

Notas Científicas

New microsatellite markers developed from an enriched microsatellite common bean library

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Abstract – The objective of this work was to develop new microsatellite markers in common bean. Ninety nine new microsatellite *loci* were developed from a microsatellite enriched library for (CT)₈ and (GT)₈ motifs, from CAL-143 line. The majority of microsatellite sequences (51%) was related to cellular metabolism. The remaining sequences were associated to transcription functions. Only 17.2% of the sequences presented some level of similarity with other plant species genes.

Index terms: *Phaseolus vulgaris*, molecular markers, plant breeding, QTL, SSR.

Novos marcadores microssatélites desenvolvidos a partir de uma biblioteca genômica enriquecida em feijão-comum

Resumo – O objetivo deste trabalho foi desenvolver novos marcadores microssatélites para feijão-comum. Noventa e nove novos locos de microssatélites foram desenvolvidos a partir de uma biblioteca enriquecida com motivos (CT)₈ e (GT)₈, proveniente da linhagem CAL-143. A maioria dos microssatélites (51%) esteve relacionada ao metabolismo celular. As demais seqüências estiveram associadas a funções de transcrição. Apenas 17,2% das seqüências apresentaram alguma semelhança com genes de outras espécies.

Termos para indexação: *Phaseolus vulgaris*, marcadores moleculares, melhoramento de plantas, QTL, SSR.

Microsatellites have to be continuously isolated for new species, as cross-species amplification is not always possible. Once developed, microsatellites are ideal markers, as they are stable and easy to assay by polymerase chain reaction. Several important genes, such as resistance ones, may be linked to microsatellite motifs and, therefore, they are quite relevant for studies such as germplasm characterization, mapping and marker-assisted selection.

When compared to commodity crops, the number of available microsatellites for common bean is insufficient, and success of mapping with limited number of markers is reduced (McClellan et al., 2004).

Polymorphisms showed by microsatellite markers have been restricted to the narrow genetic based elite population. More microsatellites for common bean represent a step beyond the constraint of polymorphism

and better tools to identify useful genetic variation (Acosta-Gallegos et al., 2007).

Campos et al. (2007) identified and characterized twenty microsatellite *loci* in common bean, from an 'IAC-UNA' microsatellite enriched library. These new informative microsatellite *loci* can be used for selecting promising common bean segregant populations (Pereira et al., 2007).

Nevertheless, the number of microsatellites available for common bean is still insufficient, and requires the development of additional markers, so as to accelerate the analytical capacity of genetic studies in this crop.

The objective of this work was to develop new microsatellites from a 'CAL-143' enriched library.

A microsatellite enriched library (Billotte et al., 1999) was developed for common bean line CAL-143. This library was developed together with other 'IAC-UNA'

libraries (Benchimol et al., 2007; Campos et al., 2007), as part of a broadened mapping project. CAL-143 is an Andean line with red stringed seeds (cream coat color) and calima type. It is susceptible to anthracnose (*Colletotrichum lindemuthianum*) and resistant to some races of angular leaf spot (*Phaeoisariopsis griseola*).

Genomic DNA was extracted from leaf tissue using CTAB method (Hoisington et al., 1994). The genomic libraries were screened by picking 2 μ L of frozen white colonies and their amplification in a PCR reaction. Each amplification reaction contained 25 μ L of 1x reaction buffer, 2 mM MgCl₂, 0.5 μ M of *RsaI* primer, 200 μ M of total dNTP mixture, and 0.5U *Taq* DNA polymerase. Amplifications were performed in a PTC-100 MJ Research thermocycler, programmed with a hot start of 4 min at 95°C; followed by 30 cycles of 30 s at 94°C, 45 s at 52°C, 1 min 30 s at 72°C, followed by 8 min at 72°C. PCR products were separated onto 3% agarose gels. Plasmid DNA was isolated according to Maniatis et al. (1982).

Sequencing of the inserts was performed using the ABI 377 Big Dye Terminator. Microsat software CIRAD (Risterrucci et al., 2005) was used for excising adaptation sequences and finding possible *RsaI* sites. Reads were processed by Phred version 0.000925.c base calling program (Ewing et al., 1998) and vector sequences, poly-A tail, and adaptators were trimmed after cross-match analysis. Clustering was performed using CAP3 software, with default parameters (Huang & Madan, 1999).

