Polymorphism of Microsatellite Markers in Papaya (*Carica papaya* L.)

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Abstract A set of 81 new microsatellite markers for Carica papaya L. previously identified by data mining using freely available sequence information from Genbank were tested for polymorphism using 30 germplasm accessions from the Papaya Germplasm Bank (PGM) at Embrapa Mandioca e Fruticultura Tropical (CNPMF) and 18 landraces. The data were used to estimate pairwise genetic distances between the genotypes. A neighbor-joining based dendrogram was used to define clusters and infer possible genetic structuring of the collection. Most microsatellites were polymorphic (73%), with an observed number of alleles per locus ranging from one to eleven. The levels of observed and expected heterozygosity for 51 polymorphic loci varied from 0.00 to 0.85 and from 0.08 to 0.82, averaging 0.19 and 0.59, respectively. Forty-four percent of microsatellites showed polymorphism information content (PIC) higher than 0.50. The compound microsatellites seem to be more informative than dinucleotide and trinucleotide repeats in average alleles per locus and PIC. Among dinucleotides, AG/TC or GA/CT repeat motifs exhibited more informativeness than TA/AT, GT/CA and TG/AC repeat motifs.

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J. G. Pádua Embrapa Recursos Genéticos e Biotecnologia (CENARGEN), Brasília, DF, Brazil The neighbor-joining analysis based on shared allele distance could differentiate all the papaya accessions and landraces as well as differences in their genetic structure. This set of markers will be useful for examining parentage, inbreeding and population structure in papaya.

Keywords Germplasm · Microsatellites · Papaya · Polymorphism · Breeding

Introduction

The genus *Carica* is monotypic and only includes papaya (*Carica papaya* L.). Papaya belongs to the small family Caricaceae which consists of six genera and 35 species (Badillo 2000). Most of them are diploid (2n=18) and a relatively small genome of 372 Mb was found in *C. papaya* (Arumuganathan and Earle 1991; Parasnis et al. 1999; Kim et al. 2002; Liu et al. 2004). There are three distinct types of *C. papaya* plants: (1) dioecious papayas have male and female flowers on some trees and bisexual (hermaphrodite) flowers on others, and (3) trioiceous papayas have male, female, and hermaphrodite flowers in different plants.

Many landraces and cultivars present hermaphrodite plants, bearing perfect flowers and producing fruits shaped from long-cylindrical to ellipsoidal, which are preferred for commercial production. The economic importance of papaya resides largely in the fruit production, and Brazil is the world's major producer (FAOSTAT 2007).

Papaya exhibits considerable phenotypic variation for many morphological and horticultural traits, including fruit size, fruit shape, flesh color, flavor and sweetness, length of juvenile period, plant stature, stamen carpellody, and carpel abortion (Kim et al. 2002; Ocampo Pérez et al.

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2006). Nevertheless, low levels of genetic variation for resistance to major fungal and virus diseases were observed in *Carica* genus (Nishijima 1994). On the other hand, resistance to several diseases which affect papaya are identified in the *Vasconcellea* genepool, including resistance to PRSV-P (*V. cundinamarcensis, V. candicans, V. stipulata, V. cauliflora* e *V. quercifolia*; Horovitz and Jimenez 1967; Alvizo and Rojkind 1987), *Asperisporium Caricae* (*V. cundinamarcensis*; Drew et al. 1998); *Fusarium* and *Meloidogyne* (*V. weberbaueri*; Scheldeman et al. 2003), phytoplasma (*V. parviflora*; Drew et al. 1998), and *Phytophthora* (*V. goudotiana*; Drew et al. 1998).

In recent years, molecular markers have been widely used in practical plant breeding to access the genetic variability available in germplasm banks, manage and develop core collections, target crosses, classify germplasm into interest groups and identify duplicate accessions (Rafalski and Tingey 1993; Manifesto et al. 2001; Martins-Lopes et al. 2007). Additionally, available molecular markers linked to important genes have proved to be useful for early selection of different desirable traits (Deputy et al. 2002; Dillon et al. 2005). These molecular markers have also been used to develop genetic maps, and for analysis of qualitative and quantitative inheritance (Luo et al. 2001; Jansen 2005; Milczarski et al. 2007). Other applications, such as marker-assisted backcrossing can reduce the number of generations needed to obtain a genotype with 98% or 99% genetic similarity to the recurrent parent for a fixed sample size (Liang et al. 2004; Oliveira et al. 2005). The first study of genetic diversity in papava was made by Tan and Weinheimer (1976) using isozyme. Genetic relationships between papaya and related wild species have been investigated using isozyme (Morshidi 1998), Random Amplification of Polymorphic DNA (RAPD) (Sondur et al. 1996; Stiles et al. 1993; Jobin-Décor et al. 1997), Restriction Fragment Length Polymorphism (RFLP) (Aradhya et al. 1999), Amplified Fragment Length Polymorphism (AFLP) markers (Kim et al. 2002; Van Droogenbroeck et al. 2002), PCR-RFLP (Van Droogenbroeck et al. 2004), SSR (Kyndt et al. 2005; Kyndt et al. 2006) as well as Inter-Simple Sequence Repeats (ISSR) (Carrasco et al. 2009).

