DEVELOPMENT AND CHARACTERIZATION OF MICROSATELLITE MARKERS FOR THE MEDICINAL PLANT *SMILAX BRASILIENSIS* (SMILACACEAE) AND RELATED SPECIES

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**Key words:** medicinal plant; microsatellites; sarsaparilla; *Smilax*; transferability.

The Smilacaceae is grouped within the Monocotyledoneae of the Liliales and has only two genera: *Smilax* L., with 300 species, and *Heterosmilax* Kunth, with 15 species (Angiosperm Phylogeny Group III, 2009). The family is distributed worldwide and is composed mainly of herbaceous vines and shrubs, and rarely of subshrubs and dioecious species. In Brazil, *Smilax* comprises 31 species, 14 of which are exclusively Brazilian (Andreata, 1997). *Smilax* species, which are popularly known as sarsaparilla and used in folk medicine as a tonic, antirheumatic, and antisyphilitic, is sold in Brazilian pharmacies, and its origin and effectiveness are not subject to quality control (Andreata, 1997). The quality control of herbal drugs should be more stringent, and molecular markers may be useful tools for the identification of species sold in pharmacies. Thus, the aim of the current study was to isolate and characterize microsatellite markers to identify *Smilax* species.

**Methods and Results:** A new set of microsatellite or simple sequence repeat (SSR) markers were developed for *Smilax brasiliensis*, which is popularly known as sarsaparilla and used in folk medicine as a tonic, antirheumatic, and antisyphilitic. *Smilax brasiliensis* is sold in Brazilian pharmacies, and its origin and effectiveness are not subject to quality control. The family is distributed worldwide and is composed mainly of herbaceous vines and shrubs, and rarely of subshrubs and dioecious species. In Brazil, *Smilax* comprises 31 species, 14 of which are exclusively Brazilian (Andreata, 1997). *Smilax* species, which are popularly known as sarsaparilla and used in folk medicine as tonics, antirheumatics, and antisyphilitics and are sold in Brazilian pharmacies without any quality control over their origin and effectiveness (Andreata, 1997). The quality control of herbal drugs should be more stringent, and molecular markers may be useful tools for the identification of species sold in pharmacies. Thus, the aim of the current study was to isolate and characterize microsatellite markers to identify *Smilax* species.

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**Methods and Results:**

Genomic DNA was extracted from fresh leaves of *S. brasiliensis* Spreng., *S. campestris* Griseb., *S. cissoides* Mart. ex Griseb., *S. fluminensis* Steud., *S. goyazana* A. DC., *S. polyantha* Griseb., *S. quinquenervia* Vell., *S. rufescens* Griseb., *S. subsessiliflora* Duhamel, and *S. syphilitica* Humb. & Bonpl. ex Willd. using the cetyltrimethylammonium bromide (CTAB) protocol described by Doyle and Doyle (1990) with modifications. The plant samples were registered (Appendix 1) and added to the plant collection of the Herbarium of the Escola Superior de Agricultura “Luiz de Queiroz” (ESA) of the Universidade de São Paulo, Brazil, and the Herbarium “Coleção de Plantas Medicinais e Aromáticas” (CPMA) of the Universidade Estadual de Campinas, Brazil.

A microsatellite-enriched library was obtained using protocols adapted from Billotte et al. (1999). Genomic DNA from one individual of *S. brasiliensis* (Campina Verde, Minas Gerais) was digested with *AfaI* (Invitrogen, Carlsbad, California, USA) and enriched in microsatellite fragments using (CT)ₙ and (GT)ₙ motifs. Microsatellite-enriched DNA fragments were ligated into pGEM-T Easy Vectors (Promega Corporation, Madison, Wisconsin, USA), which were used to transform Epicurian Coli XL1-Blue Escherichia coli competent cells (Promega Corporation). Positive clones were selected using the β-galactosidase gene and grown overnight with ampicillin. The sequencing reactions were performed in a thermal cycler (MJ Research, BioRad, Hercules, California, USA) under the following conditions: 2 min at 96°C for the first denaturation followed by 26 cycles of 45 s at 96°C, 30 s at 50°C, and 4 min at 60°C. The PCR products were precipitated with isopropanol (65%), centrifuged, and washed with 70% ethanol. Ninety-six positive clones were sequenced on an ABI 3700 automated sequencer (Applied Biosystems).

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