Development and characterization of EST-SSR markers for *Camellia reticulata*

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Camellia reticulata Lindl. (Theaceae) is an economically important ornamental flowering shrub or small tree that grows in Yunnan Province, southwestern Sichuan Province, and western Guizhou Province of China (Ming et al., 2000). As an ornamental plant, *C. reticulata* has over 1000 years of history of cultivation, and many outstanding cultivars have been selected or bred from wild *C. reticulata* for many centuries in China (Xia et al., 1994; Gu, 1997). *Camellia reticulata* is notable for its large flowers, brilliant colors, numerous cultivars, and long flowering duration (Xia et al., 1994; Ming et al., 2000). Furthermore, it is valued not only as a flowering ornamental but also as a source of oils. Its seeds have a high content of oil that is rich in unsaturated fatty acids, oleic acid, vitamin E, and other physiologically active substances, making it a valuable economic crop.

In recent years, there has been a decline in the number and size of natural populations of *C. reticulata* because of overharvesting and habitat destruction. This alarming situation necessitates an in-depth understanding of the current status of the genetic diversity of this species. Studies have been conducted on the genetic diversity of *C. reticulata* via inter-simple sequence repeat markers, chloroplast microsatellites, and amplified fragment length polymorphisms (Wang and Ruan, 2012; Tong et al., 2013; Yao et al., 2016; Xin et al., 2017). Yao et al. (2016) designed 20 expressed sequence tag–simple sequence repeat (EST-SSR) primer pairs based on the transcriptome of diploid *C. reticulata*. Of these, 18 were successfully amplified, detecting seven polymorphic loci in 24 *C. reticulata* individuals. We further tested these 20 markers in four natural populations, showing four loci with polymorphisms. These are not sufficient for inclusive studies on *C. reticulata*. Therefore, in this study, we aimed to develop new microsatellite markers that will help to investigate the reproductive...
characteristics of *C. reticulata*, evaluate its evolutionary potential, and develop effective strategies for the conservation, development, and utilization of wild natural populations. In addition, we tested the cross-species transferability of these markers in three other species of *Camellia*: *C. saluenensis*, *C. pitardii*, and *C. yunnanensis*, which are thought to be involved in the polyploidy of *C. reticulata*.

**METHODS AND RESULTS**

Fresh healthy leaves collected from 90 individuals of *C. reticulata* sampled from four wild populations from Yunnan Province, China, were freeze-dried or silica-dried. Forty samples from three populations of *C. saluenensis*, *C. pitardii*, and *C. yunnanensis* were also collected to test the cross-amplification of the markers. Voucher specimens were deposited at the Kunming Institute of Botany, Chinese Academy of Sciences (KUN) (Appendix 1). Genomic DNA was extracted from 20–30 mg of dried leaf tissue using a modified cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1987).

We obtained 50,287 EST sequences from the National Center for Biotechnology Information (NCBI) expressed sequence tags database (dbEST) (accessed on June 2019) (Boguski et al., 1993). To obtain a nonredundant EST data set for SSR identification and primer design, vectors were removed from EST sequences using SeqTrim (Falgueras et al., 2010) and poly(A) tails were trimmed using downstream applications (PREMIER Biosoft International, Palo Alto, California, USA) as a reference. The ploidy level of the sampled populations was unknown, but multiple copy bands per locus due to polyploidy were not observed. Of the 70 primer pairs tested, 50 yielded clear and reproducible amplicons in *C. reticulata*; the others were unstable or gave no product. Eleven loci showed polymorphisms (Table 1), and 39 loci were monomorphic (Appendix 2). These 11 polymorphic primers were used in 90 individuals of *C. reticulata* (four populations) for the population genetic analyses using the same protocol as the initial test. The polymorphic SSR loci were analyzed with POPGENE 32 software (Yeh et al., 2000) and GenALEX (Peakall and Smouse, 2006) for the number of alleles per locus, observed heterozygosity, and expected heterozygosity (Table 2). Hardy–Weinberg equilibrium by 1000 randomizations and linkage disequilibrium were estimated using POPGENE 32 software (Yeh et al., 2000).