A large number of different molecular techniques are at present available and each of them differs in its informational content. Although in principle all types of markers would be suitable for our purpose, microsatellites (Single Simple Repeat (SSR)) are especially useful for diversity studies (Baumung et al. 2004).

Multi-locus approaches, such as RAPD, AFLP, and ISSR, may be convenient but have some technical and analytical drawbacks, such as dominance. Multi-locus data are typically analyzed as pairwise comparison of complex patterns that only have meaning relative to others in the same study, thus results are to a limited extent comparable among studies. By contrast, single-locus markers, such as SSR, are usually characterized by co-dominance and thus are more flexible and supply more robust and comparable data (Brondani et al. 1998; Rallo et al. 2000; Karp 2002).

Despite the present availability of molecular marker techniques, papaya have just recently received molecular attention, especially by development of a large number of SSR markers with different motifs, by data mining analysis from DNA databases (Oliveira et al. 2008a) and by screening of sequence data from bacterial artificial chromosomes (BAC) ends and complementary DNA (Eustice et al. 2008). The utility of SSR as genetic markers to investigate relationships among plants has been clearly established (Zhou et al. 2003). Microsatellites have also been extensively exploited for fingerprinting, phylogenetic studies, genetic and QTL mapping for a wide range of species. The genetic analysis based on SSR made it possible to investigate the occurrence and variability of simple sequence repeats at the whole genome level in germplasm accessions of papaya. The objective of this investigation was to test the suitability of SSR for genomic analysis in C. papaya.

Material and Methods

Plant Material

Thirty papaya accessions and eighteen landraces collected from Muritiba, Bahia-Brazil, were used to screen for SSR polymorphisms (Table 1). These papaya plants were maintained in the Papaya Germplasm Bank (PGM) at Embrapa Mandioca e Fruticultura Tropical (CNPMF), in Cruz das Almas, BA, Brazil. Among the 30 papaya accessions, two were cultivars, 12 were improved (but not released) breeding lines, and 16 were unimproved germplasm. All landraces were unimproved germplasm (Table 1).

DNA Extraction

Young papaya leaves were harvested and stored at -80° C for long-term storage. Genomic DNA was extracted based on the procedure described by Doyle and Doyle (1990). DNA quantification was carried out in an agarose gel (1.0% *w*/*v*) by comparing the fluorescent intensity of the sample stained with ethidium bromide (1.0 mg/mL), relative to a dilution series of Lambda DNA (Invitrogen, Carlsbad, CA) as standard of known concentration.

PCR Amplification

A set of 100 SSR primers developed by Oliveira et al. (2008a) was tested for amplification and polymorphism.

 Table 1 Germplasm accessions (code CMF) of Carica papaya and landraces (code M) used to analyze levels of microsatellite polymorphism including origin of variety and mating system

Accession	Germplasm	Origin	Mating system
CMF008	Improved	Malaysia	Gynodioecious
CMF011	Unimproved	Costa Rica	Gynodioecious
CMF017	Unimproved	Taiwan	Gynodioecious
CMF023	Unimproved	Malaysia	Gynodioecious
CMF024	Cultivar	Costa Rica	Gynodioecious
CMF031	Unimproved	Costa Rica	Gynodioecious
CMF038	Improved	Brazil	Gynodioecious
CMF054	Unimproved	Hawaii	Gynodioecious
CMF058	Unimproved	Brazil	Gynodioecious
CMF068	Improved	Brazil	Gynodioecious
CMF082	Improved	South Africa	Gynodioecious
CMF101	Unimproved	Brazil	Gynodioecious
CMF102	Unimproved	Brazil	Gynodioecious
CMF108	Improved	South Africa	Gynodioecious
CMF115	Improved	South Africa	Gynodioecious
CMF123	Improved	Thailand	Gynodioecious
CMF125	Improved	Hawaii	Gynodioecious
CMF128	Cultivar	Taiwan	Gynodioecious
CMF129	Unimproved	Brazil	Gynodioecious
CMF134	Unimproved	Brazil	Dioecious
CMF135	Unimproved	Brazil	Gynodioecious
CMF138	Improved	Brazil	Gynodioecious
CMF142	Improved	Brazil	Gynodioecious
CMF143	Improved	Brazil	Gynodioecious
CMF147	Improved	Brazil	Gynodioecious
CMF157	Unimproved	Brazil	Dioecious
CMF165	Unimproved	Brazil	Dioecious
CMF188	Unimproved	Brazil	Dioecious
CMF189	Unimproved	Brazil	Dioecious
CMF191	Unimproved	Brazil	Dioecious
M01	Unimproved	Brazil	Dioecious
M02	Unimproved	Brazil	Dioecious
M03	Unimproved	Brazil	Dioecious
M06	Unimproved	Brazil	Dioecious
M09	Unimproved	Brazil	Dioecious
M11	Unimproved	Brazil	Dioecious
M12	Unimproved	Brazil	Dioecious
M13	Unimproved	Brazil	Dioecious
M14	Unimproved	Brazil	Dioecious
M15	Unimproved	Brazil	Dioecious
M16	Unimproved	Brazil	Dioecious
M17	Unimproved	Brazil	Dioecious
M25	Unimproved	Brazil	Dioecious
M27	Unimproved	Brazil	Dioecious
M33	Unimproved	Brazil	Dioecious
M34	Unimproved	Brazil	Dioecious
M38	Unimproved	Brazil	Dioecious
M39	Unimproved	Brazil	Dioecious

Each PCR reaction was prepared as follows: 20 ng DNA template, 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 0.3 mM of each primer, 1.5 mM MgCl₂, 0.2 mM dNTPs, and 0.5 U *Taq* DNA Polymerase (Invitrogen, Carlsbad, CA) in a total volume of 20 μ L.

