Development and characterization of genomic SSR markers for *Tamarix chinensis* (Tamaricaceae)

Ruhua Zhang¹,4, Qiang Wen², and Li-an Xu³

Belonging to the family Tamaricaceae, *Tamarix chinensis* Lour. is an alkali- and salt-tolerant, loosely branched deciduous shrub or 3–6-m-tall tree. It is naturally distributed from the temperate to subtropical zones in China, Korea, and Japan, inhabiting riverbeds, sandy floodplains, deserts, and coastal tidal flats (Baum, 1978) and has been naturalized in much of the western United States since its introduction in the mid-nineteenth century (Whiticrt et al., 2007). The level of genetic diversity and mating system of the species are not well known. A few studies have focused on the genetic diversity and population genetic structure of *T. chinensis* using random amplified polymorphic DNA (RAPD) (Zhao et al., 2008) and inter-simple sequence repeat (ISSR) markers (Jiang et al., 2011). Codominant simple sequence repeat (SSR) markers are powerful tools for population variation analysis and for the estimation of gene flow through genotypic exclusion in a number of tree species (Vahdati et al., 2015). To date, expressed sequence tag (EST)–SSR markers have been developed through mining of EST databases of *Tamarix* L. spp., and the transferability across species including *T. chinensis*, *T. gallica* L., *T. aphylla* (L.) H. Karst, *T. jordania* Bois., *T. nilotica* (Ehrenb.) Bunge, and *T. tetragyna* Ehrenb. was tested (Terzoli et al., 2013). A set of 10 genomic microsatellites of *T. ramosissima* Ledeb. was isolated using the biotinylated-oligonucleotide capture method (Gaskin et al., 2006), two of which showed polymorphism in Chinese *T. chinensis* material (Zhang, 2011). Although microsatellite primers have been developed for some *Tamarix* spp., none have yet been identified specifically for *T. chinensis*. We obtained a large number of DNA sequences based on high-throughput sequencing and characterized SSRs distributed in the genome of *T. chinensis*. A set of 10 polymorphic SSR molecular markers of *T. chinensis* were developed and the transferability was tested in its congener *T. ramosissima*.

**METHODS AND RESULTS**

Fresh leaves of one *T. chinensis* individual from a natural population of Binzhou City, Shandong Province, China (Appendix 1), were collected for DNA extraction and Illumina sequencing. Genomic DNA was extracted using a DNeasy kit (QIAGEN, Venlo, The Netherlands). Genomic libraries were constructed using the method for RAD sequencing as described by Baird et al. (2008). A Qubit 2.0 kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA) was used to evaluate the quality of the libraries, and the Agilent 2100 bioanalyzer (Agilent Technologies, Santa Clara, California, USA) was used to check the sizes of the libraries after they were diluted to 1 ng/μL. Sequencing was conducted on the
HiSeq 2500 high-throughput sequencing system (Illumina, San Diego, California, USA) to generate 125-bp paired-end reads by a commercial company (Novogene Co. Ltd., Beijing, China). Raw data were cleaned up by trimming the adapters and low-quality reads with a custom script by the company who did the sequencing, and by filtering reads with read depth of <10 and >400 using CD-HIT-EST (Li and Godzik, 2006) to avoid false positives.

Assembly of paired reads was performed using Velvet version 1.1.06 (Zerbino and Birney, 2008). Contigs with a length of less than 125 bp were deleted. Raw sequences were deposited to the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA accession PRJNA492209). A total of 818,142 contigs were generated with an average length of 323 bp and an N50 length of 474 bp. The MISA perl script (Thiel et al., 2003) was used to search for SSRs with mono-, di-, tri-, tetra-, penta-, and hexanucleotide motifs with a minimum repeat number of 14, eight, six, five, four, and four, respectively. Compound microsatellites were defined as having two or more motifs separated by an interval of ≤100 bp. A total of 31,140 SSRs were identified using the MISA perl script for 28,454 contigs. There were 2567 contigs that contained more than one SSR, and there were 2027 compound SSRs. Di- and trinucleotide motifs were the most abundant, comprising 41.53% and 41.24%, respectively. SSR primers were designed with Primer3 version 1.1.4 (Rozen and Skaletsky, 1999) with the following qualifications: primer length range from 18 to 22 bp and annealing temperature 55–60°C. Twenty-four primers were synthesized by a commercial company (GenScript, Nanjing, China).