### TABLE 1. Characteristics of 11 polymorphic microsatellite loci developed in *Camellia reticulata*.a

| Locus   | Primer sequences (5′−3′) | Repeat motif | Expected allele size (bp) | *T∗* (°C) | Putative function (Organism) | GenBank accession no. |
|---------|--------------------------|--------------|---------------------------|-----------|------------------------------|------------------------|
| CSSR2   | F: GGAAATGCGCTCGGTAGGT  | (TTG)2        | 186                       | 54        | Hypothetical protein TEA_012945 (*Camellia sinensis* var. *sinensis*) | FS951581.1             |
|         | R: CCTCTGCTCCTTCACCAATCTAC |            |                           |           |                              |                        |
| CSSR5   | F: GCTTAGGGGCAAAGATGAA  | (GGTGCT)5     | 196                       | 55        | Glycine-rich cell wall structural protein 1.8-like (*Camellia sinensis*) | FS952802.1             |
|         | R: CACTCCACTCTCCATATCCA  |            |                           |           |                              |                        |
| CSSR7   | F: GCCCTACCTCCCTCCTGTA  | (AG)13        | 123                       | 56        | Growth-regulating factor 4-like isoform X2 (*Camellia sinensis*) | FS950234.1             |
|         | R: CTATGCGGTAGGTCTTCTT  |            |                           |           |                              |                        |
| CSSR8   | F: AGGGAGAGAGGAGAGAGAGA | (CCTCA)5      | 130                       | 50        | NONE                         | GH710908.1              |
|         | R: TTTGGGAGGGGCACTTGGC  |            |                           |           |                              |                        |
| CSSR9   | F: TCGCTGCTCTCCTCACTCT  | (CTT)4        | 114                       | 56        | Hypothetical protein TEA_017838 (*Camellia sinensis* var. *sinensis*) | FS945416.1             |
|         | R: TCTACATGGGAGACTGACCTTAG |           |                           |           |                              |                        |
| CSSR35  | F: GCTCATGCGCATGCTACCC  | (CTT)3        | 167                       | 55        | sm-like protein LSM1B (*Camellia sinensis*) | GH710926.1             |
|         | R: TACCCCTTATACACCTGGT  |            |                           |           |                              |                        |
| CSSR36  | F: ATGCGAG ACAAGAAGAAGA | (TGA)3        | 105                       | 55        | Probable E3 probable E3 ubiquitin-protein ligase XERO (*Camellia sinensis*) | FS947941.1             |
|         | R: GGAGGAGATGGTAGATGGA  |            |                           |           |                              |                        |
| CSSR45  | F: AGGTCTGGTGTGATAGGTGT | (TC)14       | 117                       | 55        | Histone H1-like protein (*Camellia sinensis*) | JK711494.1             |
|         | R: ACCTCCAATCTCCACCAAC  |            |                           |           |                              |                        |
| CSSR48  | F: GCTATGGCGCTAATGGGAC  | (TGA)5        | 117                       | 55        | Protein PAT1 2 like (*Actinidia chinensis* var *chinensis*) | FS949009.1             |
|         | R: CCAGAAACATCACAACCAACA |           |                           |           |                              |                        |

Note: *T∗* = annealing temperature.

a All values are based on 90 samples representing populations from southwestern China (*N* = 18–27 for each); see Appendix 1 for locality and voucher information.
### TABLE 2. Genetic variation in the 11 polymorphic EST-SSR markers in four *Camellia reticulata* populations.a

