Development and characterization of new microsatellites for walnut (*Juglans regia*)

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**ABSTRACT.** The expressed sequence tag (EST) database represents a potentially valuable resource for the development of simple sequence repeat (SSR) markers for use in evolutionary studies. EST-SSRs reveal polymorphisms not only within the source taxon, but in related taxa as well. In this paper, we describe a case study in which the publicly available walnut (*Juglans regia*) EST database was used to develop SSR markers for use in the genetic analysis of the widespread *Juglans nigra* and *Carya cathayensis* and an endangered species *Annamocarya sinensis*. A total of 7262 unigenes, including 1911 contigs and 5351 singletons, were obtained from 13,559 ESTs retrieved from the NCBI database. The 7262 unigenes were further reduced to 706 EST-SSR sequences containing 805 SSR loci. Then, 309 EST-SSR primers were randomly designed, and 77 were identified with five high across-species transferability cross-species: namely, *J. regia*, *J. nigra*, *C. cathayensis*, *Carya dabieshanensis*, and *A. sinensis*. Thirteen highly polymorphic EST-SSRs were further used for genetic analyses in these above five species.

**Keywords:** EST-SSR; *Juglans regia*; *Juglans nigra*; *Carya cathayensis*; *Carya dabieshanensis*; *Annamocarya sinensis*