The genus *Zanthoxylum* L. (Rutaceae) includes approximately 200 or more species around the world (Wu et al., 2008), but only about six species occur in Korea. Two of these species, *Z. schinifolium* Siebold & Zucc., and *Z. piperitum* (L.) DC., are the most representative common species in this genus in Korea. *Zanthoxylum schinifolium* is a deciduous shrub distributed in China, Japan, Taiwan, and Korea (Hassler, 2016). This species has a unique aromatic flavor on its fruits and leaves and has therefore been traditionally used as an edible or medicinal plant. Recently, significant effectiveness in the antibacterial and anticancer activity of some of the compounds of this species has been scientifically confirmed (Choi et al., 2008; Li et al., 2013). Consequently, this species is attracting attention as a promising medicinal plant.

In many cases, the increasing economic value of a plant species can lead to drastic reductions in wild populations because of extensive use; thus, conservation efforts of genetic resources as breeding materials should be increased (Shippmann et al., 2003). *Z. schinifolium*, a medicinal species that breeders have recently started to cultivate, still has ample natural populations. Therefore, it can be used as a model species to identify the impact of harvest pressure on the genetic diversity patterns of wild populations of medicinal plants. However, most studies on this species have focused on the identification of medicinal compounds. In our review of the literature, the only genetic studies found for the species were studies of molecular identification based on ribosomal DNA sequence information (Sun et al., 2010) and phylogenetic relationships with cpDNA markers (Feng et al., 2016). However, these markers are unsuitable for analyzing the genetic variation of the species for conservation and management (Wan et al., 2004). Microsatellite markers are preferred in studies of genetic variation of individuals or populations due to their high level of polymorphism, codominance, biparental inheritance, and reproducibility of results (Varshney et al., 2005).

We have developed and evaluated microsatellite markers for further genetic studies of *Z. schinifolium*, and tested their cross-amplification in the related species *Z. piperitum*.
Microsatellite primer design and validation—Putative microsatellites were mined using MISA software (Thiel et al., 2003) based on the following criteria: more than three repeats for dinucleotides and hexanucleotides and a gap within 100 bp in composite types. Amplonc size (85–350 bp) and annealing temperature (57–60 °C) were the main consideration in primer design. A total of 104 primer sets were synthesized by Biomedic Co. Ltd. (www.ibiomedic.co.kr; Bucheon, South Korea) and used for preliminary screening. The preliminary screening of markers was performed by conventional PCR using the gDNA from species, so these markers could be used to distinguish between the two species. Out of these 11, six primers showed polymorphism with two or more alleles, but the remaining five primers had only one allele in the 30 samples analyzed. In most primers, the size range of alleles overlapped between the two species, but three primers (Zs3027, Zs3038, and Zs3029) showed completely different size ranges between the two species, so these markers could be used to distinguish between the two species.

Evaluation of genetic properties for use as polymorphic markers—The genetic properties of the 15 polymorphic primers for Z. schinifolium were evaluated using 102 samples from three populations (Table 2). Population genetic

| Locus   | Primer sequences (5′–3′) | Repeat motif | Allele size range (bp) | GenBank accession no. |
|---------|--------------------------|--------------|------------------------|-----------------------|
| Zs3069* | F: CAGCATTACTCTTACTAACCACA | (TTGT)$_{4}$ | 282–360 | — | 62 | KU884789 |
| Zs4034  | R: GCCTCTGCCAGACTGACTT  | (ATG)$_{4}$  | 188 | — | 62 | KU884813 |
| Zs3005* | F: GGAGATCAAGGTTGGTTGTTTGT | (AAGA)$_{10}$ | 222–250 | 222–226 | 62 | KU884748 |
| Zs3006-1* | R: TCACTCTGCTTGAATCGCTAC | (TTG)$_{4}$ | 314–323 | — | 58 | KU884749 |
| Zs3006-2* | F: TGGCGTGGGTTGGTGCTGTGT | (TTG)$_{4}$ | 196–202 | 202 | 62 | KU884749 |
| Zs3026* | R: AGCAGAGTCTAAAAAGAGG | (TTG)$_{4}$ | 186–190 | 174–190 | 57 | KU884763 |
| Zs3027* | F: TTTAGGAGCCTGCAAGACT | (TTG)$_{4}$ | 318–322 | 341–349 | 62 | KU884764 |
| Zs3035* | R: GGAAGGTAAGAAAGTG | (GAT)$_{4}$ | 164–188 | 182–188 | 64 | KU884769 |
| Zs3038* | F: ATGCGGTGGCTCAAACCTTACCT | (TTG)$_{4}$ | 163–183 | 128 | 62 | KU884770 |
| Zs4007* | R: ATCGTGGCTCAACCTTACCTC | (TTG)$_{4}$ | 207–211 | — | 62 | KU884793 |
| Zsm2010* | F: AATTCGACATGTTGAGAAGCAG | (AGT)$_{4}$ | 193–197 | 189–193 | 62 | KU884856 |
| Zsm4032  | R: CCAGCTGCTCACTGCTC | (AA)$_{4}$ | 146 | 146 | 62 | KU884883 |
| Zsm3029* | R: ATCGTGGCTCAACCTTACCT | (TC)$_{4}$ | 251–259 | 155 | 58 | KU884866 |
| Zsm4032* | R: AGAAGTACAACAGAGGAGGAAT | (AGT)$_{4}$ | 109–118 | 118 | 64 | KU884878 |
| Zs2011* | R: AAGAAGTACAACAGAGGAGGAAT | (TC)$_{4}$ | 232–250 | 233–246 | 64 | KU884709 |
| Zs2032* | R: CAGAAGTCTCTAAGAACAAGGA | (TC)$_{4}$ | 161–183 | — | 62 | KU884722 |

Note: — = information not available; $T_{u}$ = annealing temperature.

