCG	GCCAAGTTACCCAACCCAC	55	ACC
K-SSR-12	AGACGATGACGAATCTTGGC	CATTTCGTTTCATCGGTCG	53	AG
K-SSR-13	GACAATGAAGATGATGGCGA	TGTCCAAGAACATGAATGGG	52	AAG
K-SSR-14	CTGCTAGTACTCCTGGCGCT	TAACCTTTGACCCACTTGGC	56	AG
K-SSR-15	CTTGAAGAACTCGAAACGGG	CTGGTCATCCTCTGTCCCTC	56	AG
K-SSR-16	TCACGCACTAACAAACCCAA	ACCTCCAATCACAAACCACC	52	AG
K-SSR-17	GCAGGTTTGCTATTATCGTCG	GGAAATGGTCACTCTCTCCG	55	AC
K-SSR-18	CCTTAGATTCTTCATCGCCG	TGGCTCTCAATTGTCTCCCT	54	AC
K-SSR-19	CCTTACTGCGATTAACCCGA	CTCTTATTGACGACCGCTCC	53	AAT
K-SSR-20	TCATCCATTTACTGTCTGCGA	CAAGGCTTCATCAGAAGGGT	54	AG
K-SSR-21	GATCAGATTCCTGCTGCGA	TACACAATGCAGAAGACGGC	54	ACG
K-SSR-22	CAACACGATCTCCATTGGC	AACAACAATCGCAAAGAGGG	53	ACG
K-SSR-23	GTTGGGATAATAGGCTGCCA	AGGGTGTGTAACTTGAGCGAA	54	AT
K-SSR-24	AAACTCGCTAAGAAGCAGCG	GTTCAAGCTTCTATTGCGGC	54	ATC
K-SSR-25	ATCTCTTTCTTCCGTTTGCG	AAGCAGAACGAGAACGGGT	53	AG
K-SSR-26	TACTTCTCCTCGAACCGGAA	TGTAGCGTACTCGAAATCGAAA	53	AAC
K-SSR-27	CTTGAGGTTTCCATCTTCGC	CCCTCTCTCCCTATCTTCCG	55	AAG
K-SSR-28	TCTGCATAGTTGTTCCGCTG	CACTCAGTCTCTTCTCCCGC	56	AG
K-SSR-29	GAAACACCTCTTTGAGGCCA	GGTGTGGTAATTGGACTGCC	55	AG
K-SSR-30	CCATTGAAAGTAGTCGGCGT	CAGCATGGATTGACTTGGG	54	AG
K-SSR-31	CGAGTCTGATTCTGACGGGT	TCACTTCACAAATCCACCGA	54	ATC
K-SSR-32	TTTCTTTCTTAGGTGCAAGCG	GATTATCTTCAGTTGCCCGC	53	AG
K-SSR-33	TAACATCGTTTGTTGGCCG	GGTATGCGCTTTAATGGTGG	53	AT
K-SSR-34	AGGTGAGAGACGGTAGCGAA	ACCTGTTCTCGTGTTCCCAG	56	AG
K-SSR-35	GTGTAGTTTCCAACACCGGC	AGCTTTACACCATCAACGCC	54	AT
K-SSR-36	AGTCCATTTCCAAATGCCAA	TTCTTCGGAGAATCAGCACC	52	AG
K-SSR-37	ACTCGAACCTTTAGACCGCA	CTCAAATCGTCGAACTCGGT	53	AAC
K-SSR-38	AGAAACTTCCAAGACATGCCA	GACAGACTGGCTCAGCTCG	55	AG
K-SSR-39	AAAGGAACATCAAAGACGGG	ACTCAGTCAATCCGAGTCCC	53	AG
K-SSR-40	CCTTCCATGTTCATGCCCT	GTGGGTCAACTTCAACGAGG	54	ACC
K-SSR-41	TGACAGGAAGCACTACAGCG	ATGCCTTAGTGGACACGACC	55	AG
K-SSR-42	TTTCTTCTTCAGGTACGGCG	AGGTGGAGGAGTTCTTCGCT	55	AGG
K-SSR-43	ATATCCTCCTCAATTCCCGC	AAGAAAGTTCCCGATTGGCT	53	AGG
K-SSR-44	CAACTCTTACCCACACCGCT	AAGCTAAATTGTGTTGCGGG	54	AG

