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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 microsatelitte *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 (McClean 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 µL of frozen white colonies and their amplification in a PCR reaction. Each amplification reaction contained 25 µL of 1x reaction buffer, 2 mM $MgCl_2$, 0.5 µM of *Rsa*I primer, 200 µM 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 95ºC; followed by 30 cycles of 30 s at 94ºC, 45 s at 52ºC, 1 min 30 s at 72ºC, followed by 8 min at 72ºC. 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 *Rsa*I 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 60ºC; 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	60	267	(TG)2 GA (TG)2 AG (GT)2
	TTGGGAAAGGTGAGAGGTAT			
SSR-IAC125	CCCCCATAAAAGTTCACA	50.2	165	(AC)4 (TC)3 (CA)4 CG (CA)2
	ATTCAAAAGCTCATCCTCT			
SSR-IAC126	AACGAGGAGAAGAACGAT	51.4	297	(CA)5 CT (CA)3 (CT)2
	ACATAAGAAACGGTGGTC			
SSR-IAC127	GAGGCTAGCCCAACTTA	63.3	199	(TA)3 T (TGA)3 G (TA)3
	AGCGCAAGACTTTACTACTC			
SSR-IAC128	TCTTGAAGGTTGTTTCTCC	56,7	191	(AC)7 GGA (TC)2
	GGATTTGAATCTCCGTAAC			
SSR-IAC129	CACCCTTTTCTAGCTTATTTTT	56.7	294	(TG)2 G (CT)2 TCT (GA)2
	CTAGATGTTGCAGGTGACGA			
SSR-IAC130	TTGAACGTGGGTGAATC	56.7	299	(TG)5
	CCCAGAAGAAGGAAAGAG			
SSR-IAC131	AACGCTTTAATGCTTTGAAC	56.7	253	(AC)6
	GGAACACCGTGATTGGAT			
SSR-IAC132	ACAACACAACAAATGGT	54	235	(GA)3 A (GT)6 GA (GT)3
	GATATGGAAAGGTTAGC			
SSR-IAC133	GATGATGACCACAAAACAGAGA	59.7	232	(AG)3 CA (CTG)3
	AGTAAAAAGGGATCAAGAGGTG			
SSR-IAC134	TGGAAACAGCAGAGCGATACT	56.7	251	(AC)6
	TTGGTCCCTAAATTCTTCTCAT			
SSR-IAC135	ATTTGATTTTAACTGGTGTA	54	231	(AC)6 (AT)4 G (TA)4
	ACTTAACTTCCCTTGGTAT			
SSR-IAC136	GCTCTTAGGTATGGACGATTGT	56.7	233	(CA)7 (AT)5
	CAGATGGGGTTAGGAAAGTTG			
SSR-IAC137	GCAGCAGCAAATACAAC	60	169	(AT)2 T (TG)2 C (CT)2
	CCCCTAAACAATACAGC			
SSR-IAC138	GTGATGGCAAAGCGACAAC	56.7	173	(GT)3 GC (GT)3 T (CA)2 (CT)2 GCA (AT)2 GA (CA)2 AC (AT)2
	GATCAAAAAGAAAAGCCCTCAT			
SSR-IAC139	AATTTCAGGGTAACATTTT	46.5	233	(TC)10
	TCTAAATAGATCAATAATGTAT			
SSR-IAC140	GAGAGTATAGGAAAAGAGA	52	175	(CT)3 (AT)2 (TG)2 (TGT)3 (GT)3
	TGTAATCAAACGAAGGT			
SSR-IAC141	ACCCTCAGGTTTGGACTCTTCC	59.4	171	(TCT)3 A (CT)13
	GGCGGCGGTGGTTCTTG			
SSR-IAC142	AATTCTAAAAGTTCAGTGG	60	157	(CA)7
	CTCATTTTATTTTATTTATTCT			

Continue...

Tabl 1. Continuation...

