The genus *Atractylodes* DC., which consists of important medicinal plants in northeastern Asia, belongs to the Asteraceae and is generally known to consist of five species: *A. japonica* Koidz., *A. macrocephala* Koidz., *A. lancea* (Thunb.) DC., *A. koreana* (Nakai) Kitam., and *A. carlinoides* (Hand.-Mazz.) Kitam. (Shi, 1981; Kunio et al., 1997; Peng et al., 2012). There is considerable taxonomic debate regarding this; *A. japonica* has been treated as a synonym for *A. lancea* in some reports (Shi and Greuter, 2011; Peng et al., 2012). However, the Korean and Japanese Pharmacopoeia not only distinguish the two species but also treat them as distinct herbal medicines (Ministry of Food and Drug Safety, 2008; Pharmaceuticals and Medical Devices Agency of Japan, 2016). Among these, *A. japonica* and *A. macrocephala* produce the “white *Atractylodes* rhizomes” used in traditional medicine in Korea and Japan (Lee et al., 2002). *Atractylodes japonica* is a perennial herb that reaches 30–100 cm in height. Unlike *A. macrocephala* and *A. lancea*, which are native to China, *A. japonica* grows naturally in the Republic of Korea (Lee, 2006). Although the two species are similar in appearance, *A. japonica* is monocious and produces a white flower, whereas *A. macrocephala* is gynodioecious and produces a claret flower (Peng et al., 2012; Jeong et al., 2018). The mass of a seed of *A. japonica* is half of that of *A. macrocephala*; thus, it takes longer for *A. japonica* seeds to germinate (Rural Development Administration, 2018). Despite challenges in its propagation and cultivation, *A. japonica* is of particular interest in medical applications. It has a high content of sesquiterpenoids, including atractylon and atractylenolides (Yun et al., 2013; Jeong et al., 2018), which are useful in the treatment of stomach disorders, inflammation, and obesity (Kim et al., 2011; Chen et al., 2016).

Compound simple sequence repeat (SSR) markers were previously developed from a related species, *A. macrocephala* (Zheng et al., 2012). However, this earlier study did not test the cross-amplification in *A. japonica*. We initially tested these markers in *A. japonica*, but without much success. In this study, we developed 18 polymorphic SSR markers from the genome of *A. japonica* and tested them for analysis of genetic diversity in various populations and related species.

**METHODS AND RESULTS:** We obtained a total of 175,825 simple sequence repeat (SSR) loci using the Illumina HiSeq 2500 system. Eighteen polymorphic SSR primer pairs were selected to determine heterozygosity levels and allele numbers in 80 individuals from four *A. japonica* populations. The levels of observed and expected heterozygosity ranged from 0.000 to 1.000 and from 0.133 to 0.892, respectively. Cross-amplification in the related species *A. macrocephala* and *A. lancea* was successful in 15 and 14 of the 18 markers, respectively.

**CONCLUSIONS:** These microsatellite markers will be useful for future studies involving *A. japonica* population genetics and breeding.

**KEY WORDS**: Asteraceae; *Atractylodes japonica*; genetic diversity; microsatellite markers; population genetics; simple sequence repeat (SSR).
A total of 157,825 SSRs were identified using the stand-alone version of SSRIT (Temnykh et al., 2001) with the following parameters: SSRs were defined as di-, tri-, tetra-, penta-, and hexanucleotide repeats with ≥4 repeats; and no variation (mutation) in repeat motifs was permitted. Forty-eight polymorphic SSR loci with at least a 4-bp motif containing a minimum of four repeats were selected by the comparison of the specific SSR loci of the four sequenced individuals using CLC Main Workbench (version 6.8.4, QIAGEN) according to Gil et al. (2017). SSR primers were designed using Primer3 (Untergasser et al., 2012) using the following conditions: length 18–26 bp, GC content 50–70%, and melting temperature 55–62°C. The PCR products ranged between 150 and 300 bp. Preliminary PCR analysis of the 48 primers was performed on one A. japonica individual collected from the Jeol Mountain population. Forty-four pairs of primers amplified the targets successfully. Four individuals per population were then tested with the selected primer sets and analyzed with the Fragment Analyzer Automated CE system (Advanced Analytical Technologies, Ankeny, Iowa, USA). Eighteen pairs of primers were selected based on the amplification efficiency and the number of alleles, and the forward primer of each set was labeled (Table 1). The PCR reaction mixture (total

TABLE 1. Characteristics of the 18 SSRs developed for Atractylodes japonica.