PCR cycling consisted of 94°C for 4 min, followed by 35 cycles of 94°C for 40 s, (annealing temperature of 55°C, 60°C, or 62°C according to each SSR primer (Table 2)) for 40 s, and 72°C for 1 min, with a final extension at 72°C for 2 min, on a PTC-100 thermal cycler (MJ Research, Inc., Watertown, MA). After cycling, fragments with size difference shorter than 10 base pairs (bp) were electrophoresed on a 6% (w/v) denaturing polyacrylamide gel in a Hoefer SQ3 DNA sequencer gel electrophoresis unit (Pharmacia Biotech Inc., San Francisco, CA) at 70 W for 2.5 h. The gels were stained with silver nitrate, according to Creste et al. (2001). Previous analysis in polyacrylamide gel showed some primers that produces fragments longer than 10 bp in size difference. These loci were electrophoresed on a 3% agarose 1,000 gel (Invitrogen, Carlsbad, CA) at 130 V for 3.5 h. The 50-bp ladder (New England Biolabs, Inc., Beverly, MA) was used as a molecular weight standard to estimate the size of microsatellite alleles.

Data Analysis

Genetic variability was measured as allelic richness determined by the total number of the detected alleles and the number of alleles per locus (N_A) , observed heterozygosity (H_O) , expected heterozygosity (H_E) and polymorphism information content (PIC). We defined rare alleles as those whose individual frequency is lower than 1% in the investigated materials. Common alleles are those that occur with a frequency between 1% to 20%, while those whose frequency is higher than 20% are classified as most frequent alleles. Genetic distances between individuals were estimated by shared allele distance (SAD). All these analysis were carried out using the software POWER-MARKER version 3.25 (Liu and Muse 2005). The matrix of genetic distance was used to construct the neighborjoining tree, using the MEGA 4.1 package (Tamura et al. 2007). To assess confidence in the nodes of a tree, bootstrap values were obtained from 1,000 replicates by re-sampling microsatellite loci.

Results

Polymorphism of Microsatellites

All 100 SSR loci were screened for patterns of amplification using two individuals of the PGM-CNPMF. Eighty-one primer pairs were selected according to strength, clarity of

