


# Development of novel EST-SSR markers for *Ephedra sinica* (Ephedraceae) by transcriptome database mining

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**PREMISE OF THE STUDY:** *Ephedra sinica* (Ephedraceae) is a gymnosperm shrub with a wide distribution across Central and Eastern Asia. It is widely cultivated as a medicinal plant, but its wild populations are monitored to determine whether protection is needed.

**METHODS AND RESULTS:** Thirty-six microsatellite markers, including 11 polymorphic markers, were developed from *E. distachya* RNA-Seq data deposited in the National Center for Biotechnology Information dbEST database. Among 100 genotyped *E. sinica* individuals originating from five different population groups, the allele number ranged from three to 22 per locus. Levels of observed and expected heterozygosity ranged from 0 to 0.866 (average 0.176) and 0 to 0.876 (average 0.491), respectively. Allelic polymorphism information content ranged from 0.000 to 0.847 (average 0.333). Cross-species amplifications were successfully conducted with two related *Ephedra* species for all 11 di- or trinucleotide simple sequence repeats.

**CONCLUSIONS:** This study provides the first set of microsatellite markers for genetic monitoring and surveying of this medicinal plant.

**KEY WORDS** *Ephedra sinica*; Ephedraceae; expressed sequence tag–simple sequence repeat (EST-SSR) marker; genetic diversity; gymnosperm; medicinal plant.

*Ephedra sinica* Stapf (also known as Chinese ephedra or ma huang; Ephedraceae), a gymnosperm shrub, is distributed across southern Siberia, Mongolia, and China, and is found in arid areas and highlands, occurring on slopes, dry river beds, sandy places, or fields in mountainous areas (Lin et al., 2002). The species is reported as dominant in some areas, but little is known about its entire population size. *Ephedra sinica* has been used in Chinese herbal medicine for thousands of years (Fabricant and Farnsworth, 2001). The stems of most members in the genus *Ephedra* L. contain the alkaloid ephedrine, which is used for treatment of asthma and other respiratory ailments (Liu, 1989; Nam et al., 2003). Recently, *E. sinica* has become extensively exploited in a large market developed for nutritional supplements and stimulants involving this plant. *Ephedra sinica* is recorded on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (Bell and Bachman,

2011). The IUCN lists the species as Least Concern; however, wild populations still need to be monitored to determine whether protection is required, as a species of Least Concern may still be critically endangered within a particular region where numbers are very small or declining.

Recently, 29 polymorphic microsatellite loci were developed for a distantly related species, *E. gerardiana* Wall. ex C. A. Mey., by mining the whole-genome-skimming data from Illumina MiSeq sequencing (De et al., 2017). However, no DNA markers have been developed for *E. sinica*, limiting our ability to monitor its population dynamics and employ conservation genetic measures. The present study developed a crucial set of di- or trinucleotide microsatellite markers by mining an *E. distachya* expressed sequence tag (EST)–derived database. The EST–simple sequence repeat (SSR) markers developed here will enrich the genetic marker set for *Ephedra* species.