| Locus | Primer sequences (5’-3’) | Repeat motif | Allele size range (bp) | Fluorescent labelb | GenBank accession no. |
|-------|--------------------------|--------------|------------------------|---------------------|-----------------------|
| AJSSR001 | F: AACATCAGATGAGTTGGACCA  
                 R: ATAGGAGGAATGGGATGGAAGA | (ATGT)₅ | 157–189 | VIC | MN107252 |
| AJSSR002 | F: AAGGAGGAGAGGCTGGTTA  
                 R: GCAATTGAGCATCGACATA | (ACCAA)₅ | 216–281 | FAM | MN107253 |
| AJSSR003 | F: CAACCTCGCTGCAATTTTCG  
                 R: GAAGAGCCGAGCTGAGTTTA | (GGTTT)₆ | 261–291 | VIC | MN107254 |
| AJSSR004 | F: CAGGTTACCTCGCAATTTA  
                 R: ACCTTCTCCCTGTAATTCAAC | (AACCA)₆ | 118–161 | FAM | MN107255 |
| AJSSR005 | F: ATGGGCAACAGGGTGAAGT  
                 R: GGCGTACGGGATAGGGTG | (ACCA)₆ | 220–250 | FAM | MN107256 |
| AJSSR006 | F: TTACCCCGGACACATCTAA  
                 R: GCCACCAGTGGTACGATT | (GGTTT)₆ | 293–318 | VIC | MN107257 |
| AJSSR007 | F: TCTAAGACGTACGTCTGTTT  
                 R: TGACCTACCACACAAACATTG | (GGTTT)₆ | 270–295 | VIC | MN107258 |
| AJSSR008 | F: TGCTGTACCCGCAACCTCAT  
                 R: TGGCTGTCATTGCTGTTG | (AGGAGT)₅ | 344–404 | VIC | MN107259 |
| AJSSR009 | F: TTTTCTGCACTCTCACAACA  
                 R: CACACGAGATGCCAACAACA | (CTCTTC)₅ | 195–240 | PET | MN107260 |
| AJSSR010 | F: CCTGTTGTTTTCTGAGGAT  
                 R: TGATTTTGACTTACAGGGGA | (GGTTT)₅ | 217–259 | VIC | MN107261 |
| AJSSR011 | F: GTGCAAGCTTCCATGTCATG  
                 R: TAAGGCTGGTACATCCAT | (AAACCA)₅ | 148–178 | PET | MN107262 |
| AJSSR012 | F: TGGGTGTTTATACGGGTTC  
                 R: TCCCTGACCTTTACGACAA | (AATAAA)₄ | 208–230 | PET | MN107263 |
| AJSSR013 | F: GCAAATGGAGGCACTACATG  
                 R: AGCGTTCTCTCTACAAAGG | (GGTTT)₅ | 380–405 | PET | MN107264 |
| AJSSR014 | F: ATGTTGTTGCTGTCCTACCT  
                 R: GCTGTCTGGTCGGAGGTG | (GGGA)₄ | 238–262 | VIC | MN107265 |
| AJSSR015 | F: GGCTATTAGACTCTTCCCA  
                 R: CTCGTGCCCTGAGCTTAAA | (CAGT)₅ | 228–263 | VIC | MN107266 |
| AJSSR016 | F: GTATTGCTTGTGATGTGTAT  
                 R: ATGTAAAGGAGGGTGCTTC | (CTTCTC)₅ | 303–327 | NED | MN107267 |
| AJSSR017 | F: GAGAATGATCTCCTGCTGCG  
                 R: TTTCACTGGCATTCCAGGAA | (ATGT)₈ | 294–334 | FAM | MN107268 |
| AJSSR018 | F: TGAGTGGGATTTGAAAATGGGA  
                 R: GAGATGAGGCCCATGCTTT | (AAACCA)₅ | 147–192 | NED | MN107269 |

ₐAnnealing temperature was 56°C for all loci.

₏Fluorescent labeling was applied to forward primers.
We developed 18 polymorphic SSR markers from A. japonica and successfully used them to analyze genetic diversity in different populations and in two related species. These markers will be useful for the development of A. japonica cultivars and potentially for differentiation among Atractylodes species.

CONCLUSIONS

We developed 18 polymorphic SSR markers from A. japonica and successfully used them to analyze genetic diversity in different populations and in two related species. These markers will be useful for

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TABLE 2. Genetic properties of 18 polymorphic SSR markers in four Atractylodes japonica populations. (A = number of alleles; H_e = expected heterozygosity; H_o = observed heterozygosity; n = number of individuals.)