In total, 58 individuals of *T. chinensis* from four natural populations and 24 individuals of *T. ramosissima* from one population were sampled (Appendix 1). We collected no more than 20

**TABLE 1. Characteristics of 21 novel genomic microsatellite markers developed for *Tamarix chinensis.***

| Locus* | Primer sequences (5′–3′) | Repeat motif | Allele size range (bp) | GenBank accession no. |
|--------|--------------------------|--------------|------------------------|-----------------------|
| TC1    | F: ATGTGGGGAGGTGGAGTG   | (CTT)$_{10}$ | 115–127 T. chinensis   | MG856343 |
|        | R: AATGTAGGACGACAAGAGT  |              | 115–121 T. ramosissima  |          |
| TC3    | F: AAACCGGAGTGGAGTGA    | (TTA)$_{11}$ | 150–204 T. chinensis   | MG856344 |
|        | R: ACACCCCTAATGCCATAAC  |              | 153–201 T. ramosissima  |          |
| TC4    | F: ATCCCCAGGTTGTTAAAT   | (AAT)$_{11}$ | 162–201 T. chinensis   | MG856345 |
|        | R: GCTGCTGGTACCCCTAACA |              | 162–186 T. ramosissima  |          |
| TC5    | F: GTCTGCTAAGAAGTCGC    | (TCTT)$_{8}$ | 189–221 T. chinensis   | MG856346 |
|        | R: CGAAATAAACGAGAAGAT  |              | 186–218 T. ramosissima  |          |
| TC6    | F: GATAAGCTTGTACGATT    | (ATA)$_{11}$ | 158–236 T. chinensis   | MG856347 |
|        | R: TCTAGTACACCTACCCC   |              | 158–230 T. ramosissima  |          |
| TC7    | F: GGTCTTTTATGTTCTTCTC | (TATT)$_{6}$ | 194–214 T. chinensis   | MG856348 |
|        | R: TATGGCTCTAATCTTCTT  |              | 194–206 T. ramosissima  |          |
| TC8    | F: TTGAGCTTGAGTGAGTA    | (AAT)$_{11}$ | 208–244 T. chinensis   | MG856349 |
|        | R: GATGACCGGTGTTTAGT    |              | 208–238 T. ramosissima  |          |
| TC12   | F: TAAGAAAGGTTAGAGGAGA | (AAG)$_{11}$ | 281–341 T. chinensis   | MG856350 |
|        | R: TAATCAAAGTTCAACAGG   |              | 281–338 T. ramosissima  |          |
| TC17   | F: AGTAGGCGCAAGGTATAT   | (TG)$_{10}$  | 335–347 T. chinensis   | MG856351 |
|        | R: CTCAGAAGCTTCCTAGA    |              | 332–344 T. ramosissima  |          |
| TC19   | F: GAGGCTGGGCAAGAAATG   | (TTA)$_{9}$  | 369–409 T. chinensis   | MG856352 |
|        | R: TGGAGCAGCAAGATGTA    |              | 369–409 T. ramosissima  |          |
| TC2    | F: CAGTGGTATGAGGGT      | (AAT)$_{10}$ | 151 T. chinensis       | MG856353 |
|        | R: GATGCGGTGACGGATG     |              | 151 T. ramosissima     |          |
| TC9    | F: CGAACTAAATACCTCAA    | (TTA)$_{10}$ | 205 T. chinensis       | MG856354 |
|        | R: CTATCCCGAAGACTCAA    |              | 205 T. ramosissima     |          |
| TC10   | F: CAACTTTTACCCCTCTTCT | (AT)$_{11}$  | 239 T. chinensis       | MG856355 |
|        | R: ATTCGAGGCTACCTACA    |              | 239 T. ramosissima     |          |
| TC11   | F: CAGTGGTATAGGAGGTT    | (TTA)$_{10}$ | 256 T. chinensis       | MG856356 |
|        | R: GATGCGGATTGGAGG      |              | 256 T. ramosissima     |          |
| TC13   | F: TTCTAACCCTAAACTCCT   | (TATT)$_{9}$ | 273 T. chinensis       | MG856357 |
|        | R: A T G A A A T C T C T G T  |              | 273 T. ramosissima     |          |
| TC14   | F: AAATGTGGTGCTTGTG     | (AAG)$_{10}$ | 290 T. chinensis       | MG856358 |
|        | R: TTTATAGCTTCTTGGG     |              | 290 T. ramosissima     |          |
| TC15   | F: CTTAGCCCTAGCCTTGG    | (TAT)$_{11}$ | 317 T. chinensis       | MG856359 |
|        | R: TAACCTCCCTCTTACCC    |              | 317 T. ramosissima     |          |
| TC16   | F: CTCTGGGCTTGGATAC     | (CAG)$_{10}$ | 320 T. chinensis       | MG856360 |
|        | R: TAAAGCTGGCTTGGGAG    |              | 320 T. ramosissima     |          |
| TC21   | F: ATATCTCCACCTCGGACAA  | (ATT)$_{11}$ | 382 T. chinensis       | MG856361 |
|        | R: AACACCATCCTACCTACATC |              | 382 T. ramosissima     |          |
| TC22   | F: TTCCTTACCTTTCTTGC    | (AAT)$_{10}$ | 383 T. chinensis       | MG856362 |
|        | R: ATTCGAGCTCCACACACA   |              | 383 T. ramosissima     |          |
| TC24   | F: TTATGCTGGAGTTGAGT    | (ATT)$_{10}$ | 409 T. chinensis       | MG856363 |
|        | R: GTGGTAATGTTGACGAAT   |              | 409 T. ramosissima     |          |