BLASTN search utility program (available in NCBI; <http://blast.ncbi.nlm.nih.gov/Blast.cgi>) was used to identify similarities between 'CAL-143' new sequences and 'IAC-UNA' sequences (Benchimol et al., 2007; Campos et al., 2007), in order to avoid redundancies among the microsatellites. BLASTX search utility program was used to identify similarities of 'CAL-143' sequences within known genes represented in the GenBank non-redundant database (Altschul et al., 1990).

Only sequences containing five or more repeated units were considered, regardless if the sequences were perfect or not. The free software SSRIT – simple sequence repeat identification tool (Temnykh et al., 2001), available at <http://www.gramene.org/db/searches/ssrtool> – was used to identify, count, and localize the microsatellite motifs inside the sequences. Complementary primers to the single sequences flanking the microsatellites were designed with Primer Select

software from Lasergene program, with the following conditions: amplification DNA size from 150 to 350 pb; GC content between 40 and 60%; annealing temperature (Ta) between 45 and 60°C; primer length between 18 and 22 pb; no hairpins or dimmers.

This analysis showed that 45 sequences, containing compound microsatellite motifs of 'CAL-143', were redundant to a unique 'IAC-UNA's library contig. A total of 1,440 clones were identified and sequenced. Seventy percent (1,002) of these clones were putative positive (one unique insert cloned, having neither contamination of other clones, nor showing double bands).

Among all sequences, 256 microsatellites were found: 176 (68.8%) presented dinucleotide compound motifs, 74 (28.9%) presented dinucleotide simple repetitions and six (2.3%) presented trinucleotides. Therefore, dinucleotide compound (perfect and imperfect) motifs were the most frequent kind of microsatellite found in this Andean variety enriched library. The same was also reported for 'IAC-UNA' studies (Benchimol et al., 2007; Campos et al., 2007). The AC/TG motifs were the most frequent, followed by the GT/CA ones. This result is different from the one reported for 'IAC-UNA' (Mesoamerican cultivar), in which most of microsatellites found contained GT/CA motifs followed by GA/CT motifs (Benchimol et al., 2007). Gaitán-Solís et al. (2002) reported that GA repeat was the most common dinucleotide detected, accounting for 62.7% of the microsatellites found among three Andean accession libraries. Yu et al. (1999) have searched GenBank database for microsatellites sequences and found a higher frequency for AT dinucleotide repeats in *P. vulgaris* and *Vigna* sp., followed by GA repeats.

Only ninety-nine primer pairs were designed for the 256 microsatellites found (Table 1). It was not possible to develop primer pairs for all the microsatellites, because: it was impossible to draw primers with an amplification product over 350 pb, as there was no available ladder (the one of 10 pb has the largest fragment at 330 pb); there were complementarity and hairpin formation among some of the primers; and, in some cases, many were found at the initial or at the end of the sequenced sequences. These ninety-nine microsatellites are being used to accomplish the mapping of resistance *loci* associated to anthracnose and angular leaf spot, and for evaluating genetic diversity among 'Carioca' beans.

The results of BLASTX revealed that only 17.2% of the microsatellite sequences presented some level of

Table 1. Primer sequences of microsatellite markers obtained from line CAL-143.