| Locus   | TC (n = 18) | XD (n = 26) | SM (n = 19) | CX (n = 27) | Total (n = 90) |
|---------|-------------|-------------|-------------|-------------|---------------|
|         | A | H<sub>o</sub> | H<sub>e</sub> | H<sub>E</sub> | A | H<sub>o</sub> | H<sub>e</sub> | H<sub>E</sub> | A | H<sub>o</sub> | H<sub>e</sub> | H<sub>E</sub> | A | H<sub>o</sub> | H<sub>e</sub> | H<sub>E</sub> |
| CSSR2   | 3 | 0.222 | 0.541 | 0.032 | 3 | 0.462 | 0.503 | 0.027 | 2 | 0.000 | 0.102 | 0.000 | 3 | 0.000 | 0.140 | 0.000 |
| CSSR5   | 2 | 0.167 | 0.322 | 0.029 | 3 | 0.462 | 0.585 | 0.000 | 3 | 0.053 | 0.363 | 0.000 | 3 | 0.296 | 0.319 | 0.008 |
| CSSR11  | 4 | 0.222 | 0.630 | 0.000 | 4 | 0.115 | 0.664 | 0.000 | 5 | 0.000 | 0.677 | 0.000 | 4 | 0.037 | 0.505 | 0.000 |
| CSSR17  | 4 | 0.722 | 0.589 | 0.618 | 5 | 0.846 | 0.686 | 0.000 | 3 | 0.053 | 0.317 | 0.000 | 4 | 0.111 | 0.357 | 0.000 |
| CSSR18  | 3 | 0.722 | 0.643 | 0.026 | 5 | 0.846 | 0.787 | 0.000 | 4 | 0.421 | 0.563 | 0.000 | 2 | 0.296 | 0.377 | 0.144 |
| CSSR19  | 2 | 0.000 | 0.508 | 0.000 | 2 | 0.462 | 0.498 | 0.705 | 2 | 0.000 | 0.102 | 0.000 | 2 | 0.148 | 0.201 | 0.136 |
| CSSR35  | 2 | 0.056 | 0.056 | 1.000 | 3 | 0.039 | 0.112 | 0.000 | 2 | 0.000 | 0.102 | 0.000 | 3 | 0.148 | 0.322 | 0.000 |
| CSSR36  | 3 | 0.111 | 0.641 | 0.000 | 4 | 0.039 | 0.612 | 0.000 | 3 | 0.000 | 0.199 | 0.000 | 3 | 0.037 | 0.238 | 0.000 |
| CSSR38  | 3 | 0.222 | 0.298 | 0.000 | 4 | 0.192 | 0.250 | 0.000 | 2 | 0.000 | 0.398 | 0.000 | 4 | 0.111 | 0.357 | 0.000 |
| CSSR45  | 3 | 0.778 | 0.560 | 0.267 | 4 | 0.769 | 0.719 | 0.012 | 4 | 0.158 | 0.289 | 0.000 | 3 | 0.444 | 0.444 | 0.000 |
| CSSR48  | 3 | 0.722 | 0.532 | 0.220 | 2 | 0.000 | 0.462 | 0.000 | 2 | 0.000 | 0.102 | 0.000 | 2 | 0.074 | 0.352 | 0.000 |
| Mean    | 2.909 | 0.359 | 0.484 | 3.546 | 0.385 | 0.534 | 2.909 | 0.062 | 0.292 | 3.091 | 0.155 | 0.328 | 4.182 | 0.242 | 0.457 |

Note: A = number of alleles sampled; H<sub>o</sub> = observed heterozygosity; H<sub>e</sub> = expected heterozygosity; H<sub>E</sub> = Hardy–Weinberg equilibrium; n = number of individuals sampled.

*aLocality and voucher information are provided in Appendix 1.

### TABLE 3. Cross-amplification and genetic diversity statistics of EST-SSR markers developed for *Camellia reticulata* in three related species.a

| Locus   | JCPT (n = 13) | JCPL (n = 15) | JCYN (n = 12) |
|---------|--------------|--------------|--------------|
|         | A | A<sub>e</sub> | H<sub>o</sub> | H<sub>e</sub> | A | A<sub>e</sub> | H<sub>o</sub> | H<sub>e</sub> | A | A<sub>e</sub> | H<sub>o</sub> | H<sub>e</sub> |
| CSSR2   | 3 | 1.476 | 0.154 | 0.335 | 2 | 1.385 | 0.067 | 0.287 | 2 | 1.180 | 0.000 | 0.159 |
| CSSR5   | 2 | 1.257 | 0.077 | 0.212 | 2 | 1.220 | 0.067 | 0.186 | 1 | 1.000 | 0.000 | 0.000 |
| CSSR11  | 3 | 1.660 | 0.154 | 0.394 | 3 | 1.312 | 0.067 | 0.246 | 3 | 1.405 | 0.083 | 0.301 |
| CSSR17  | 3 | 1.733 | 0.077 | 0.440 | 3 | 1.495 | 0.133 | 0.343 | 2 | 1.280 | 0.083 | 0.228 |
| CSSR18  | 2 | 1.451 | 0.077 | 0.323 | 2 | 1.220 | 0.067 | 0.186 | 2 | 1.280 | 0.083 | 0.228 |
| CSSR19  | 2 | 1.451 | 0.077 | 0.323 | 2 | 1.220 | 0.067 | 0.186 | 1 | 1.000 | 0.000 | 0.000 |
| CSSR35  | 3 | 1.751 | 0.154 | 0.446 | 3 | 1.402 | 0.133 | 0.297 | 3 | 1.412 | 0.000 | 0.304 |
| CSSR36  | 3 | 1.808 | 0.231 | 0.465 | 3 | 1.495 | 0.067 | 0.343 | 3 | 1.412 | 0.000 | 0.304 |
| CSSR38  | 2 | 1.257 | 0.077 | 0.212 | 2 | 1.220 | 0.067 | 0.186 | 2 | 1.180 | 0.000 | 0.159 |
| CSSR45  | 3 | 1.660 | 0.000 | 0.394 | 3 | 1.226 | 0.067 | 0.191 | 2 | 1.180 | 0.000 | 0.159 |
| CSSR48  | 3 | 1.257 | 0.077 | 0.212 | 2 | 1.142 | 0.000 | 0.129 | 1 | 1.000 | 0.000 | 0.000 |
| Mean    | 2.546 | 1.514 | 0.105 | 0.342 | 2.455 | 1.303 | 0.073 | 0.235 | 2.000 | 1.212 | 0.023 | 0.168 |