Table 2 Characteristics of 81 microsatellite loci developed for Carica papaya

	Marker	Repeat motif	Genbank accession (gi)	Pimer Sequence (5'-3')	Ta (°C)	
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$				Forward	Reverse	
	CP02	(AGG) ₉	33898396	aggcgaaatcggaagagag	ctggtaaaacgacgatgacg	59
CP04 (AGA) ₁ =(AG) ₂ ; 33898550 agg.gram.gram.grag.grag.gr cc.ataccccturg.gottcal 57 CP05 (AT) ₁₇ 33898578 gt.cccaat.ccg.agg.atat cataccccturg.gottcat 57 CP06 (TGCCA) ₄ 33898574 gt.cccaat.ccg.agg.tat gt.agg.tm.agg.tt.gt.tt 60 CP07 (AT) ₁ , (T) ₁ /3 33898074 cccaat.ccg.agg.tt.agt.tt.gt.tt.ccat.cc.tat 58 CP10 (ACA) ₁ +(T) ₁ /17), 33898074 asaat.cca.gcg.st.gt.tt.gt.gt.tt.ccat.cc.tat 57 CP11 (AT) ₁ +(T) ₁ /17), 33898403 gg.gg.gg.gt.gt.gt.gt.tt.gt.tt.gt.tt.tt.ccat.cc.tat 57 CP13 (AT) ₁ +(TA) ₁ , 14211817 tg.ccg.tt.gt.gt.agt.tt.gt.gt.tt.tt.gt.tt.tt.ccat.cc.tat 57 CP14 (AC) ₁ /A ₁ /4(TA) ₁ , 33898403 tg.gg.gt.gt.gt.gt.gt.tt.gt.tt.tt.gt.tt.t	CP03	(AATA) ₇	33898427	gaaggcccgtgtaagtgc	tggtgaaaattggaaaggag	58
CP05 (AT) $_{17}$ 33898578 genetation graph of the structure of the	CP04	$(AGA)_5 + (AG)_{21}$	33898550	aagggagaagagaagcagagt	ctccagtttgcctccaaag	57
CP06 CF0C (Λ_{1} , 33898764 tigccaccaggettat qsgftacgiftatrig 58 CP07 (Λ_{1})rigCt(Π_{1}) 33898964 ccaaticitigcaatic gccaatiticacaticacacc 60 CP09 (Λ_{1}) _g +(Λ_{1}) _L 33899071 cccaaticitigtcaatic gittgaccaatiggecaatagge 58 CP10 (Λ_{1}) _g +(Λ_{1}) _L +(Λ_{1}) 14211817 tigcgataggaataga teteccaaaastteatt 57 CP11 (Λ_{1}) _g +(Λ_{1}) _L +(Λ_{1}) 33898403 atggaataggaataga teteccaaastteatt 57 CP13 (Λ_{1}) _h (Λ_{1}) _L (Λ_{1}) 33898502 teaatitteccaggaatag 58 CP14 (Λ_{1}) _h (Λ_{1}) _h 33898503 teaatatteccagaat 57 CP16 (Λ_{1}) _h (Λ_{1}) _h 33898503 teacatifticccagetag 58 CP14 (Λ_{1}) _h (Λ_{1} 33898503 teacatifticcagetag 58 CP19 (Λ_{1}) _h (Λ_{1} 33898503 teagaatifticgticgtiftig gcaacetagtaatcagaat 58 CP10 (Λ_{1}) _h (Λ_{1} 33898700 teagaatifticgticgtiftig gcaacetagtaat	CP05	(AT) ₁₇	33898578	gtcctcaatccgaagcat	catacacccttgtggcttct	57
CP07 (GT), gen(CT) _{1,2} 33898964 ccapacitycutygicy gencalitacitacitacitacitacitacitacitacitacitac	CP06	(TGCCA) ₄	33898764	ttgcccaccaggettaat	tgacgttacggtttcatctg	58
CP09 $(\Lambda_{1n}^{-1}(\Gamma_{1n}^{-1}(\Lambda_{1-2}))$ 33899071cccantcalcalcacaccalcaccatatgtgacaacaagaagcaa59CP10 $(\Lambda_{1n}^{-1}+(\Gamma_{1n})_{2n}^{-1}+(\Gamma_{n})_{3}$ 14211817tyccalcalcactaltagettgamattacaanagggaanaa58CP12 $(\Lambda_{1n}^{-1}+(\Gamma_{n})_{2n}^{-1}+(\Gamma_{n})_{3}$ 13898403gggaggattgagccttttiggattaccaccatcat57CP13 $(\Lambda_{1n}^{-1}+(\Gamma_{n})_{4n}^{-1}(\Gamma_{n})_{3}$ 33898502totattagtccaagcaggatta59CP14 $(\Lambda_{C})_{A}^{-1}(\Gamma_{n})_{4n}^{-1}(\Gamma_{n})_{3}$ 33898502totattagtccaagcaggatttotagtcgattagtcaagcat57CP15 $(\Gamma_{0n}^{-1}(\Gamma_{n})_{4n}^{-1}(\Gamma_{n})_{3}$ 33898502ccgattagtcgattagtcat58CP16 $(\Lambda_{1n}^{-1}(\Gamma_{n})_{3}^{-1}(\Gamma_{n})_{3}^{-1}^{-1}(\Gamma_{n})_{3}^{-1}^{-1}(\Gamma_{n})_{3}^{-1}^{-1}(\Gamma_{n})_{3}^{-1}^{-1}(\Gamma_{n})_{3}^{-1}^{-1}(\Gamma_{n})_{3}^{-1}^{-1}(\Gamma_{n})_{3}^{-1}^{-1}^{-1}(\Gamma_{n})_{3}^{-1}^{-1}^{-1}(\Gamma_{n})_{3}^{-1}^{-1}^{-1}(\Gamma_{n})_{3}^{-1}^{-1}^{-1}(\Gamma_{n})_{3}^{-1}^{-1}^{-1}(\Gamma_{n})_{3}^{-1}^{-1}^{-1}^{-1}^{-1}^{-1}^{-1}^{-1$	CP07	$(GT)_{12}gct(GT)_{13}$	33898964	cctagcattgccttgaggtc	gcccactattcacattcacacc	60