Table S2: Continue

No.	PRIMER_LEFT_SEQUENCE	PRIMER_RIGHT_SEQUENCE	TM (°C)	MOT_TRANS
K-SSR-45	TGTCTAAAGCATTGAATCGGG	CAGACCGTTAGGTAGCTGGC	53	AG
K-SSR-46	CACCAGATTCAGACAGCCAA	CCGCAAGGAATTAATCGAAA	52	CG
K-SSR-47	CAATAGACCCAATAGCTGGCA	GGAGTCAGAGAAGCACCACC	57	ACC
K-SSR-48	ACCACTGAGTCCAAAGCCAA	ATGCTTCCTTGTTCACTCCG	54	AAG
K-SSR-49	AACATGTTCAAATCCTCCGC	CTTCTCTGAGTCGTCACGCA	56	AG
K-SSR-50	CATAGACACCCTCAACGCCT	CTCCACTTCACTCCTCCCAA	56	AG
K-SSR-51	TGGAATTGTACATCCTCGCA	TCTTCTTCAATCGCAATCCC	53	ATC
K-SSR-52	GTGAGAGAGTGAAGGAGCCG	GGTGTCTCCATTGAAGTCCG	57	AT
K-SSR-53	ACCCTCTCCTTTCTATCCCG	CCTTTCTCTTGGTCTGCCAC	56	AG
K-SSR-54	AACCTGTGTATGAGACCGCC	CGTCTTCTTCAACACACCTCC	56	AT
K-SSR-55	GAAGAGAGAAGGCTCTGCGA	CTCATCATCACATAGCTCCCAA	55	AG
K-SSR-56	AAGGAAATGATGGGTTCGG	TTCAAAGACCATCAATCCAGC	52	AG
K-SSR-57	GCCTTCCTCATGTACTTGCC	TTGTCGTACTCCACTTGGCTT	55	AG
K-SSR-58	CGTATACAGTAGCCGACAGCG	TAGTTCTTCCTTCACCGCCA	57	AT
K-SSR-59	AATCGTCTGTACAAACCGGC	TCGTTCCTTTCTTGAAACCG	52	AAG
K-SSR-60	TGTTGTGTAGTTCAACCGCC	TTACTCGGTGGTTGTTTCCC	52	AG
K-SSR-61	TGGATTGCATACACAAAGCG	ACAAATAAGTGCGGTGCGTT	52	AG
K-SSR-62	CGTCGTAGACGTACTCGGCT	TACAACCAATCCAATGGCCT	55	AGG
K-SSR-63	TGTTTGGAATACGATTTCGG	TTTCATTTCCTCTCCATCCC	51	AAG
K-SSR-64	GACGATGGATTCAAATTGGG	TTCAACCACAACAACTTCGC	52	AAC
K-SSR-65	TCATCATCATAATCACCGCC	TTTCTCTTCTTCCAAGAGCCA	53	AAT
K-SSR-66	AATCCTTGAACGACTTTCGC	GGAACACACAACACACCACC	55	AC
K-SSR-67	CTTCTTCTTGCTCCTCGTCG	CGATACCTTTACCAATGCCAA	54	ATC
K-SSR-68	GCATGTTTACAGACCTCGCA	GAGACGATCACGAGAGGAGC	56	AGG
K-SSR-69	CTGTTGAGGTGTCAAGCGAA	TCAGAAACTCCACCTCCACC	55	ACC
K-SSR-70	TTTATCGTCATCATCCTGCG	TGGAGATGGATGTCTCCTCC	53	CCG
K-SSR-71	TGTAGCTGTGATAATGGCCG	ACTACCCAACACCAACAGGC	55	AAC
K-SSR-72	TCTATTTCACGTGATCTGGGC	CATGGACTGACTCACTCTCCC	56	AG
K-SSR-73	GACGTCGACATAATTGGCG	TTGGGTAGCAAGAGAGGGAA	54	AG
K-SSR-74	AGGTACTCACTGCAACGGCT	TGCACTGATTGCTATGGAGG	55	ACC
K-SSR-75	AAGAGAGAGTGCAAACCCGA	AAGCCAGCCTGTAACCAGC	55	AAG
K-SSR-76	ATAGCAAGTAAACCACCGGC	AAATTGTTGTCATTGAAGCCC	52	AG
K-SSR-77	GTGGTGCAGTAGTGAGGGCT	CTTGATCTTCTCGCTCCGAC	58	AG
K-SSR-78	CCCAAGTCTGAATCTACGGC	AGTGGGCTAGTTGTTGCCTC	56	AG
K-SSR-79	GAAATGCCAGTAGAGAGGCG	GTTGTTCGAAGAGTCGGGAG	56	AG
K-SSR-80	AAAGTTCTGTGAGAAGCCGC	CACCAATAACCCAAACCACC	56	AG
K-SSR-81	GACAAGATCAGCACTGCCAA	TTCTCCATTTCTGTCTCCGC	54	AG
K-SSR-82	GGAAATAATGGATCCAGGGC	CTATGCTCATGGAAATCCCG	53	AT
K-SSR-83	TACAGGTGGTTGAAATGCCA	TTTACCTGAGGGTTCTCCACC	53	AGG
K-SSR-84	GATCGGAGTTCTTGCGCTT	AAGCTTTCTCTGTTTCGGCA	51	AG
K-SSR-85	AAACAGTTGACAGTCGCCCT	CTCTTCACTCTCAGCGACCC	55	AG
K-SSR-86	ACATATCATGTTGCCAAGCG	AACTTGCTTTCGTTTCCAGC	51	AG
K-SSR-87	CAACAATCTTGTTCCTCCCAA	CTTTCCCTCTCTCTCCCGTT	53	AG
K-SSR-88	ACTGCTTTCTCAAAGTCGCC	AACGTGTCCTCTGAACCGAT	54	AG
K-SSR-89	GATGCTGTAGCTTAACCGCC	ACGGATAAATTCCTTTCCGC	54	AG
K-SSR-90	AGAACTCCCTGTTGACCCAA	GCTGTATGTAGCCTGAGCCC	56	AG
K-SSR-91	AGGAGAAGGAGAAGAAGCCG	TTTCCTCTTCAACACCTCCG	54	AGC
K-SSR-92	GGCTTGTGAAATTGTTGCG	GCTTAAGCATCTTCTGCGAAA	53	AAG
K-SSR-93	TTGTCGTTCTTGTTGTTGGC	ACGAAGTAATCGCAGAAGGC	52	AG
K-SSR-94	CTGAAATTTCGGAGAAAGCG	AGCTTGAACCTTATTCAGGGC	53	AAG
K-SSR-95	AAATTTATGGCAACCAAGCG	CAATCACACTCACACACGCA	52	AG
K-SSR-96	ACGAGGTAAGAGTGCTTGCG	CATCTGTCGGCTATGGCAA	54	AG