| Locus     | Jeol Mountain (n = 21) | Sageum Mountain (n = 20) | Jiri Mountain (n = 18) | Cheonbul Mountain (n = 21) |
|-----------|------------------------|--------------------------|------------------------|-----------------------------|
| AJSSR001  | 3                      | 0.476 (0.381)            | 3                      | 0.833 (0.505)               |
| AJSSR002  | 4                      | 0.619 (0.552)            | 5                      | 0.592 (0.500)               |
| AJSSR003  | 8                      | 0.714 (0.796)            | 5                      | 0.752 (0.718)               |
| AJSSR004  | 5                      | 0.905 (0.638)            | 5                      | 0.751 (0.771)               |
| AJSSR005  | 5                      | 1.000 (0.728)            | 5                      | 0.752 (0.701)               |
| AJSSR006  | 5                      | 0.333 (0.434)            | 3                      | 0.751 (0.674)               |
| AJSSR007  | 5                      | 0.235 (0.786)            | 5                      | 0.751 (0.627)               |
| AJSSR008  | 8                      | 0.810 (0.813)            | 7                      | 0.751 (0.676)               |
| AJSSR009  | 11                     | 0.905 (0.829)            | 9                      | 0.751 (0.676)               |
| AJSSR010  | 7                      | 0.762 (0.621)            | 6                      | 0.751 (0.676)               |
| AJSSR011  | 6                      | 0.667 (0.734)            | 5                      | 0.751 (0.676)               |
| AJSSR012  | 5                      | 0.333 (0.736)            | 3                      | 0.751 (0.676)               |
| AJSSR013  | 6                      | 0.810 (0.659)            | 5                      | 0.751 (0.676)               |
| AJSSR014  | 2                      | 0.143 (0.133)            | 7                      | 0.751 (0.676)               |
| AJSSR015  | 8                      | 0.450 (0.836)            | 3                      | 0.751 (0.676)               |
| AJSSR016  | 4                      | 0.154 (0.524)            | 7                      | 0.751 (0.676)               |
| AJSSR017  | 6                      | 0.381 (0.528)            | 5                      | 0.751 (0.676)               |
| AJSSR018  | 6                      | 0.667 (0.692)            | 7                      | 0.751 (0.676)               |
| Mean      | 5.78                   | 0.576 (0.634)            | 5.33                   | 0.465 (0.612)               |

Note: A = number of alleles; H_e = expected heterozygosity; H_o = observed heterozygosity; n = number of individuals.

The amplified DNA products (0.2 μL) were mixed with 9.8 μL of Hi-Di formamide (Applied Biosystems), 1 μL of 10 μM of each forward and reverse primer, and 5 μL of gDNA. The PCR reaction conditions were: initial denaturation at 95°C for 5 min; 34 cycles at 95°C for 30 s, 55°C for 25 s, and 72°C for 1 min; and a final extension at 72°C for 30 min. The amplified DNA product (0.2 μL) was mixed with 9.8 μL of Hi-Di formamide (Applied Biosystems) and 1 μL of GeneScan 500 LIZ size standard (Applied Biosystems). The mixture was denatured at 95°C for 5 min and kept on ice before being separated by capillary electrophoresis on an ABI 3730 DNA analyzer (Applied Biosystems). The amplified fragments were analyzed by size using GeneMapper version 4.1 software (Applied Biosystems). The allele count, levels of expected and observed heterozygosity, and Hardy–Weinberg equilibrium (Emigh, 1980) of each locus were calculated using PowerMarker software (version 3.23) (Liu and Muse, 2005).

The 18 SSR primer pairs were then tested in all collected A. japonica individuals, and the genetic diversity was calculated for each, as described above. The number of alleles per locus varied from two to 14 (Table 2). The levels of observed and expected heterozygosity per locus ranged from 0.000 to 1.000 and from 0.133 to 0.892, respectively. Some markers showed significant deviation from Hardy–Weinberg equilibrium (Table 2). For the applicability test of the developed markers, we applied the markers to five individuals each of A. japonica cultivars and potentially for differentiation among Atractylodes species.
DATA AVAILABILITY

Sequencing reads have been deposited in the National Agricultural Biotechnology Information Center (NABIC) Sequence Read Archive (BioProject ID: NN-5968, NN-5970, NN-5971, and NN-5972). Primer sequences have been deposited to the National Center for Biotechnology Information’s GenBank database; accession numbers are listed in Table 1.

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APPENDIX 1. Locality and voucher information for Atractylodes japonica and the two related species used in this study.

| Species | Collection locality | Voucher no. | Geographic coordinates | n |
|---------|---------------------|-------------|------------------------|---|
| A. japonica | Jeol Mountain, Hwacheon-gun, Gangwon-do | MPS005754 | 38°05′57″N, 127°44′57″E | 21 |
| A. japonica | Sageum Mountain, Samcheok-si, Gangwon-do | MPS005755 | 37°10′22″N, 129°10′56″E | 20 |
| A. japonica | Jiri Mountain, Gurye-gun, Jeollanam-do | MPS005756 | 35°20′14″N, 127°29′28″E | 18 |
| A. japonica | Cheonbul Mountain, Naaju-si, Jeollanam-do | MPS005757 | 34°55′30″N, 126°52′11″E | 21 |
| A. macrocephala | Eumseong-gun, Chungcheongbuk-do | MPS004740 | 36°56′34″N, 127°45′02″E | 2 |
| A. macrocephala | Mungyeong-si, Gyeongsangbuk-do | MPS004741 | 36°37′05″N, 127°59′54″E | 3 |
| A. lancea | (Thunb.) DC. | MPS000723-1 | 36°56′54″N, 127°45′02″E | 5 |

Note n = number of individuals sampled

* Locality and Korean province

These samples represent cultivated materials in Korea without known geographical sources from China.

The sample was collected in a cultivation area.