Locus/GeneBank Accession	Primers (5' → 3')	T _a (°C)	Predicted size (pb)	Core motifs
SSR-IAC124	CCTTTATTGTTTTGGTGAGTAG TTGGGAAAGGTGAGAGGTAT	60	267	(TG)2 GA (TG)2 AG (GT)2
SSR-IAC125	CCCCATAAAAGTTCACA ATTCAAAAGCTCATCCTCT	50.2	165	(AC)4 (TC)3 (CA)4 CG (CA)2
SSR-IAC126	AACGAGGAGAAGAACGAT ACATAAGAAACGGTGGTC	51.4	297	(CA)5 CT (CA)3 (CT)2
SSR-IAC127	GAGGCTAGCCCACTTA AGCGCAAGACTTTACTACTC	63.3	199	(TA)3 T (TGA)3 G (TA)3
SSR-IAC128	TCTTGAAGGTTGTTCTCC GGATTTGAATCTCCGTAAC	56.7	191	(AC)7 GGA (TC)2
SSR-IAC129	CACCCTTTCTAGCTTATTTTT CTAGATGTTGCAGGTGACGA	56.7	294	(TG)2 G (CT)2 TCT (GA)2
SSR-IAC130	TTGAACGTGGGTGAATC CCCAGAAGAAGGAAAGAG	56.7	299	(TG)5
SSR-IAC131	AACGCTTAAATGCTTTGAAC GGAACACCGTGATTGGAT	56.7	253	(AC)6
SSR-IAC132	ACAACACAACAAATGGT GATATGGAAAGGTTAGC	54	235	(GA)3 A (GT)6 GA (GT)3
SSR-IAC133	GATGATGACCACAAAACAGAGA AGTAAAAAGGGATCAAGAGGTG	59.7	232	(AG)3 CA (CTG)3
SSR-IAC134	TGGAAACAGCAGAGCGATACT TTGGTCCCTAAATCTTCTCAT	56.7	251	(AC)6
SSR-IAC135	ATTTGATTTAACTGGTGTA ACTTAACTCCCTTGGTAT	54	231	(AC)6 (AT)4 G (TA)4
SSR-IAC136	GCTCTTAGGTATGGACGATTGT CAGATGGGGTTAGGAAAGTTG	56.7	233	(CA)7 (AT)5
SSR-IAC137	GCAGCAGCAAATACAAC CCCCTAAAACAATACAGC	60	169	(AT)2 T (TG)2 C (CT)2
SSR-IAC138	GTGATGGCAAAGCGACAAC GATCAAAAAGAAAAGCCCTCAT	56.7	173	(GT)3 GC (GT)3 T (CA)2 (CT)2 GCA (AT)2 GA (CA)2 AC (AT)2
SSR-IAC139	AATTCAGGGTAACATTTT TCTAAATAGATCAATAATGTAT	46.5	233	(TC)10
SSR-IAC140	GAGAGTATAGGAAAAGAGA TGTAATCAAACGAAGGT	52	175	(CT)3 (AT)2 (TG)2 (TGT)3 (GT)3
SSR-IAC141	ACCCTCAGGTTTGGACTCTTCC GGCGGCGGTGGTTCTTG	59.4	171	(TCT)3 A (CT)13
SSR-IAC142	AATTCATAAAAGTTCAGTGG CTCATTATTTATTTATTCT	60	157	(CA)7

Continue...

Tabl 1. Continuation...

Locus/GeneBank Accession	Primers (5'→3')	T _a (°C)	Predicted size (pb)	Core motifs
SSR-IAC143	CCCTTCACATCCACTTCACAC GCTGCTTCATCGCTTCTG	63.3	171	(TC)2 T (TC)2 T (TC)2
SSR-IAC144	TATAAATTCCCCCTTCACATCC GCTGCTTCATCGCTTCTG	56.7	181	(CT)10
SSR-IAC145	AGATGACAATGACAACCACA TAAGGCAATCCATCTCC	56.7	233	(TA)2 AC (TA)2 TC (AT)2 (AT)2 AA (AT)9 (AC)9
SSR-IAC146	CTGGGAGAATATGTGAGGA TCGTAGACCAAAATGAACT	56.7	255	(AC)8
SSR-IAC147	CTTTAACGCTTAAATGCTTTGA GGAACACCGTGATTGGATG	56.7	252	(CA)5
SSR-IAC148	CAAGCATGTAAGGGTGAGT AAGGTTTGGTGGGTTTAG	56.7	188	(CAT)3 (CA)3 (TA)2
SSR-IAC149	TTGGGATCTCTGCCTCGTC CCCCATTAAGTATCGGAACAG	60	273	(TC)8
SSR-IAC150	CAAGACCAAAGAAAATGTA CTGGAGTGGTGGATAATA	58.4	190	(GA)12
SSR-IAC151	CGTGCTTTGAGGTTACGAACAA CTCAAGTCAGCCAGCCAGAA	65	161	(AC)5
SSR-IAC152	GTAGCCTTTCTCTTCTCACC ACTACTCTTTGCTTTCCACTAT	60	178	(GT)2 GA (GT)5
SSR-IAC153	AGGGAAGAAAGAGATACACAA TGCTGAGCCAAAGGAAG	59.7	178	(AC)3A (AC)2
SSR-IAC154	AATGGCATAGGATAACTTTCTC ATCAACAACCCATACTTTTAG	56.7	164	(TG)2 (TC)3
SSR-IAC155	AAGGTCACAGGAGCCAGTCT TTTTGAATTCTCCGCACCTA	56.7	181	(AG)9
SSR-IAC156	ATTTGTGCATCAGGTA TTCAAAAAGCAGTTAGAG	56.7	230	(TC)3 TG (GC)2
SSR-IAC157	TTTAAATTTGTGCCACTTCTC TGCACACGTTTCCCTATTCTAT	56.7	165	(GA)8 GT (GA)3
SSR-IAC158	AATACCACAAAACGCAAAAA TTAAATAAATGGAAGGGAACAC	56	292	(GA)7 AT (CA)3
SSR-IAC159	AAAACCAACCAAACAT ACGCCAATAAATCTAAAG	56.7	298	(AC)6 (AC)4 C (CT)2
SSR-IAC160	TGGAATCACATCACGGAAGGT GCGGAGAATGTTGGAATAGG	56.7	173	(TG)2 (TA)2 (TG)5
SSR-IAC161	TACACCAAGAGCTACATCCAG TGTATTCTATTCTTTCTTCTC	55.3	233	(AC)3 AT (CA)3
SSR-IAC162	GTCACAACCAAACAAAACAAT CAGGGAGAAGGATGTGAATG	56.7	190	(CG)2 TG (CT)2
SSR-IAC163	TTTGGGAACATTTGTAGGAGAT ACAGGATTGGTTCACTTTCAC	59.4	193	(GT)6 GA (GT)2