CTAB method[4]. The PCR amplification was performed using a Thermal Cycler (Bio-Rad T-100™ series, USA) and a reaction mix consisting of 25 μL 2×PCR reaction mixture (New England Biolabs, Ipswich, M A, USA), 2 μL genomic DNA (10-20 ng), 1 μL each of forward and reverse primer (10 μM) and 21 μL ddH_2O in a final volume of 50 μL. The PCR conditions were divided into two steps. First, initial denaturation at 94°C for 3 min, three cycles of 94°C for 30 sec, 50-60°C (T_m+1, T_m+0.5 and T_m, where T_m is the annealing temperature of the primers) for 30 sec, 72°C for 40 sec. Then 35 cycles of 94°C for 30 sec, 50-60°C for 30 sec, 72°C for 40 sec and a final extension at 72°C for 7 min. The PCR products were separated by polyacrylamide gel electrophoresis and agarose gel electrophoresis and they were observed using white and UV light, respectively.

Capillary electrophoresis: The primers were selected that showed stable amplification and redesigned the forward primers. Three primers were used: A 5′ M13-tailed forward primer (primer A), a reverse primer (primer B) and a fluorescently labeled M13 primer (primer C). The reaction mix was as follows: 12.5 µL 2×PCR reaction mixture (New England Biolabs, Ipswich, MA, USA), 2 µL genomic DNA and primer A, 0.5 µL primer B, 5 µL primer C and 3 µL ddH_2O in a final volume of 25 µL. The PCR conditions were the same as already discussed in this study.

Statistical analysis: The raw data was analyzed using the GeneMarker software (ver. 2.2.0) and the observed heterozygosity (H_o) and expected heterozygosity (H_e), the allelic Polymorphism Information Content (PIC) were calculated using GenAlEx (ver. 6.5)[14] and CERVUS (ver. 3.0)[15], respectively.

RESULTS

Characterization of EST-SSRs in *K. paniculata* Laxm.: In total, 2871 unigenes having putative EST-SSRs were discovered when we screened the transcriptome of *K. paniculata* Laxm. Among them, dinucleotide repeats, which constituted the largest group of repeated motifs, accounted for more than half of the total EST-SSR content (64.51%), followed by trinucleotide (34.38%), tetranucleotide (0.90%) and pentanucleotide (0.21%) repeats (Fig. 2). Dinucleotides and trinucleotides formed most of EST-SSRs, with the remaining repeats constituting only 1.11% of EST-SSRs.

The number of EST-SSR repeats ranged widely, from 5-12, in dinucleotide repeats in *K. paniculata* Laxm. and the frequencies of EST-SSR repeats decreased with increased motif length. Additionally, AG/TC accounted for the highest proportion (67.71%) of dinucleotide repeats, followed by AT/TA (21.00%), AC/TG (10.21%) and CG/GC (1.08%; Fig. 3a). Among trinucleotide repeats, AAG/TTC was the most abundant (25.43%), followed by ACC/TGG (16.21%), ATC/TAG (14.79%), AGG/TCC (11.14%) and AAC/TTG (10.13%; Fig. 3b).