	CP09	$(AT)_{10} + (CT)_{14} (AT)_{12}$	33899071	cccaattcatgtccaaatcc	atgttgaccaaaggaagcaa	59
	CP10	(TACA) ₄ (TA) ₉ (GA) ₁₀	33899084	aaaaatcacagcacgtatggtt	gaaattacaaatgggcaaaaag	58
CP12 (AT)r 3389403 gggggggtgtggctt ttggnttccctacta 57 CP13 (AT)r+(TA) _A (TG) _k 33898463 attgggaaccactutg tcacaccgegggatta 59 CP14 (AC) _b (AT), a 33898502 tcattftcctgetagtigt tgggatgftcacattg 56 CP15 (TG) _b (TA), tg(TA), e, (AT), 33898593 tcattftccccgegata ccattccttgtcaaagtf 60 CP18 (TA) ₁₂ 33898500 cggggtdgtgcgtcata agcaggctaaaaccattgg 57 CP19 (AT) ₁ (AT) ₈ 33898700 tagggetdgtgtgtcgtcata agcaggtaaaccattggtdtgtgttgt 59 CP21 (GT) ₁ 2 33898744 tgtgggtgtgtcgtcata agcagctaaaaccattggtagtg 60 CP22 (TA) ₁₆ 33899038 ctgcgrtggtctagtgt tgaacactctgaaaccattg 60 CP24 (GA) ₇ 33899039 ctgcgatgtgtgtcagtgt ttgaattgtcagtgaaccaccattgt 60 CP27 (AT) ₁₇ 33899070 aagagacggcatctgtgaat ttgaattgccacggaaa 58 CP26 (CA) ₁₆ (A) ₁₁ 33899079 ggaaagagaggcctgtggggt	CP11	$(AT)_9 + (TA)_{12} + (TA)_8$	14211817	tgccgtatgagaaggaattaga	tetetectecaaacatteattt	58
CP13(AT) ₂ +(TA) ₀ (TG) ₈ 33898463atgggaccaaccattcgtcaccaccgcaggatatna59CP14(AC) ₈ (AT) ₈ 33898502tcangttretycgatagttcacgaccgaggatatta56CP15(TG) ₈ (TA) ₁ g(TA) ₆ +(AT) ₈ 33898503tcaactattcaccgaaggattcatggttrecananggt57CP16(AT) ₁₃ 33898509tcaactattcaccgaaggatcactcctgtcaanaggt58CP19(AT) ₁₄ (AT) ₈ 33898700tagggttgtggtcgtcataagcaggctanaactgtgca58CP20(AT) ₁₄ (TG) ₈ 33898744tytgaanttgtctgtgtgtggacccganaatcanaact59CP20(AT) ₁₆ (A)33898744tytgaanttgtctgtgtgtccatccaccacaa59CP20(AT) ₁₆ (A)33898743acgacgggagagtactcaatcaccaccacaa59CP20(GT) ₁₂ 33898043acgacggtctactagttcaatcaccaccacaaa59CP23(GT) ₁₄ 33899038cgacgattcacgtctactcaccaccacaa58CP24(GAA) ₇ 33899039cctcgcatgatattgaaacccgataact60CP25(TTC) ₈ 33899040anagagggctctgtgatttgaaactccccacaaa58CP24(GA) ₁₇ 33899080tgtcgttcactgtcaatggaaacacccattgtgat58CP25(AT) ₂₀ +(AT) ₁₄ 33899189ggaagaaggctctgtgatttgaatccccgttgtgat58CP24(AG) ₁₇ 33890908tgtcgttcactgtcaaggaagaaggctctgttgat58CP35(AT) ₁₀ 3757757ggaagaagagcttgtaggtactgaadtccccgttg58CP36(AT) ₁₀ 3757757gg	CP12	(ATT) ₇	33898403	gggagggattgtagctcttt	ttggattttcccctacctaa	57
CP14(AC) ₉ (AT) ₈ 33898502teatgttcgtcgtagtetgggatgtgcaattggt56CP15(TG) ₈ (TA) ₉ (TA) ₆ +(AT)33898503teactcacgcgaagagttectgttcgttcaaagtet57CP16(AT) ₁₃ 33898503teactacttrecccgaatacactcctgtcgtatcaaggt58CP18(TA) ₁₂ 33898704tgtgggttgtggtcaatagcagctaaaacagte58CP20(AT) ₁ (TG) ₈ 33898704tgtgaggttgtggttggttgaaccaggtaaagaga59CP21(GT) ₁₂ 33898893gttgcgtgttactagtgttgaaccagtaaagagaagaga59CP23(GT) ₁₁ 33899033caacattaggageagagatcettccaaccata57CP24(GA) ₇ 33899034cggcccggattcaagt60CP25(TTC) ₈ 33899039cctcgatgtccaatagat12CP24(GA) ₁₇ 33899039cctcgatgtctcaactaggacccacttttgtt58CP24(GA) ₁₇ 33899039cctgcatggtctcaaca32CP26(CA) ₁₆ (TA) ₈ 33899070gaacgtcatcgtgtactttataactcccagaagt59CP27(AT) ₁₇ 33899070ggagaggtgcttgtactttataactcccacgatagt58CP28(CA) ₆ (TA) ₁₁ 33899070ggagaggtgcttgtacaggactatctgttgtact58CP29(AT) ₁₀ (TA) ₁₆ 3757757ggagagaggctgttgtgtgaattactcgttgtagt58CP30(AG) ₁₇ 3757758ggagagaggcttgttgtggaattactcgttggtt59CP31(AT) ₈ (AS) ₈ 37577568ttaccactgtataggacaagcattgttgttgtgttgttgtgt59CP36<	CP13	$(AT)_7 + (TA)_6 (TG)_8$	33898463	attgggaaccaaccattcg	tcaccaaccgcaggatataa	59
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	CP14	(AC) ₉ (AT) ₈	33898502	tcaatgttctcgtcgatagtc	tgggatagtgcaaattggt	56