Locus/GeneBank Accession	Primers (5´→ 3´)	T_a (°C)	Predicted size (pb)	Core motifs
SSR-IAC143	CCCTTCACATCCACTTCACAC	63.3	171	(TC)2 T (TC)2 T (TC)2
	GCTGCTTCATCGCTTTCTG			
SSR-IAC144	TATAAATTCCCCCTTCACATCC	56.7	181	(CT)10
	GCTGCTTCATCGCTTTCTG			
SSR-IAC145	AGATGACAATGACAACCACA	56.7	233	(TA)2 AC (TA)2 TC (AT)2 (AT)2 AA (AT)9 (AC)9
	TAAGGCAATCCATCTCC			
SSR-IAC146	CTGGGAGAATATGTGAGGA	56.7	255	(AC)8
	TCGTAGACCAAAATGAACT			
SSR-IAC147	CTTTAACGCTTTAATGCTTTGA	56.7	252	(CA)5
	GGAACACCGTGATTGGATG			
SSR-IAC148	CAAGCATGTAAGGGTGAGT	56.7	188	(CAT)3 (CA)3 (TA)2
	AAGGTTTGGTGGGTTTAG			
SSR-IAC149	TTGGGATCTCTGCCTCGTC	60	273	(TC)8
	CCCCATTAAAGTATCGGAACAG			
SSR-IAC150	CAAGACCAAAGAAAATGTA	58.4	190	(GA)12
	CTGGAGTGGTGGATAATA			
SSR-IAC151	CGTGCTTTGAGGTTACGAACAA	65	161	(AC)5
	CTCAAGTCAGCCAGCCAGAA			
SSR-IAC152	GTAGCCTTTCTCTTCTCACC	60	178	(GT)2 GA (GT)5
	ACTACTCTTTGCTTTCCACTAT			
SSR-IAC153	AGGGAAGAAAGAGATACACAA	59.7	178	(AC)3A (AC)2
	TGCTGAGCCAAAGGAAG			
SSR-IAC154	AATGGCATAGGATAACTTTCTC	56.7	164	(TG)2 (TC)3
	ATCAACAACCCCATACTTTTAG			
SSR-IAC155	AAGGTCACAGGAGCCAGTCT	56.7	181	(AG)9
	TTTTGAATTCTCCGCACCTA			
SSR-IAC156	ATTTTGTGCATCAGGTA	56.7	230	(TC)3 TG (GC)2
	TTCAAAAAGCAGTTAGAG			
SSR-IAC157	TTTAAATTTGTTGCCACTTCCT	56.7	165	(GA)8 GT (GA)3
	TGCACACGTTTCCCTATTCTAT			
SSR-IAC158	AATACCACAAAACGCAAAAA	56	292	(GA)7 AT (CA)3
	TTAAATAAATGGAAGGGAACAC			
SSR-IAC159	AAAACCCAACCAAACAT	56.7	298	(AC)6 (AC)4 C (CT)2
	ACGCCAATAAATCTAAAG			
SSR-IAC160	TGGAATCACATCACGGAAGGT	56.7	173	(TG)2 (TA)2 (TG)5
	GCGGAGAATGTTGGAATAGG			
SSR-IAC161	TACACCAAGAGCTACATCCAG	55.3	233	(AC)3 AT (CA)3
	TGTATTCTATTCCTTTCTTCCT			
SSR-IAC162	GTCACAACCAAACCAAAACAAT	56.7	190	(CG)2 TG (CT)2
	CAGGGAGAAGGATGTGAATG			
SSR-IAC163	TTTGGGAACATTTGTAGGAGAT	59.4	193	(GT)6 GA (GT)2
	ACAGGATTGGTTCACTTTCACT			

Continue...

Table 1. Continuation...

Locus/GeneBank Accession	Primers (5′→ 3′)	T_a (°C)	Predicted size (pb)	Core motifs
SSR-IAC164	CAGTGAAAGTGAACCAAT ACAACAGTAGCTAGATGAAGT	59.4	207	(TC)2 (TA)2 A (AC)2
SSR-IAC165	CTTAACCAAGACCTACAT TCAAGTTTTTCCAAGAT	46.1	294	(TG)5
SSR-IAC166	TCCTCCTTTTTCTTATTCGTGT CATCAGATTCCCAAAGGTTATT	56.7	199	(CA)2 AA (AC)3 (TA)2 GAC (TG)3
SSR-IAC167	AGACCAAGAAACAATGAGAAT AAGTTTCGTTAGTCTGTGATTT	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 AGTGCCCACCGATTCTCCA	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 TTAGGAGGGATTATGATTTG	53.2	230	(AT)3 A (AT)2 (AC)7 TTT (CA)3
SSR-IAC175	AGAAAAGTTACTGGTTGA TAGAAGCCTTTTATTTTG	45	251	(AC)6
SSR-IAC176	CTTCTTCCCTTAACCGTATTC TTTTCCTGCTAAGTCATCAA	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 TGTGTTGTATGCAATGTTTCTGT	63.3	205	(AC)3 T (CA)3 TAA (AC)3 (AC)3 G (CA)2
SSR-IAC181	AGGTTCCTTTCTTTCTACT CTACTTTTTCCCACAGC	58.4	216	(AT)2 AC (AT)3 (AG)5 TAA (AG)2 C (AG)2
SSR-IAC182	CTGGTTTTTGTTTGCTTTTG TGCTGACATAACCCATTTTG	56	176	(GA)7
SSR-IAC183	CGCATTTATAAGAGAAGCAACA CCGATGGAAACACCTGAAA	56	195	(AG)18 A (AC)4
SSR-IAC184	ACAACTGTAGCAACCAAAAT ATGCAATGTGACTTAGAAAATA	56.7	218	(AT)2 T (TG)2 C (CT)2 (TC)2 ACA (CT)2 GA (AC)6

Continue...