Polymorphism analysis and fingerprint construction: Through the SSR amplification of samples from *K. paniculata* Laxm., 92 pairs of primers gave clear bands on PCR amplification; these accounted for 95.83% of the total primers tested, demonstrating the utility of our primers (Fig. S1, S2). Of the microsatellite loci that had clear amplification results, we selected 13 pairs for the construction of fingerprints with capillary electrophoresis (Fig. 4). In these 13 loci, the number

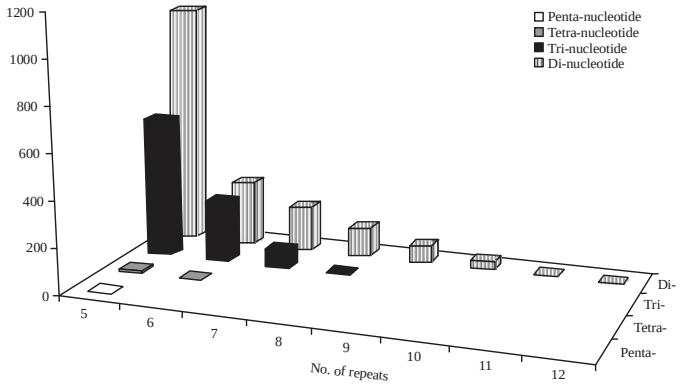

Fig. 2: Frequency distributions of various *K. paniculata* Laxm. EST-SSRs with differing numbers of repeats

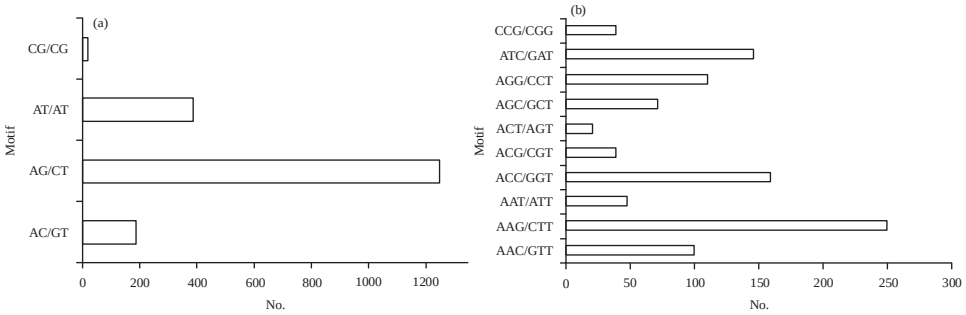

Fig. 3(a-b): No. of various (a) Dinucleotide and (b) Trinucleotide repeat motifs in *K. paniculata* Laxm.