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Table 1. Continuation...

Locus/GeneBank Accession	Primers (5'→3')	T _a (°C)	Predicted size (pb)	Core motifs
SSR-IAC164	CAGTCAAAGTGAACCAAT ACAACAGTAGCTAGATGAAGT	59.4	207	(TC)2 (TA)2 A (AC)2
SSR-IAC165	CTTAACCAAGACCTACAT TCAAGTTTTTCCAAGAT	46.1	294	(TG)5
SSR-IAC166	TCCTCCTTTTCTTATTCGTGT CATCAGATCCCAAAGGTTATT	56.7	199	(CA)2 AA (AC)3 (TA)2 GAC (TG)3
SSR-IAC167	AGACCAAGAAACAATGAGAAT AAGTTTCGTTAGTCTGTGATT	56.7	176	(TG)7 (CG)3
SSR-IAC168	GATGCTTTGATGTCTCCA GGCTTAAAATTCACCTGT	56.3	226	(TTG)3 (TG)5
SSR-IAC169	TCCATAACTATTCATCGTAACC TGGGAGACATCAAAGCATC	59.2	193	(CA)4 CT (CA)6
SSR-IAC170	GCATGTTCTACTGCTTTT TTGCCATGTGAATAGTT	47.3	206	(TG)3 G (CT)2 (TC)11
SSR-IAC171	TCTGAACTTTGATGATGCGT TGTTGTTTTTACTGCTCTTTCT	53.0	151	(TC)2 (AG)2 C (AG)2
SSR-IAC172	GGCCAGCGAATCGTTGAAAT AGTGCCACCGATTCTCCA	56.7	127	(AG)4 TG (GT)3 G (GT)2
SSR-IAC173	ACTGATAACAATGAACAACAAA CTAGAACGAGAGCACTGAAT	57.4	172	(AC)6 (TCT)3 (GT)2
SSR-IAC174	GTTTAGTTAAGGCACCACAC TTAGGAGGGATTATGATTG	53.2	230	(AT)3 A (AT)2 (AC)7 TTT (CA)3
SSR-IAC175	AGAAAAGTTACTGGTTGA TAGAAGCCTTTTATTTG	45	251	(AC)6
SSR-IAC176	CTTCTCCCTTAACCGTATTC TTTTCTGCTAAGTCATCAA	56.7	151	(CT)8
SSR-IAC177	ACGGTTGGAGAAGATGATGA ACCAATACAGGAAAGGGAGTT	52.8	238	(AC)9 (TC)4 G (CT)2
SSR-IAC178	CAAATGAACTTGGATAAACTT GCCTTGGCAATAATGATA	50	210	(AC)6
SSR-IAC179	TTTCGCTATCACTTCGGCTATC TGAAGAACAGGAGGAGGTCGTA	63.3	203	(AC)2 CTTT (AC)2 CTA (TC)5
SSR-IAC180	CATTCACCCACACCTATCCA TGTGTTGTATGCAATGTTCTGT	63.3	205	(AC)3 T (CA)3 TAA (AC)3 (AC)3 G (CA)2
SSR-IAC181	AGGTTCCCTTCTTTCTACT CTACTTTTTCCACAGC	58.4	216	(AT)2 AC (AT)3 (AG)5 TAA (AG)2 C (AG)2
SSR-IAC182	CTGGTTTTTGTGTTTTG TGCTGACATAACCCATTTG	56	176	(GA)7
SSR-IAC183	CGCATTATAAGAGAAGCAACA CCGATGGAAACACCTGAAA	56	195	(AG)18 A (AC)4
SSR-IAC184	ACAACCTGTAGCAACCAAAAT ATGCAATGTGACTTAGAAAATA	56.7	218	(AT)2 T (TG)2 C (CT)2 (TC)2 ACA (CT)2 GA (AC)6

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Table 1. Continuation...