*Annealing temperature for all loci was 55°C.*
TABLE 2. Level of polymorphism of 10 microsatellite loci developed for Tamarix chinensis in four T. chinensis populations and one T. ramosissima population.*

| Locus | T. chinensis (KL, N = 18) | T. chinensis (HK, N = 13) | T. chinensis (LJ, N = 13) | T. chinensis (FS, N = 14) | T. ramosissima (CY, N = 24) |
|-------|--------------------------|---------------------------|--------------------------|--------------------------|----------------------------|
|       | H_o | H_e | PIC | H_o | H_e | PIC | H_o | H_e | PIC | H_o | H_e | PIC |
| TC1   | 3   | 0.558 | 0.517 | 0.469 | 3   | 0.364 | 0.310 | 0.478 | 3   | 0.556 | 0.526 | 0.594 | 3   | 0.300 | 0.455 | 0.566 |
| TC3   | 10  | 0.812 | 0.794 | 0.803 | 5   | 0.677 | 0.593 | 0.612 | 7   | 0.524 | 0.616 | 0.673 | 8   | 0.626 | 0.723 | 0.717 |
| TC4   | 4   | 0.414 | 0.507 | 0.516 | 3   | 0.337 | 0.415 | 0.468 | 4   | 0.384* | 0.497 | 0.404 | 5   | 0.414* | 0.535 | 0.487 |
| TC5   | 6   | 0.667 | 0.583 | 0.632 | 6   | 0.660 | 0.678 | 0.669 | 7   | 0.750 | 0.726 | 0.723 | 4   | 0.556 | 0.562 | 0.605 |
| TC6   | 11  | 0.607 | 0.733 | 0.645 | 7   | 0.541 | 0.501 | 0.627 | 9   | 0.653 | 0.582 | 0.605 | 11  | 0.549 | 0.645 | 0.653 |
| TC7   | 4   | 0.632 | 0.568 | 0.603 | 3   | 0.548 | 0.337 | 0.606 | 4   | 0.621 | 0.678 | 0.717 | 3   | 0.523 | 0.585 | 0.568 |
| TC8   | 8   | 0.717 | 0.634 | 0.622 | 6   | 0.562 | 0.667 | 0.636 | 7   | 0.613 | 0.546 | 0.608 | 8   | 0.701 | 0.757 | 0.628 |
| TC12  | 8   | 0.596 | 0.515 | 0.502 | 6   | 0.659 | 0.553 | 0.597 | 6   | 0.549 | 0.512 | 0.504 | 7   | 0.624 | 0.657 | 0.663 |
| TC17  | 3   | 0.222 | 0.204 | 0.264 | 3   | 0.283* | 0.434 | 0.310 | 2   | 0.182 | 0.165 | 0.201 | 3   | 0.231 | 0.210 | 0.385 |
| TC19  | 5   | 0.706 | 0.630 | 0.643 | 3   | 0.846* | 0.541 | 0.512 | 3   | 0.538 | 0.494 | 0.584 | 4   | 0.636 | 0.645 | 0.693 |
| Mean  | 6.2 | 0.593 | 0.543 | 0.570 | 4.3 | 0.548 | 0.542 | 0.572 | 5.2 | 0.537 | 0.534 | 0.561 | 5.6 | 0.516 | 0.577 | 0.597 |