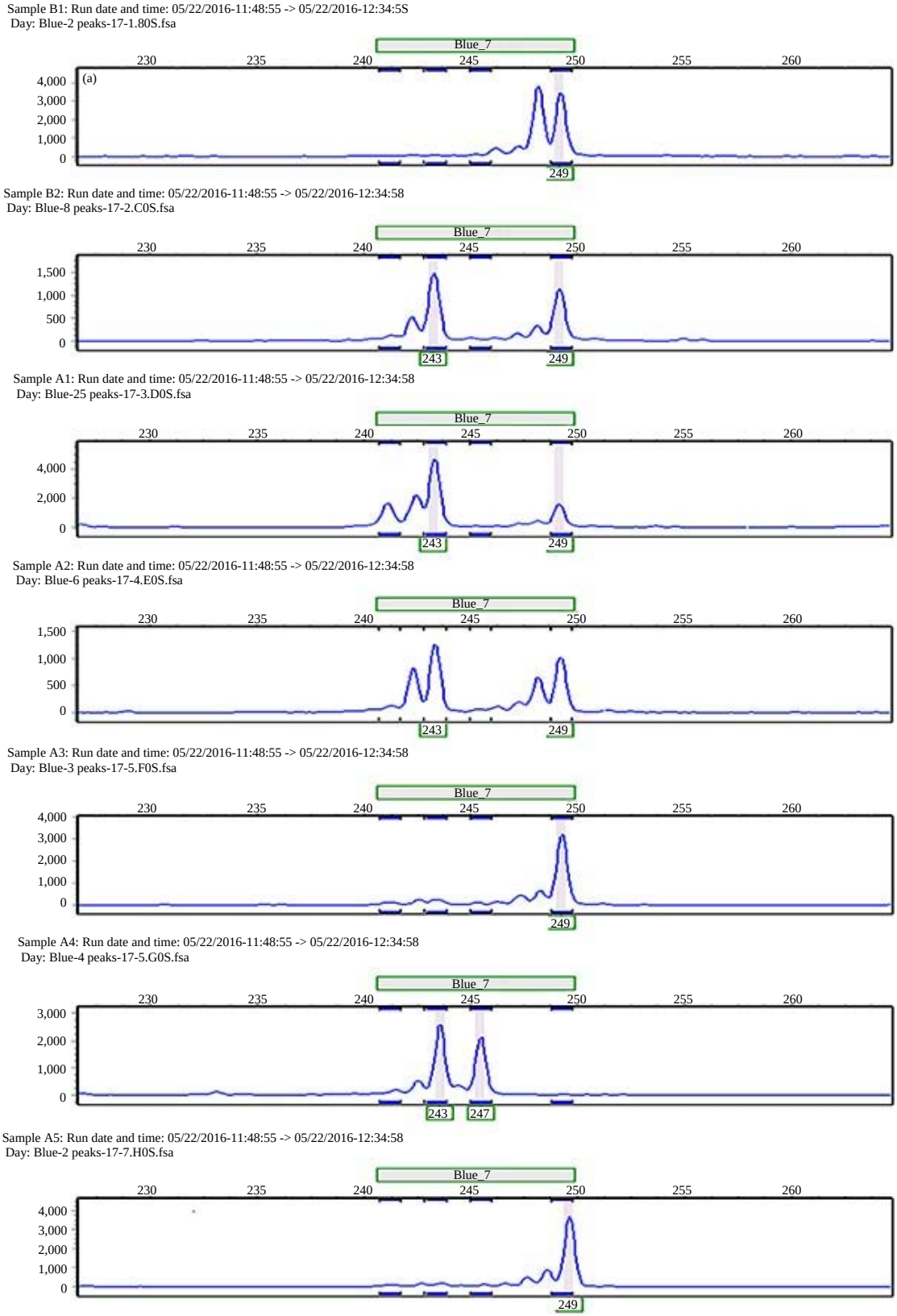

Fig. 4(a-c): Continue

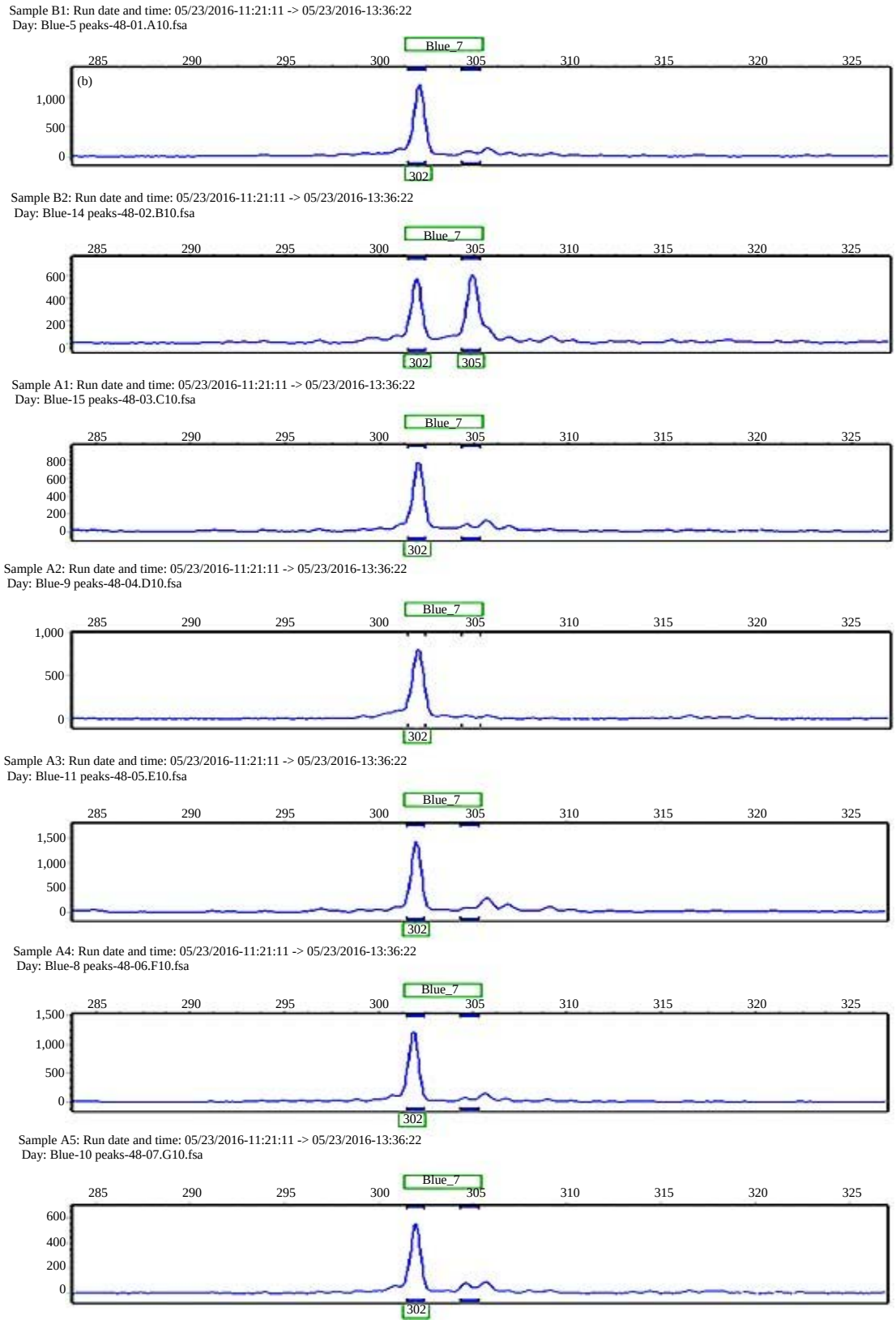

Fig. 4(a-c): Continue

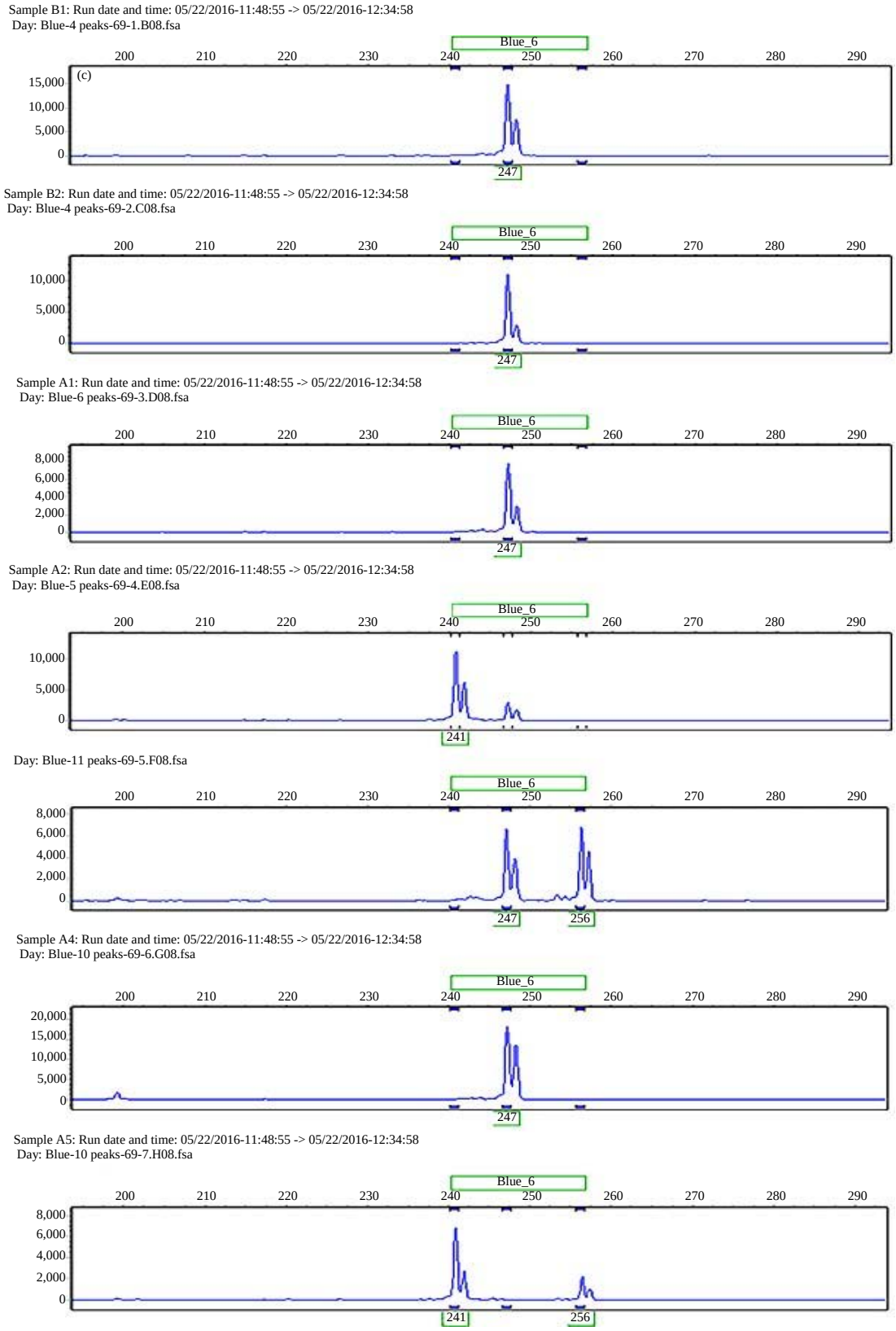

Fig. 4(a-c): Capillary electrophoresis results with primers, (a) 17 (b) 48 and (c) 69 for all samples (B1, B2, A1, A2, A3, A4 and A5)

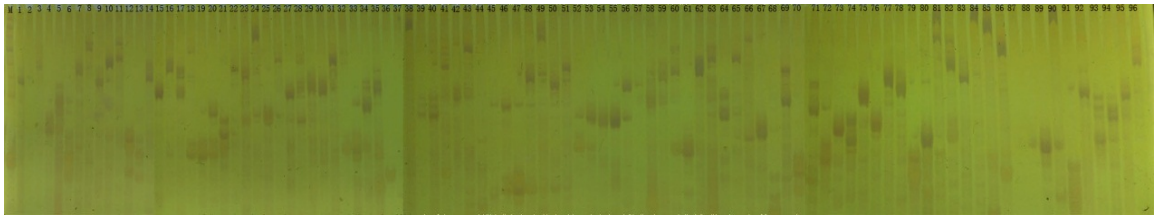

Fig. S1: Polyacrylamide gel electrophoresis results for all primers
Mixed DNA was used in the amplification