CP16(AT)1233898598teaactattteccegetalcactectigtecaaggtt60CP18(TA)1233898700teggetttgegtttacattectigtigtttgg57CP19(AT)c(TA)1833898700teggetttgegttogcatatcaaactggtca58CP20(AT)c(TO)233898744teggagttgegtettaggtggacgetaaaactggcaag59CP21(GT)1233898842atcgaccgagaggtagteaaaacccataggetage59CP23(GT)113389903getgegtgtettacgtftigcaactgaaatcccaacaa57CP24(GAA)73389903cetgeatgattetfagatgetgaaatcccacatage60CP25(TTC)83389903cetgeatgatetcaactgetgaaatcccacatage60CP26(CA)10(TA)633899070aaagagegcatetgactteattccaccacgaagtgt60CP27(AT)733899070aagaagaggagettgattigtanttccccgacaat58CP28(CA)6(TA)1133899080tgtegttccactgtaggacattatcgttgaccgttgt58CP29(AT)737577349ggaagaagaggectgtatteaaactcccaccta58CP30(AG)1737577568tgccccaattagetatigtigtegctecattg59CP34(AAT)1137577690tgacagaagaacagtgctgagatigtagtgcatggtgg59CP35(TA)123757768tgcccaattag5856CP37(AAT)1137577690tttttggttatccatcgaggacagtagtagtaggaggggggggggggggggggggggg	CP15	$(TG)_5(TA)_7tg(TA)_6+(AT)_9$	33898543	atgcactcagcgaaaggat	tcctggtctgttcaaaagtct	57
CP18(TA)1233898620ccgtcagttittectittcatatctcgtgattetigg57CP19(AT)r(AT) ₈ 33898700tagggttgtgetjcataaagcaggtaanaactggta58CP20(AT)c(AT) ₈ 33898744tgtgaattgttgttgtggtggggctcgaanatcaanacta58CP21(GT)123389842atcaccaggaggtagteanaacccattgattgtgtgtg59CP22(TA)1633899013aacaattaggaagcagcadteanaacccattgattaggtag60CP23(GT)13389013aacaattaggaagcagcadteanaacccattgattaggtag60CP24(GA)A73389039cctcgarggatctcaacaaggacccatttitgtatt58CP25(TTC) ₈ 3389041gcocccgataaagtatgtatactcccacgatgte60CP27(AT)173389070aaaagacggcattgtgacttttataccaccgcaaa58CP28(CA) ₉ (TA)1,13389080gtgtatgtccatgcaaattgaattatccgattggacaa58CP29(AT)2,+(AT)1,43389080gggtgcatgttaacgaattteaaacctccaccctta58CP30(AG)1737577349ggaagaggcctgttaggattggattgcgatgg58CP31(AT)A ₃ ,+(TAA) ₉ 3757758ggacagacaacattgattggtgaagatgctcatgg59CP34(AT)1,13757768tgacagatgacaacattgtatgagattgctatggt59CP34(AT)1,137577685ttcccgetgcatagtacaagatgcggtagg59CP34(AT)1,13757768ttccagtgtctgatg58CP34(AT)1,23757768ttcccgetgcatagtgacagatgactgatgg59CP34	CP16	(AT) ₁₃	33898598	tcaactatttcccccgcata	cacctccttgtccaaaggtt	60
CP19 (AT) $_{7}(AT)_{8}$ 3389700 tagggttgtgtgtgtectata ageagetaaaactgatca 58 CP20 (AT) $_{5}(CO_{8}$ 33898744 tgtagattgtrtgttgttg gggctgaaaactaaaacat 58 CP21 (GT) $_{12}$ 33898842 atcgaccgaggaggtagt tgaaaccattgagtetge 60 CP22 (TA) $_{6}$ 3389908 egtegcutctaccitegt tgaaacctgaaaccaaaa 57 CP24 (GAA) 33899038 cagacattgagacgagt egtaaatccccaaaa 57 CP25 (TC)_8 33899039 cctogcatggatctcaaac aggaccccgattggt 60 CP26 (CA) $_{10}(TA)_{6}$ 3389903 cctogcatggatctaaaa aggacaccagtgggt 67 CP26 (CA) $_{10}(TA)_{6}$ 33899070 aaaagacggcattgtact tgaatatcccaccgaaca 58 CP27 (AT) $_{17}$ 33899080 tgtagatgtccatgtaaat ttatatcccaccgatagt 58 CP30 (AO) $_{17}$ 33899080 tgtagatgtccatgtagat ggatatatctgttgtagatgtcatgtg 58 CP31 (AT) $_{20}$ 757758 ggaagagaggtgttgttgagat 58 <td>CP18</td> <td>$(TA)_{12}$</td> <td>33898620</td> <td>ccgtcatgttttcgctttt</td> <td>caattctcgttgattcttgg</td> <td>57</td>	CP18	$(TA)_{12}$	33898620	ccgtcatgttttcgctttt	caattctcgttgattcttgg	57
CP20(AT) ₈ (TG) ₈ 33898744igtgagtigtigtigtigtigtigtigtigtigtigtigtigtig	CP19	$(AT)_7 t(AT)_8$	33898700	taggggttgtgcgtccata	agcaggctaaaaactggtca	58