* Polymorphic microsatellite loci.

http://www.bioone.org/loi/apps
### Table 2: Genetic properties of 15 polymorphic microsatellite loci of Zanthoxylum schinifolium and Z. piperitum

| Locus       | Z. piperitum | Z. schinifolium |
|-------------|--------------|-----------------|
|             | f | A | H<sub:o</sub> | f | A | H<sub:o</sub> |
| Zs3006-2    | 36 | 2 | 1.3 | 0.091* | 32 | 2 | 1.1 |
| Zs3026      | 35 | 2 | 1.7 | 0.457 | 32 | 2 | 1.2 |
| Zs3035      | 31 | 2 | 1.9 | 0.419 | 32 | 3 | 2.2 |
| Zs4007      | 36 | 2 | 1.4 | 0.333 | 32 | 1 | 1 |
| Zsm2010     | 36 | 5 | 3.3 | 0.528* | 33 | 5 | 2.7 |
| Zsm3029     | 36 | 3 | 1.7 | 0.278* | 32 | 3 | 1.8 |
| Zsm4023     | 31 | 3 | 1.4 | 0.156* | 30 | 3 | 1.5 |
| Zs2011      | 36 | 5 | 1.5 | 0.306 | 32 | 6 | 2.6 |

Note: H<sub:o</sub> = observed heterozygosity; A = number of individuals genotyped; PIC = polymorphism information content. 

**Table 3:** Comparison of genetic diversity parameters (i.e., number of alleles [A], number of effective alleles [A<sub>e</sub>], and observed [H<sub:o</sub>] and expected [H<sub>e</sub>] heterozygosities) were estimated using GenAIEX version 6.41 software (Peakall and Smouse, 2006). The Hardy–Weinberg equilibrium (HWE) at each locus for each population was tested based on <i>χ²</i> tests using GenAIEX version 6.41 software (Peakall and Smouse, 2006). Polymorphic information content (PIC) and nonexclusion probability (NEI; identity) were calculated by CERVUS version 3.0.3 (Kalinski et al., 2007). The test for null allele presence was performed using MICRO-CHECKER (van Oosterhout et al., 2004). Over all samples, A ranged from two to eight, H<sub:o</sub> ranged from 0.070 to 0.677 and 0.093 to 0.688, respectively. The near-zero NEI value (0.0000005) indicated that the developed markers in this study are useful for individual identification. A ranged from one to four for <i>Z. piperitum</i>, and H<sub:o</sub> and H<sub>e</sub> at six polymorphic loci were in the respective ranges 0.077–1.000 and 0.211–0.536. Significant deviations (P < 0.05) from HWE were detected for some primers within each population. Because agreement with HWE depends on certain assumptions including an infinite population size, simple Mendelian inheritance in a diploid organism, discrete generations, and random mating, the test results could not be interpreted without information such as the mating system in the tested populations. Unexpected genotype patterns in the microsatellite data set were reported, of which null allele presence has been frequently mentioned as an explanatory cause (Dakin and Avise, 2004). The null test results indicated a significant possibility of the presence of null alleles at some loci in some populations (Table 2). In particular, Zs2011 showed a significant possibility of the presence of null alleles in all three tested populations; this locus should be used carefully in further genetic studies. Additional testing of known parent–offspring relationships should be used to confirm these results, as they might be affected by the presence of null alleles.

**CONCLUSIONS**

In this study, 15 polymorphic and three monomorphic microsatellite markers were developed for <i>Z. schinifolium</i>. In the cross-amplification test of the developed markers for <i>Z. piperitum</i>, a related species in the same genus, 61% (11/18) were successfully amplified. Three of the 11 cross-amplified primers could be useful for distinguishing between the two species because the amplified fragments have completely different size ranges. These developed markers can be useful for individual identification within species as well as for conservation and management of the genetic resources of <i>Z. schinifolium</i>.

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### Appendix 1

| Species | Locality | Geographic coordinates | N | Accession no. (DNA) | Voucher accession no. |
|---------|----------|------------------------|---|-------------------|----------------------|
| *Zanthoxylum schinifolium* Siebold & Zucc. | Chungju-si, Chungcheongbuk-do, South Korea | 36°52′22.86″N, 127°58′17.34″E | 36 | 0300-13-070792–0300-13-070827 | 0300-06-04679–0300-06-04681 |
| | Jincheon-gun, Chungcheongbuk-do, South Korea | 36°49′22.07″N, 127°29′46.14″E | 34 | 0300-13-070828–0300-13-070861 | 0300-06-04682–0300-06-04684 |
| | Namyangju-si, Gyeonggi-do, South Korea | 37°43′50.00″N, 127°10′21.00″E | 32 | 0300-13-070862–0300-13-070893 | 0300-06-04685–0300-06-04687 |
| *Zanthoxylum piperitum* (L.) DC. | Youngcheon-si, Gyeongsangbuk-do, South Korea | 35°59′41.49″N, 128°46′34.86″E | 30 | 0300-13-070894–0300-13-070923 | 0300-06-04688–0300-06-04690 |

*Note: N = number of samples.*

*All DNA, leaf samples, and plant vouchers were deposited in the Gene Bank of the National Forest Seed and Variety Center (NFSV), Chungju, South Korea.*