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 TCTACTCACCAAAACAAT	41.7	221	(TA)3 CCA (CT)2 (TG)2 (AT)4
SSR-IAC190	CCTTTATTGTTTTGGTGAGTA GCTTATAATATTGCTTTTGTGA	59.2	279	(GT)2 CT (CA)3 (TG)2 CT (TG)2 AG (GT)2
SSR-IAC191	ATTTCACTCTACTTCATTAC AGATTTACAACTTCCATT	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 GCTTTGTGGTGGTAGTTATTCT	63.3	191	(AC)7 (AT)7 (TA)2
SSR-IAC195	TGGACATCAAACAAACAAAAA TGCATCGGCAGTTCATCA	63.3	203	(TC)2 TT (TC)8
SSR-IAC196	TTCCTCAAATATGCCTAAC TTTTCTGTATTACTCCCTTTT	43.0	202	(GT)8
SSR-IAC197	TTCTCCTTGTTATTATTGT TCAGAACTTACCTTAGATAG	45.7	200	(CT)3 (GA)2 TTA (CT)2
SSR-IAC198	TGAATGCAAAGATGAACT AGACGGAGCAGAATGAA	56	209	(AC)7
SSR-IAC199	ATAAACAAAACAAGCATCTCAT TGCCATCTCTTACTTCTTTCTC	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	GTCCTCAACTAACCACTGA GTATTCTATTCCTTTCTTCCT	56.7	199	(AC)4 CA (CT)2
SSR-IAC203	AAAAATAACAACCCAGAAAAT ATATGATGCGAAGGTTGAAG	46.0	294	(AT)6 G (AT)2 (CT)2
SSR-IAC204	GGGCCGCACCAGGAGGAGA CTGTGAAGGGCCCCAAAGACC	65	210	(GGT)3 (GA)2
SSR-IAC205	ATTTATTTCACTCTCATTCTCT TGTGTTCATCTTCCTGTCT	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	CAATTCCCTCACCACCACTTAT	53.4	285	(AAG)4
	CGATTAGATTTTGAGGGGACTG			
SSR-IAC207	GAATGGGTGAAAGTGTTGTTAT	56.7	197	(GT)8
	AGGGAAAGGTGGTTGAAAT			
SSR-IAC208	TGCCGCAGTTATTCAAAGAT	56.7	259	(AT)2 (AG)2 G (TA)2
	ATGCAGGGTCAAGATTAGTCC			
SSR-IAC209	TCCTCAAATTTATAACGAAGAA	56.7	273	(AC)2 (TG)3
	TTATGGATGGTATTGGATTGT			
SSR-IAC210	GCAGGGAGGAAACAAAG	56	219	(CA)3 ACCG (TC)4
	AGAAACCTGGGCAACATA			
SSR-IAC211	ATTCTTCTTCCCTCCTA	43.8	176	(CA)10 (TA)8
	GATAATCACTACCAAAAT			
SSR-IAC212	TTAACTACCAAATACACAACAT	46.9	301	(TG)6
	GATAGCAAAGTCAAACAAAG			
SSR-IAC213	TGCTTGATTGGTCTTTTACATT	61.5	216	(TG)3 C (TG)2 T (CA)2
	ATACGATAGTGCAACAGGACAT			
SSR-IAC214	TGGCTGTTCATTTGTCATCAC	51.0	279	(CT)2 (TC)2 (TA)2 TC (AT)2
	CTTCCCAGTAGCCATTCTTTG			(AT)2 CTA (AT)2 (TC)2
SSR-IAC215	AAAAATCTGATCAAAAACACAA	56.7	191	(TA)5
	AAGCCTGCACCCACATT			
SSR-IAC216	GGTGCAGGCTTGGTCTT	63.3	188	(CT)2 GA (GT)4 (GA)3 (GA)5
	ATTAATTTGCGGGTTTCTCT			
SSR-IAC217	GTCAGCCAGCAAGAAACACCAA	55.2	234	(GT)2 ATC (TG)6
	TTTAAATAGCGCGGAGAAGTCG			
SSR-IAC218	AAACGACTACACCTGATTGA	56.7	264	(AC)2 TTAA (CT)3
	TCTGTTATAAGTTGGGTTTCTA			
SSR-IAC219	TTGAATTAATGAGACTGTT	42.5	152	(GT)7 AG (AT)2 A (AT)2
	GTGCACTAGATGATGGT			
SSR-IAC220	AGTTTTCTTTCTCTTTTCTTA	56.7	202	(TG)7
	CAACAACAGTGGATGATG			(TC)3 AAG(CT)2
SSR-IAC221	GGGATGGAAGAGATTTTG	44.8	126	(AT)8 (GT)9
	CTTAACATGCACATTTCACTT			
SSR-IAC222	TCATTAGGTCCATTTCATTCAC	63.3	225	(TCC)4
	GAGCGGCCATCTTCCTA			

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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