PRIMER NOTE

DEVELOPMENT OF MICROSATELLITE MARKERS BASED ON EXPRESSED SEQUENCE TAGS IN ASPARAGUS COCHINCHINENSIS (ASPARAGACEAE)\textsuperscript{1}

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• **Premise of the study:** Transcriptome-derived simple sequence repeat (SSR) markers were developed in *Asparagus cochinensis* (Asparagaceae). Due to its application in traditional medicine, its wild populations are threatened by over-collection even in protected areas, requiring immediate conservation efforts.

• **Methods and Results:** Based on transcriptome data of *A. cochinensis*, 96 primer pairs with two to seven alleles per locus were selected for initial validation; of those, 27 primer pairs amplified across all samples, resulting in 15 polymorphic and 12 monomorphic microsatellite markers. The usefulness of these markers was assessed in 60 individuals representing three populations of *A. cochinensis*. Observed and expected heterozygosity values ranged from 0.050 to 0.950 and 0.049 to 0.626, respectively. Cross-species amplification of the 27 markers was tested in the related species *A. rigidulus* and *A. schoberioides*.

• **Conclusions:** These polymorphic, transcriptome-derived SSR markers can be used as molecular markers to study population genetics and ecological conservation in *A. cochinensis* and related taxa.

**Key words:** Asparagaceae; *Asparagus cochinensis*; EST-SSR markers; genetic diversity; medicinal plant.

The genus *Asparagus* L. (Asparagaceae) comprises approximately 200 species distributed worldwide. The genus includes highly valuable plant species that have therapeutic properties and are also consumed as food (Shasney et al., 2003). *Asparagus cochinensis* (Lour.) Merr. is distributed in northeastern Asia (Xiong et al., 2011) and has been used in traditional medicine in Korea and China (Lee et al., 2009). The tuberous roots of this plant have various medicinal properties including anti-inflammatory (Lee et al., 2015), antibacterial, and anti-inflammatory qualities (Samad et al., 2013). In addition, previous research has also demonstrated that *A. cochinensis* has antitumor properties, particularly targeting lung cancer (Zhang and Jin, 2016). Such uses have led to a great demand for this plant, increasing the risk of extinction in this species due to over-collection of its wild populations (Jiang et al., 2010). *Asparagus cochinensis* is recorded in several protected areas in China (Information Center for the Environment, 2013), but information about its population size in the existing protected areas remains insufficient (International Union for the Conservation of Nature, 2016). Therefore, the genetic diversity and population structure of *A. cochinensis* requires immediate investigation to establish a conservation strategy.

Despite the ecological and medical importance of *A. cochinensis*, the genetic diversity in wild populations of this species is yet to be evaluated. Accordingly, polymorphic microsatellite markers in *A. cochinensis* were developed based on expressed sequence tag (EST) data obtained from Illumina paired-end sequencing. Simple sequence repeat (SSR) markers derived from ESTs are a powerful molecular tool for exploring genetic diversity and high level of transferability (Xu et al., 2014; Zhou et al., 2016). To the best of our knowledge, the current study is the first to profile the leaf transcriptome of *A. cochinensis* to generate EST-SSR markers. The usefulness of these markers was assessed in 60 individuals from three populations of *A. cochinensis* in Korea, Taiwan, and Japan. Cross-amplification of polymorphic microsatellite markers was performed in two related species (n = 8, for each species), *A. rigidulus* Nakai and *A. schoberioides* Kunth.

**METHODS AND RESULTS**

Sixty individuals of *A. cochinensis* were collected from wild populations in three countries (Korea, Taiwan, and Japan). Voucher specimens were deposited in the Herbarium of the National Institute of Biological Resources (KB) and the Herbarium of Hallym University (HHU), Republic of Korea (Appendix 1). To test cross-species amplification of the markers, we sampled eight individuals of each *A. rigidulus* and *A. schoberioides* (Appendix 1).

For RNA library construction, total RNA was extracted from a leaf of a single plant collected from Korea (voucher no: NIBRVP0000556138; Appendix 1). We constructed Illumina-compatible transcriptome libraries using a TruSeq Primer Note Development of microsatellite markers based on expressed sequence tags in Asparagus cochinensis (Asparagaceae) doi:10.3732/apps.1700021


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