CP21 (GT) ₁₂ 3389842 ategacgaggagtag teaaaaccattgagteg 60 CP22 (TA) ₁₆ 3389998 gitgcgtgctctacgtgt tgacaccatgagacgaga 59 CP23 (GT) ₁₁ 33899013 accatatgagacgagcta tccattccaaccacacaa 57 CP24 (GA) ₇ 3389038 cgagacatctaccacattg tegaaaatccccgatad 60 CP25 (TTC) ₈ 33899041 gccgccgcatataagta tggatactcccacgatggt 60 CP26 (CA) ₁₀ (TA) ₆ 33899070 aaagacggcattgtgad tttctatccaccgacaa 58 CP28 (CA) ₅ (TA) ₁₁ 3899070 gagagagaggtcgtgad ggacattcgtgad 58 CP29 (AT) ₂ +(AT) ₁₄ 3899109 gggtgcgtatgtaacgaat ttcaaaactcgttgacac 58 CP30 (AG) ₁₇ 37577349 ggaagaagaggccgtatgtg gatcgatgtgccatgtg 58 CP31 (AT) ₈ (AG) ₈ 37577548 tgacccacaattggta ttegagttggccatagtg 59 CP35 (TA) ₁₂ 3757758 ttegagattgccatgt 59 CP34	CP20	$(AT)_5(TG)_8$	33898744	tgtgagattgtctgttggttg	gggctcgaaaatcaaaacat	58
CP22(TA)1633898998gttegetgetictaegtgttgacactgataaggeagag59CP23(GT)1133899013aacaataggagaagaagctatecattccaaccacaaa57CP24(GAA)733899038cgagcatctcaccattgectgacaatccccgaaact60CP25(TTC)833899039cctcgcatggatctcaccattgectgacaatccccagaagte60CP25(TA)a633899041gccgcccgcattaaatatggtatactcccacgatggte60CP27(AT)1733899070aaagaggggctgtggattitctaccacgcgacaa58CP28(CA)g(TA)1133899080tgtcagttcacactgcaaatggacattactgttgacacc56CP29(AT)2+(AT)1433899109gggaggaggaggctgtatgggatctggatgtcgcatggt58CP30(AG)1737577349ggagagaggaggctgtatgggatctggatgccatggt58CP31(AT)a(GT)103757758ggaggagacgtctcaattateggtaggcattggg59CP32(AT)2+(TA)1037577588tgaccccggaattaggtateggataacaacaataggg59CP36(AT)1237577685ttccccccgtcattagaaaaggacgggggdctcaatg59CP37(AAT)837577610gcaaggaagaagagggggggggagggaggggggggggg	CP21	(GT) ₁₂	33898842	atcgaccgaggaaggtacg	tcaaaaacccattgagtctgc	60
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	CP22	(TA) ₁₆	33898998	gttcgcgtgctctacgtgt	tgacacctgataaaggcaaga	59
CP24(GAA)733899038cgagcactetcaccatigeetgenantcccegnanet60CP25(TTC)833899039cctcgcatggatctaaacaaggacccactttigtatte58CP26(CA)10(TA)633899039cctcgcatggatctaaagtatggtatactcccacggatgte60CP27(AT)1733899070aaagacggcatctggattitctatcccacggatgte60CP28(CA)6(TA)1133899080tgtcagttcacactgcaaatggacattactcgttgacace56CP29(AT)20+(AT)1433899109gggtggccatgttaacgaattitctaccacgcacagtgt58CP30(AG)1737577349ggaagaagagcctgtatgggatctggatgtcgcatggt58CP31(AT)6(GT)1037577372aagggtagtcatggagcattgtggtagctatggtg58CP33(ATTAA)5+(TAA)937577568tgacccacagtttggtt59CP36(AT)12+(TA)1037577610geaagaagcacctggtaatggtgtagctatggtg59CP34(AAT)1137577690ttcttacattgcgctaacaactcacagtggaggatggggaggaggggggggagggggggg	CP23	(GT) ₁₁	33899013	aacaataggaagcaagctca	tccattccaacccacaaa	57
CP25(TTC)833899039cctgcatggatctcaaeaaggacccactitigtatte58CP26(CA)10(TA)633899041gccgccgcataaagtatggtatactccacagtggte60CP27(AT)1733899070aaagacggcatctggattitclatcccacgatggte60CP28(CA)6(TA)1133899080tgtcagttcacatgcaatggacattactcgttgaccc56CP29(AT)20+(AT)1433899109gggtgcatgttatcgatgtaggatttcaaacctccacccta58CP30(AG)1737577349ggaagaagggcgttgtatgggatctgatgtcgatggt58CP31(AT)6(GT)1037577372aagggtacgtatgtatggagattcaaactctcta57CP32(AT)8(AG)837577444tgcctcacaattatgetctgcaaaagtattttggtgg58CP33(ATTAA)5+(TAA)537577568tgaccccagtttcgatteagacatgattttatcg59CP35(TA)1237577610gcaagaagcacctataggteagacatgatttatcgtt59CP36(AT)1437577690tttttacattgctcaacaagtcccgttttta57CP39(AG)1237577761ttttggtattccagcggacaggaggaggaggaggaggaggaggaggaggaggagga	CP24	(GAA) ₇	33899038	cgagcactctcaccattgc	ctgcaaaatccccgaaact	60