Locus/GeneBank Accession	Primers (5'→3')	T _a (°C)	Predicted size (pb)	Core motifs
SSR-IAC185	ATTCTTCACTCCGTTCT GAGCTTAATAGGTCAAATA	45	248	(TA) ₂ TTT (AC) ₃ AA (AC) ₄ (AT) ₄
SSR-IAC186	TGTAAGGGAATAGACTCAAAG ACACAAAAGGTTACATCGTT	58.4	166	(TG) ₃ (TA) ₄
SSR-IAC187	GCATAATATAAGCAACCAAACA AAAAGGGATTAAAGTCAAACAA	48.5	190	(TTG) ₃ GT (TG) ₅ TTT (AG) ₂
SSR-IAC188	CCTGCCTTTGCCACTCCTC CTCCTTCTACCCAGCCAAACC	53.5	180	(CT) ₁₀ T (TC) ₄
SSR-IAC189	GGAAAAGAAGACAAAATA TCTACTCACAAAACAAT	41.7	221	(TA) ₃ CCA (CT) ₂ (TG) ₂ (AT) ₄
SSR-IAC190	CCTTTATTGTTTGGTGAGTA GCTTATAATATTGCTTTTGTA	59.2	279	(GT) ₂ CT (CA) ₃ (TG) ₂ CT (TG) ₂ AG (GT) ₂
SSR-IAC191	ATTTCACTCTACTTCATTAC AGATTTACAACCTCCATT	56	186	(AG) ₂ A (AC) ₂ GCA (AT) ₂
SSR-IAC192	TACTGATGCATTGCTTTGTGTT GTTTCCTTGGCTCTTTCAGTAG	61.5	180	(CA) ₇ CT (AC) ₃ (CT) ₂
SSR-IAC193	TGTAGAGTGTGAGAGGCAATGA GTTTCTGAGGACTGGATGTAGC	49.9	241	(AG) ₃ ATT (CA) ₃
SSR-IAC194	CCCCACTCGACTTATCTCA GCTTGTGGTGGTAGTTATTCT	63.3	191	(AC) ₇ (AT) ₇ (TA) ₂
SSR-IAC195	TGGACATCAAACAAACAAAA TGCATCGGCAGTTCATCA	63.3	203	(TC) ₂ TT (TC) ₈
SSR-IAC196	TTCTCAAATATGCCTAAC TTTTCTGTATTACTCCCTTTT	43.0	202	(GT) ₈
SSR-IAC197	TTCTCCTGTATTATTATTGT TCAGAACTTACCTTAGATAG	45.7	200	(CT) ₃ (GA) ₂ TTA (CT) ₂
SSR-IAC198	TGAATGCAAAGATGAACT AGACGGAGCAGAATGAA	56	209	(AC) ₇
SSR-IAC199	ATAAACAAAACAAGCATCTCAT TGCCATCTCTTACTTCTTCTC	56.7	183	(AC) ₅
SSR-IAC200	GAACATCCACGGTAGTAATAGA TCAAGAAAGAGAAAAGAGAAGA	56	247	(CA) ₇
SSR-IAC201	CATCAGTCCCATCACAAGTTCG TGCTGCAGCCCCCTCAT	56	159	(TA) ₄ CCC (AT) ₂ CAT (CA) ₂ (AC) ₄ (TC) ₃
SSR-IAC202	GTCCCTCAACTAACCCTGA GTATTCTATTCTTCTTCCT	56.7	199	(AC) ₄ CA (CT) ₂
SSR-IAC203	AAAAATAACAACCCAGAAAAT ATATGATGCGAAGGTTGAAG	46.0	294	(AT) ₆ G (AT) ₂ (CT) ₂
SSR-IAC204	GGGCCGACCAGGAGGAGA CTGTGAAGGGCCCCAAAGACC	65	210	(GGT) ₃ (GA) ₂
SSR-IAC205	ATTTATTTCACTCTCATTCTCT TGTGTTCACTTCCTGTCT	56	277	(GT) ₇ (GA) ₂

Continue...