Note: A = number of alleles sampled; A<sub>e</sub> = effective number of alleles; H<sub>o</sub> = observed heterozygosity; H<sub>e</sub> = expected heterozygosity; n = number of individuals sampled.

*aLocality and voucher information are provided in Appendix 1."
Among the 11 polymorphic loci, the number of alleles per locus ranged from two to seven with a mean of 4.182. The levels of observed and expected heterozygosity were 0.044–0.567 and 0.166–0.642, with averages of 0.242 and 0.457, respectively (Table 2). Four SSR markers were able to detect levels of expected heterozygosity above 0.5, indicating a high level of polymorphism in *C. reticulata*. All 11 polymorphic loci showed deviation from Hardy–Weinberg equilibrium within two or more populations (Table 2) as a result of heterozygosity deficits. This was most likely the result of the reproduction mode, habitat fragmentation, and inbreeding. We found no consistent deviation from linkage disequilibrium for any loci within the populations (*P* > 0.001). Cross-species amplification of the 11 polymorphic loci was tested on *C. saluenensis*, *C. pitardii*, and *C. yunnanensis*. All 11 EST-SSR markers were amplified successfully, using the same PCR protocol for *C. reticulata*, and were shown to be polymorphic (Table 3).

**CONCLUSIONS**

The EST-SSR polymorphic markers developed in this study will add to the existing resources of molecular markers and are expected to be useful for studies on population structure and genetic diversity in *C. reticulata*. The microsatellite loci described here were successfully cross-amplified in *C. saluenensis*, *C. pitardii*, and *C. yunnanensis*, suggesting that these markers may also be applicable to the study of genetic diversity in other *Camellia* species.

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**DATA AVAILABILITY**

Expressed sequence tag sequences for the newly developed primers have been deposited to the National Center for Biotechnology Information (NCBI)’s GenBank database; accession numbers are listed in Table 1 and Appendix 2.

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### APPENDIX 1. Locality and voucher information for *Camellia* species used in this study.

| Species                  | Population code | Voucher no. | Location                      | Geographic coordinates                  | Elevation (m) | N  |
|--------------------------|-----------------|-------------|-------------------------------|-----------------------------------------|---------------|----|
| *C. reticulata* Lindl.   | TC              | CR-TY-021   | Mazhan, Tengchong, Yunnan, China | 25°12′03.65″N, 98°28′34.46″E             | 1980          | 18 |
| *C. reticulata*          | XD              | CR-TY-013   | Niujie, Xundian, Yunnan, China | 25°19′21.03″N, 102°53′28.21″E             | 2121          | 19 |
| *C. reticulata*          | SM              | CR-TY-004   | Baiyi, Songming, Yunnan, China | 24°59′54.33″N, 101°25′10.77″E             | 2318          | 27 |
| *C. reticulata*          | CX              | CR-TY-018   | Fuyuan, Songming, Yunnan, China | 25°15′33″N, 102°55′18″E                   | 2147          | 13 |
| *C. saluenensis* Stapf ex Bean | JCPT          | CS-TY-01    | Fuyuan, Songming, Yunnan, China | 24°37′13.58″N, 104°9′28.4″E               | 2031          | 15 |
| *C. pitardii* Cohen-Stuart | JCPL        | CP-TY-01    | Junzi Mountain, Shizong, Yunnan, China | 25°15′53″N, 102°55′18″E                   | 18            |    |
| *C. yunnanensis* (Pit. ex Diels) | JCYN         | CY-TY-01    | Machang, Heqing, Yunnan, China | 26°28′26.68″N, 100°3′13.53″E              | 3113          | 12 |

Note: N = number of individuals sampled.