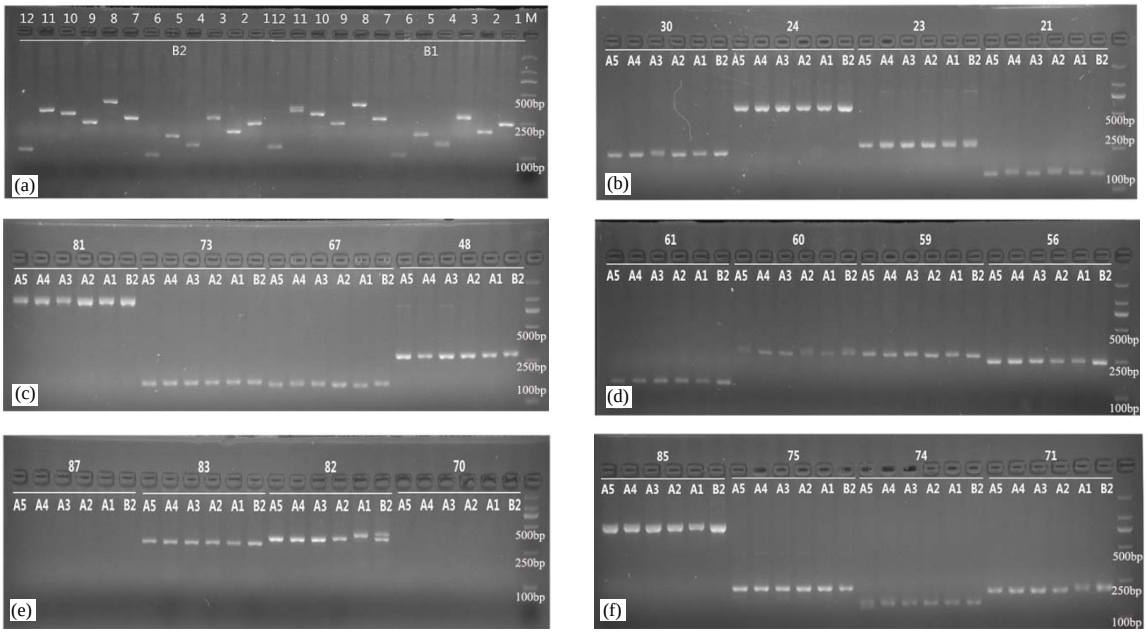

Fig. S2: Agarose gel electrophoresis results for some primers
The numbers indicate different primers and the letters are the numbers of the samples

of alleles ranged from 1-5 per locus and 10 pairs were polymorphic among individuals, whereas 3 pairs were monomorphic. The 10 pairs were used to analyze polymorphism information content and for fingerprint construction. The PIC value of the 10 pairs ranged from 0.124-0.726. The H_o and H_e levels were in the ranges of 0.143-1.000 and 0.133-0.765, respectively (Table 1). Based on the capillary electrophoresis results, the study established fingerprints for each sample (Table 2).

DISCUSSION

This is the first study about EST-SSR markers development in *K. paniculata* Laxm. and the information about EST-SSRs distribution in the species was obtained, which was similar to that reported for the rubber tree[16]. As an important biotechnology, the study about SSRs in the species could play an important role in future research and similar reports have been used in many species[17], espically in agricultural[18,19], commercial[20,21] and horticultural[22] crops. However, the lacking sequence limit the development of SSR markers in most species. The advent of next-generation sequencing technology provides resources for the development of potential SSR markers, especially non-sequenced species. For example, EST-SSRs was studied in *Amentotaxus* species to obtain amplifiable EST-SSR markers in the interspecies[5] using transcriptome; EST-SSRs developed from transcriptome can do a great favour in variety identification as well as identification of parent-offspring relationships for some species like caprifig[23] and tea[24], Dutta *et al.*[9] and Li *et al.*[7] also made a research prograss about the distribution of EST-SSRs in pigeonpea and kenaf using next generation squencing, respectively.

Koelreuteria paniculata Laxm., a unique native Chinese tree, has great value in both landscaping and pharmacy[1]. However, in the current study, it is insufficient about the

Table 1: Characteristics of 13 microsatellite loci developed in *K. paniculata* Laxm.