Note: A = number of alleles; H_o = expected heterozygosity; H_e = observed heterozygosity; N = sample size for each population; PIC = polymorphism information content.

*Indicates that H_e departs significantly from H_o under Hardy–Weinberg equilibrium (P < 0.01).

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CONCLUSIONS

This is the first report of genomic microsatellites for T. chinensis. The 10 polymorphic markers showed comparatively high genetic variation, transferability to congeneric species, little or no deviation from HWE, and were in linkage equilibrium. These properties make them especially useful for genetic analysis of population genetic structure, mating system, and gene flow in T. chinensis and its congeners.

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DATA ACCESSIBILITY

Raw sequences were deposited to the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA accession PRJNA492209). Sequence information for the developed primers has been deposited to NCBI; GenBank accession numbers accession PRJNA492209). Sequence information for the developed primers has been deposited to NCBI; GenBank accession numbers are provided in Table 1.

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APPENDIX 1. Voucher and locality information for *Tamarix chinensis* and *T. ramosissima* used in this study.\(^4\)

| Species | Locality | Geographic coordinates | Population code | N | Voucher specimen accession no.\(^2\) |
|---------|----------|------------------------|-----------------|---|------------------------------------|
| *T. chinensis* Lour. | Kenli, Shandong, China | 37°48′06″N, 119°02′17″E | KL | 18 | Tch-KL01-ZR |
| *T. chinensis* | Hekou, Shandong, China | 38°13′19″N, 118°50′30″E | HK | 13 | Tch-HK02-ZR |
| *T. chinensis* | Lijing, Shandong, China | 38°02′13″N, 118°4′30″E | LJ | 13 | Tch-LJ03-ZR |
| *T. chinensis* | Fangshan, Beijing, China | 39°63′19″N, 115°7′30″E | FS | 14 | Tch-FS04-ZR |
| *T. chinensis* \(^3\) | Binzhou, Shandong, China | 37°22′16″N, 118°03′22″E | BZ | 1 | Tch-BZ05-ZR |
| *T. ramosissima* Ledeb. | Changyi, Shandong, China | 37°05′06″N, 119°21′22″E | CY | 24 | Tra-CY01-ZR |

Note: N = number of individuals sampled.

\(^{\text{a}}\)Vouchers are deposited in Linyi University, Shandong Province, China.

\(^{\text{b}}\)All individuals were sampled from natural stands.

\(^{\text{c}}\)Binzhou, Shandong, China.

\(^{\text{d}}\)Individual used for DNA extraction and Illuma sequencing.