*All voucher specimens are deposited in the Herbarium of the Kunming Institute of Botany (KUN), Kunming, Yunnan Province, China.*

### APPENDIX 2. Characteristics of 39 monomorphic microsatellite loci developed in *Camellia reticulata*.

| Locus   | Primer sequences (5′-3′) | Repeat motif | Expected allele size (bp) | GenBank accession no. |
|---------|--------------------------|--------------|---------------------------|-----------------------|
| CSSR1   | F: CAAAGCCAAATGGAATTTGTC | (A)30        | 179                       | FS943489.1            |
|         | R: GCCAGTGAATTGTAATACGA   |              |                           |                       |
| CSSR3   | F: TTCCTCCATTTGCGTGAAA   | (AG)13       | 194                       | FS951626.1            |
|         | R: ACCGTCAGCCCTCACAATC   |              |                           |                       |
| CSSR4   | F: TGTTGACAATTCTTGTTGG   | (CTT)12      | 128                       | FS951901.1            |
|         | R: TTGGTACAGATGGAGATTG   |              |                           |                       |
| CSSR6   | F: TGTCTCTGATCCACCTTCTG  | (TCA)6       | 140                       | FS953739.1            |
|         | R: GGCACGAAATTGCCCTTGG   |              |                           |                       |
| CSSR7   | F: AAGATGAAAGTGTGGATTCC  | (TG)25       | 148                       | GH159087.1            |
|         | R: GTAACAACCATCACCAACAT  |              |                           |                       |
| CSSR8   | F: GCAGTAGTTGTTGAGTTGAG  | (A)31        | 180                       | GW863559.1            |
|         | R: CAGTGAATTGTAATACGACTC |              |                           |                       |
| CSSR9   | F: TTGTATGCTCCAAGGATCTG  | (A)20        | 201                       | GW863563.1            |
|         | R: GACTCCTGATAGGGCAGAT   |              |                           |                       |
| CSSR10  | F: TGCTGCTACACTCCCTCT    | (AG)20       | 104                       | GW342632.1            |
|         | R: GGTGCTGAGCTGCTGAT     |              |                           |                       |
| CSSR12  | F: ACCTTGGCTTTGCTCTCT    | (AAG)13      | 135                       | GO255031.1            |
|         | R: TTGACGCGGAAACCTCCT    |              |                           |                       |
| CSSR13  | F: TGCTGCTCCTACATAAGGTC  | (A)10        | 192                       | GW315083.1            |
|         | R: GCCAGTGAATTGTAATACGA  |              |                           |                       |
| CSSR14  | F: GGAAGCTGCTTGTTAGACCAT| (A)10        | 196                       | GW863601.1            |
|         | R: ACGGCCGATGGTATGTAAT   |              |                           |                       |
| CSSR15  | F: TCTTAAGCCAGCTCCTCAAC  | (A)10        | 179                       | GH734011.1            |
|         | R: GACCTCCTGATTGGGAGAT   |              |                           |                       |
| CSSR16  | F: TCACTAGCACCAGTGCTCTA  | (TG)25       | 187                       | GH734178.1            |
|         | R: GTGAAATGTAATACGGATCTC |              |                           |                       |
| CSSR20  | F: GCAGCTCTCCTCTTGCTAT   | (A)31        | 206                       | GH709471.1            |
|         | R: GCCAGTGAATTGTAATACGA  |              |                           |                       |
| CSSR21  | F: GTTGCTAATCTGCTGTCTAC  | (A)10        | 177                       | GH709922.1            |
|         | R: GCCAGTGAATTGTAATACGA  |              |                           |                       |
| CSSR22  | F: GAAATGATGACATCTCCTCA  | (CTCCAG)4    | 107                       | GH709760.1            |
|         | R: ATAGGGAGGATGTAACTTG   |              |                           |                       |
| CSSR23  | F: TTGGACACCTTGAAATGACT  | (A)20        | 114                       | GH612882.1            |
|         | R: TAGTCTGATAGCGGTCTGC   |              |                           |                       |
| CSSR24  | F: TGATGTAGTACAGCTGAG    | (A)20        | 110                       | GH613058.1            |
|         | R: TAGTCTGATAGCGGTCTGC   |              |                           |                       |
| CSSR25  | F: GCCAGGAGAATTCTTGATG   | (A)10        | 210                       | GE650217.1            |
|         | R: GCGTGAAATTGTAATACGA   |              |                           |                       |
| CSSR26  | F: TGATGCTGATAGCGGTCTGC  | (A)10        | 126                       | GH623471.1            |
|         | R: TAGTCTGATAGCGGTCTGC   |              |                           |                       |
| CSSR27  | F: CATATGTCATGGTAATTTTG  | (A)31        | 176                       | GW696815.1            |
|         | R: AGTGATACAGGCGGAGAG    |              |                           |                       |
| CSSR28  | F: CACATCCTCTCCTGTTGCTA  | (A)14        | 148                       | FS944961.1            |
|         | R: CCTCTTGTCTGCTCTTCCTC  |              |                           |                       |
| CSSR29  | F: GCTGCTGCTGTCTGACG     | (GA)12       | 163                       | FE861335.1            |
|         | R: TCTCTTCTCCTCCTCCTC    |              |                           |                       |