Locus	Primers (5'→3')	Na	Motif	Ta (°C)	H_o	H_e	PIC
K-SSR-3	F:TGTCAACTTTCATGATCGCC R:GCCTCTGGATTTCCTCCC	5	AG	53	0.571	0.765	0.726
K-SSR-13	F:GACAATGAAGATGATGGCGA R:TGTCCAAGAACATGAATGGG	4	AAG	52	1.000	0.684	0.626
K-SSR-17	F:GCAGGTTTGCTATTATCGTCG R:GGAAATGGTCACTCTCTCCG	3	AC	55	0.571	0.500	0.427
K-SSR-23	F:GTTGGGATAATAGGCTGCCA R:AGGGTGTGTAACTTGAGCGAA	2	AT	54	0.429	0.337	0.280
K-SSR-48	F:ACCACTGAGTCCAAAGCCAA R:ATGCTTCCTTGTTCACTCCG	2	AAG	54	0.143	0.133	0.124
K-SSR-60	F:TGTTGTGTAGTTCAACCGCC R:TTACTCGGTGGTTGTTTCCC	4	AG	52	0.857	0.724	0.674
K-SSR-69	F:CTGTTGAGGTGTCAAGCGAA R:TCAGAAACTCCACCTCCACC	3	ACC	55	0.286	0.520	0.464
K-SSR-71	F:TGTAGCTGTGATAATGGCCG R:ACTACCCAACACCAACAGGC	2	AAC	55	0.167	0.153	0.141
K-SSR-92	F:GGCTTGTGAAATTGTTGCG R:GCTTAAGCATCTTCTGCGAAA	2	AAG	53	0.143	0.133	0.124
K-SSR-93	F:TTGTCGTTCTTGTTGTTGGC R:ACGAAGTAATCGCAGAAGGC	3	AG	52	0.714	0.653	0.580
K-SSR-28	F:TCTGCATAGTTGTTCCGCTG R:CACTCAGTCTCTTCTCCCGC	2	AG	56	/	/	/
K-SSR-74	F:AGGTACTCACTGCAACGGCT R:TGCACTGATTGCTATGGAGG	1	ACC	55	/	/	/
K-SSR-82	F:GGAAATAATGGATCCAGGGC R:CTATGCTCATGGAAATCCCG	2	AT	53	/	/	/

Na = No. of different alleles, Ho: Observed heterozygosity = no. of hets/N, He: Expected heterozygosity = 1-Sum pi^2, PIC: Polymorphic information content

Table 2: Fingerprints of seven samples of *K. paniculata* Laxm.

Locus	Allele	A1	A2	A3	A4	A5	B1	B2
K-SSR-3	289	0	0	0	0	0	0	1
	295	0	1	0	1	1	0	0
	301	0	1	1	0	0	1	0
	305	1	0	1	0	1	0	0
	307	0	0	0	0	0	1	0
K-SSR-13	128	0	1	1	1	1	1	1
	134	1	0	0	1	1	0	0
	194	0	1	1	0	0	1	1
	203	1	0	0	0	0	0	0
K-SSR-17	243	1	1	0	1	0	0	1
	247	0	0	0	1	0	0	0
	249	1	1	1	0	1	1	1
K-SSR-23	291	0	1	1	1	0	0	0
	293	1	1	1	1	1	1	1
K-SSR-48	302	1	1	1	1	1	1	1
	305	0	0	0	0	0	0	1
K-SSR-60	309	1	0	1	0	0	1	1
	315	0	0	0	0	0	1	1
	321	0	1	0	1	1	0	0
	323	0	1	0	1	1	0	0
K-SSR-69	241	0	1	0	0	1	0	0
	247	1	0	1	1	0	1	1
	256	0	0	1	0	1	0	0
K-SSR-71	232	0	1	1	1	1	1	1
	241	0	0	0	0	0	0	1
K-SSR-92	250	0	0	0	0	0	1	0
	256	1	1	1	1	1	1	1
K-SSR-93	181	0	0	0	1	1	1	0
	187	1	1	1	1	0	0	1
	195	0	1	1	0	1	0	1

1: Presence of polymorphic alleles in the samples, 0: Absence of polymorphic alleles in the samples

research in molecular markers for the species. The markers obtained in the study have been proved to be valuable and effective. Ninety two primers were proved to have effective amplification in the species and 10 of 13 primers are with polymorphism. These primers all can be used for future genomic studies in *K. paniculata* Laxm. For example, they can be used in genomic diversity analyses as has been reported in the white poplar[11], *Jatropha curcas* L.[25], the chickpea[26] and wheat[27]. They will also be of value in genetic mapping, including linkage mapping and comparative mapping as has been described in wheat and rice[28], red raspberry[29], *Populusnigra* L.[30] and pepper[31]. Moreover, the cross-species/genera transferability of EST-SSRs can also be used in research on phylogenetic relationships[32,33]. The EST-SSR markers, which have the advantages of SSR markers are also more widely applicable. For some non-model plants, especially those with large genomes, it is more effective to develop EST-SSRs from the transcriptome. Indeed, it may be cheaper and more reliable.

CONCLUSION AND FUTURE RECOMMENDATIONS

In this study, effective EST-SSR markers were developed and 10 pairs of primers were confirmed to be polymorphic. The construction of fingerprints for four special varieties in the species provided protection for variety promotion. The study about EST-SSRs in this tree has great potential utility in future studies about the species. Abundant and highly transferable EST-SSR markers would do a great favour for future breeding of *K. paniculata* Laxm..

SIGNIFICANCE STATEMENTS

This study developed new EST-SSR markers in *Koelreuteria paniculata* Laxm. at the first time and provides information about the distribution of EST-SSRs in the species. This study will help researchers to estimate genetic diversity and population structure within this species and can be benificial for the development of a breeding strategy for *Koelreuteria paniculata* Laxm.

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