

CAPSICUM EST-DERIVED MICROSATELLITES AND THEIR POTENTIAL USE FOR MAPPING STUDIES

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The genus *Capsicum* belongs to *Solanaceae* family and includes five cultivated species: *C. annuum*, *C. frutescens*, *C. chinense*, *C. pubescens*, *C. baccatum*. *C. annuum*, known as pepper, sweet pepper or paprika is the most cultivated worldwide.

Several genetic maps have been constructed for *Capsicum spp.* in the past twenty years by using F₂, BC₁, RILs or doubled haploid (DH) populations of interspecific crosses *C. annuum* x *C. chinense* or *C. annuum* x *C. frutescens* and from intra-specific crosses of *C. annuum*.

To date there is no map of *Capsicum* that achieve the goal of completely delineating and saturating pepper chromosomes, however DNA markers linked to some agriculturally important characters have been identified and marker assisted selection (MAS) has become feasible for some traits.

Among the different classes of molecular markers available, microsatellites are particularly useful for mapping studies because of their high variability and ease of detection by PCR. Here we report on the development of EST-derived microsatellites which show the advantage of detecting variation in the expressed portion of the genome, allowing greater transferability across species, and in some cases having additional utility as anchor markers for comparative mapping.

A computer search of 8.094 sequence from *Capsicum* species in the EMBL Nucleotide Sequence Database (<http://www.ebi.ac.uk/embl>), corresponding to approximately 4.1 Mb, yielded 1325 microsatellite in 899 sequences. This corresponds to an average distance between SSRs of approximately 3.1 kb or one SSR-containing sequences every 9.0 sequences.

In order to obtain non-redundant sequences containing SSRs, a cluster analysis was performed; the contigs obtained were carefully evaluated and redundancies were removed. In total, 783 microsatellites in 576 non-redundant sequences were identified; among them 531 were mononucleotide, 103 dinucleotide and 137 trinucleotide motifs. Trimeric SSR repeats appeared to be more abundant than the dinucleotide ones.

Primer pairs could be designed for 348 out of the 576 SSR-containing sequences (60.4%). The remaining sequences contained either too little DNA sequence flanking the microsatellite or the sequences were inappropriate for primer modelling. A subset of 204 primer pairs flanking SSR loci was used for screening polymorphisms among four *C. annuum* inbred lines ('P4', 'GD1', 'Yolo Wonder' and 'Criollo de Morellos 334') and one *C. frutescens* accession (cv. Tabasco) being parents of three mapping populations. As a result, 49 EST-derived SSR-markers were found valuable for genetic mapping; 47 of them were polymorphic in the inter-specific population 'Tabasco' x 'P4', while 21 might be mapped in both the intra-specific populations 'GD1' x 'CM334' and 'Yolo Wonder' x 'CM 334'. In order to assess marker diversity and judge the utility

of these markers for fingerprinting, PIC (polymorphic information content) values were assessed using 16 *Capsicum* accessions, including 6 accessions of *C. annuum* and 10 genotypes belonging to either the species *C. frutescens*, *C. chinense*, *C. pubescens*, *C. chacoense*, *C. eximium*, *C. baccatum* var. *baccatum*, *C. baccatum* var. *pendulum*, and *C. praetermissum*. A total of 219 PCR fragments were amplified and the estimated PIC values ranged from 0.24 to 0.86, with an average of 0.62.