(Continues)
| Locus  | Primer sequences (5′–3′) | Repeat motif | Expected allele size (bp) | GenBank accession no. |
|--------|--------------------------|--------------|---------------------------|-----------------------|
| CSSR30 | F: AGAAAGAAGCTGCAAGGG    | (TTCT)₅       | 128                       | FE861638.1            |
|        | R: CTTAGATGAGGGCTGAGAG   |              |                           |                       |
| CSSR31 | F: AGCGCTGAAGTCCAAATCC   | (GCC)₆       | 145                       | CV699742.1            |
|        | R: AGTGCTCTCCGGTCTCAC     |              |                           |                       |
| CSSR32 | F: AACACTCAATCATCAGTGGTT | (A)₂₉        | 207                       | GH733834.1            |
|        | R: GTAAATACGTCCACTACATGAG|              |                           |                       |
| CSSR33 | F: GCGAAATGGGCTGATTGGTT  | (A)₂₉        | 172                       | FS959890.1            |
|        | R: GCCAGTGAATTGTTAATACGA |              |                           |                       |
| CSSR34 | F: CATGACATGCTGCTCATT    | (TC)₁₅       | 128                       | FS947495.1            |
|        | R: GATGACATGTGAACTGCTA   |              |                           |                       |
| CSSR37 | F: ACCCAAGCAAAGCAAT      | (AG)₁₃       | 103                       | FS948821.1            |
|        | R: AACTGCTGAAGATAGAGAGAGG|              |                           |                       |
| CSSR39 | F: TCCATCAAGACACACATCA   | (TTTC)₆      | 100                       | FS949741.1            |
|        | R: TCAACATCACAATACACAG    |              |                           |                       |
| CSSR40 | F: GGAATGTACTTGATGTTCCTTC| (A)₁₃        | 197                       | FS954738.1            |
|        | R: CCAGTGATTGTAATACGACTC |              |                           |                       |
| CSSR41 | F: CCTCCTCTATCTCTGTAATCAATA| (GA)₁₂     | 110                       | FS949096.1            |
|        | R: CGTAAACGCAATTCCTCTCT  |              |                           |                       |
| CSSR42 | F: GTAGCAATGGATACTCTGCA  | (A)₁₀        | 186                       | GH623925.1            |
|        | R: TCATGATTGGGAATTG     |              |                           |                       |
| CSSR43 | F: CGCTATTTATCTCGTCGGTT  | (A)₁₃        | 133                       | GH623383.1            |
|        | R: GTGTATGCCACCGAGAGTA   |              |                           |                       |
| CSSR44 | F: CCACACACGTACATCTACA   | (CAC)₁₅      | 157                       | FS949501.1            |
|        | R: GTGAGGGAGAGTTGAGTA    |              |                           |                       |
| CSSR46 | F: GCCGGAAAGATATTGGGGT   | (A)₁₄        | 149                       | GH623235.1            |
|        | R: TAGTAGGAGGCTGTGCTG     |              |                           |                       |
| CSSR47 | F: CTAGTGATAGCGTGTCTG    | (T)₁₅(T)₅₀   | 148                       | GH623319.1            |
|        | R: GTGTGATACGACCTCGA      |              |                           |                       |
| CSSR49 | F: TAAACTTATGACCTACAGT   | (A)₁₁        | 215                       | GW863554.1            |
|        | R: CAGTGATATGGTAATACGACTC|              |                           |                       |
| CSSR50 | F: TCCATAAAGGACCTCTAGC   | (CT)₁₁       | 164                       | JKS11141.1            |
|        | R: TCCACACATCTCCCAACT     |              |                           |                       |