

## **A BATTERY OF SSR MARKERS FOR GENETIC ANALYSIS OF PEPPERS (*Capsicum spp.*)**

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A large portion of the genetic diversity of the genus *Capsicum* is in Brazil, particularly in the Atlantic Forest and the Amazon. The knowledge and use of this germplasm is incipient and studies are needed so that it can be explored, preserved and utilized in breeding programs. There is a need to develop methods to accelerate the analytic capacity of genetic studies of *Capsicum* dealing with estimates of genetic diversity, population genetics and genetic mapping. Simple Sequence Repeats (SSR) are powerful genetic markers for genomic analysis. They are PCR based, codominantly inherited, multiallelic and can be semiautomated in multiplexed assays for high throughput data collection. A set of SSR based markers have been developed. Genomic DNA was digested with *Tsp509 I* restriction enzyme for a single accession of *Capsicum annuum*. Fragments between 300 and 800 bp were recovered onto a nylon membrane. An enrichment procedure was applied where fragments containing SSRs were selected by hybridization to biotinylated oligonucleotides bound to magnetic beads. This fraction was used to construct a genomic library enriched for the dinucleotide sequence motif poly AG. This library has been screened for SSR containing clones by hybridization of colony plaques with an AG (13) probe. Positive clones were further screened by anchor-PCR analysis and the selected clones sequenced by automated fluorescent cycle sequencing in the ABI Prism 377. Sequences flanking the repeats are being used to design specific primer pairs. Out of a sample of 575 SSR positive colonies screened, 302 confirmed the presence of AG-rich repeated sequences by the anchor-PCR approach. A subsample corresponding to 56% of the selected cloned SSR-rich DNA was sequenced. From those sequences 18 presented SSRs with sufficient flanking DNA to allow primer design and construction. The new SSR primers are being characterized and studied on cultivated and wild accessions of *Capsicum*.

**Key words:** *Capsicum*, SSR, Microsatellites, Molecular Marker