Table 1. Continuation...

Locus/GeneBank Accession	Primers (5'→3')	T _a (°C)	Predicted size (pb)	Core motifs
SSR-IAC206	CAATCCCTCACCACCTTAT CGATTAGATTTGAGGGGACTG	53.4	285	(AAG) ₄
SSR-IAC207	GAATGGGTGAAAGTGTGTTAT AGGGAAAGGTGGTTGAAAT	56.7	197	(GT) ₈
SSR-IAC208	TGCCGCAGTTATTCAAAGAT ATGCAGGGTCAAGATTAGTCC	56.7	259	(AT) ₂ (AG) ₂ G (TA) ₂
SSR-IAC209	TCCTCAAATTTATAACGAAGAA TTATGGATGGTATTGGATTGT	56.7	273	(AC) ₂ (TG) ₃
SSR-IAC210	GCAGGGAGGAAACAAAG AGAAACCTGGGCAACATA	56	219	(CA) ₃ ACCG (TC) ₄
SSR-IAC211	ATTCTTCTCCCTCCTA GATAATCACTACCAAAAT	43.8	176	(CA) ₁₀ (TA) ₈
SSR-IAC212	TTAACTACCAAATACACAACAT GATAGCAAAGTCAAACAAAG	46.9	301	(TG) ₆
SSR-IAC213	TGCTTGATTGGTCTTTACATT ATACGATAGTGCAACAGGACAT	61.5	216	(TG) ₃ C (TG) ₂ T (CA) ₂
SSR-IAC214	TGGCTGTTCATTTGTCAATCAC CTTCCCAGTAGCCATTCTTTG	51.0	279	(CT) ₂ (TC) ₂ (TA) ₂ TC (AT) ₂ (AT) ₂ CTA (AT) ₂ (TC) ₂
SSR-IAC215	AAAAATCTGATCAAAAACACAA AAGCCTGCACCCACATT	56.7	191	(TA) ₅
SSR-IAC216	GGTGCAGGCTTGGTCTT ATTAATTTGCGGGTTTCTCT	63.3	188	(CT) ₂ GA (GT) ₄ (GA) ₃ (GA) ₅
SSR-IAC217	GTCAGCCAGCAAGAAACACCAA TTTAAATAGCGCGGAGAAGTCG	55.2	234	(GT) ₂ ATC (TG) ₆
SSR-IAC218	AAACGACTACACCTGATTGA TCTGTTATAAGTTGGGTTTCTA	56.7	264	(AC) ₂ TTAA (CT) ₃
SSR-IAC219	TTGAATTAATGAGACTGTT GTGCACTAGATGATGGT	42.5	152	(GT) ₇ AG (AT) ₂ A (AT) ₂
SSR-IAC220	AGTTTCTTTCTCTTTTCTTA CAACAACAGTGGATGATG	56.7	202	(TG) ₇ (TC) ₃ AAG(CT) ₂
SSR-IAC221	GGGATGGAAGAGATTTTG CTTAACATGCACATTTCACTT	44.8	126	(AT) ₈ (GT) ₉
SSR-IAC222	TCATTAGGTCCATTTCAATCAC GAGCGGCCATCTTCCTA	63.3	225	(TCC) ₄

similarity with some other plant gene, especially of *Arabidopsis thaliana* and *Oryza sativa*. From these, four sequences (SSR-IAC129, SSR-IAC179, SSR-IAC204 and SSR-IAC205) presented similarities with proteins with unknown function (E-value < 10⁻¹²), and two sequences (SSR-IAC166 and contig10 - SSR-IAC209 and SSR-210) showed similarities with the retrotransposon gag protein family (E-value < 10⁻³¹).

This protein is present in some virus capsids and in plants; it may be involved with pathogenic resistance to virus, bacteria and fungi (Grandbastien et al., 1997; Benko-Iseppon et al., 2003). These results suggest that the new microsatellites reported here could be used for synteny studies, establishing the conservation of genes between other species of plants. Around 51% of sequences were involved in the cellular metabolism, such as lipid and

kinase metabolism and photosynthesis (E-value < 10⁻⁴⁹). The remaining sequences (SSR-IAC174 and SSR-IAC175) were associated to transcription functions (E-value < 10⁻¹²) related, in several aspects, to the developmental processes, which might be interesting for manipulating important agronomic traits.

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