CP26(CA) ₁₀ (TA) ₆ 33899041geogecegataagtatggtatactecacagtggte60CP27(AT) ₁₇ 33899070aaagacggcatctgtgacttitctatcccaccgcacaa58CP28(CA) ₀ (TA) ₁₁ 33899080tgtcagttacactgcaatggacattactcgttgtgaccc56CP29(AT) ₂₀ +(AT) ₁₄ 33899109gggtgcgatgtacgaattitcaaacctcccacctta58CP30(AG) ₁₇ 37577349ggaagaaggcgtgtatgggattggatgtcgcatggt58CP31(AT) ₆ (GT) ₁₀ 37577572aaggtacgtcatggagcatctgtcgccttitatacttg57CP32(AT) ₈ (AG) ₈ 37577444tgcctcacatttatgctcgcaadgactgtgtgg59CP33(ATTAA) ₅ +(TAA) ₉ 37577568tgaccacaattggtattggtgtagcgtcatgtgg59CP35(TA) ₁₂ + (TA) ₁₀ 37577610gcaagaagcacattgtatatcagacaatgctgttactge59CP34(AT) ₁₂ + (TA) ₁₀ 37577600tttttcagttgcctgcattacagtcccgttttca57CP39(AG) ₁₂ 3757761tttttggtattccagctacggacaggcagggggtttac60CP40(AC) ₁₇ 51243481atacgcatctcctgacgacaggcagggggtttac60CP44(AT) ₁₂ 90857025catagcaggagactcacagaag56CP44(AT) ₁₄ 90857042gacaggagtactacccagacatggagtggagagagaggggg56CP44(AT) ₁₂ 9085705catgaccaggagacccacagtaagataggagattggaga56CP44(AT) ₁₆ 90857042gacagatgtcaccaggaatggagtgtgattgaga56CP47(TA) ₁₄ 90857060 <td>CP25</td> <td>(TTC)₈</td> <td>33899039</td> <td>cctcgcatggatctcaaac</td> <td>aaggaccccacttttgtattc</td> <td>58</td>	CP25	(TTC) ₈	33899039	cctcgcatggatctcaaac	aaggaccccacttttgtattc	58
CP27(AT)1733899070aaagaegeatctgtgacttitctatcccacegacaa58CP28(CA)3(TA)1133899080tgtcagttcacatgcaaatggacattactcgtttgacacc56CP29(AT)20+(AT)1433899109ggtgtcgcatgttaacgaatttcaaaacctcccacctta58CP30(AG)1737577349ggaagaagaggcctgtatgggatctggatgtcgcatggt58CP31(AT)6(GT)1037577372aagggtagtcatgtaggacatctgtcgcttttataccttg57CP32(AT)8(AG)837577444tgcctcacatttagctatggcaatagtcttttgggtga58CP33(ATTAA)3+(TAA)937577588ggacaagetccacattaggtaatggtagcgtccattgtg59CP35(TA)1237577610gcaagaagcacattggtaatcagacatgattgtacgtc55CP37(AAT)837577685ttccccgcctgcattagtcagacatgggggtttac59CP38(AAT)1137577690tttttacgttccgtcataacagaggggggttttac60CP44(AT)1690856112aacagctcctcctagcgacagaagtgagggggttgtgga56CP44(AT)1290857025catagcgaagtccaatgatg59CP44(AT)1290857042gacagaagtaccattggaccagaattgggaga56CP44(AT)1290857052cctgaagcaagctacgtggcataggaggtggagaga56CP44(AT)1290857052cctgaagcaaccattictatcgctggagctatagga56CP44(AT)1290857052cctgaagcaaccattictatcgctggagctatagga56CP44(AT)1690857052cctgaagcaaccattictatcgctggagctatagga56	CP26	$(CA)_{10}(TA)_{6}$	33899041	gccgcccgcaataaagta	tggtatactcccacgatggtc	60
CP28 $(CA)_9(TA)_{11}$ 33899080tgtcagttcacat(cgcaat)ggacttactcgtitgacacc56CP29 $(AT)_{20} + (AT)_{14}$ 33899109gggtgcatgttacaat)ttaaaacctoccacctta58CP30 $(AG)_{17}$ 37577349ggaagaagaggcctgtatgggatctgatgtcgcatgt58CP31 $(AT)_6(GT)_{10}$ 37577372aagggtacgtcatggacatctgtcgcctttatactctig57CP32 $(AT)_8(AG)_8$ 37577544tgcctccaattattgctccgcaattadccttigggta58CP33 $(ATTAA)_3 + (TAA)_9$ 37577568tgaccacattattgctccgcaatcaaaccaataggg59CP36 $(AT)_{12} + (TA)_{10}$ 37577610gcaagaagacacttggtaatcagcaatgatgtttatcgt59CP37 $(AAT)_8$ 37577685ttccccgctgcattagtcagcacatgatgttgttacgg59CP38 $(AAT)_{11}$ 37577600ttcttacattgcctgcataacagtccccgttttca57CP39 $(AG)_{12}$ 37577761ttttggttatccagtagggacaggagggaggtagg56CP40 $(AC)_{17}$ 51243481atacgccattcctcatgggacaggagggagtatgagg56CP44 $(AT)_{16}$ 90856717tgacaacgaactacatgcagacctacagagtgtggag56CP44 $(AT)_{12}$ 90857042gacagagcagcatcatgcagccagaatgtaggagcattagg56CP44 $(AT)_{16}$ 90857042gacagaagcaccattctagcaatcaatttggtctc59CP49 $(AT)_{16}$ 90857042gacagaagcaccattctatcgctgaagttgaggag56CP44 $(AT)_{16}$ 90857042gacagaagcaccattctacctagaagtaggag56 </td <td>CP27</td> <td>(AT)₁₇</td> <td>33899070</td> <td>aaaagacggcatctgtgact</td> <td>tttetateccacegeacaa</td> <td>58</td>	CP27	(AT) ₁₇	33899070	aaaagacggcatctgtgact	tttetateccacegeacaa	58
CP29 $(AT)_{20}+(AT)_{14}$ 33899109gggtegcatgttaacgaatttcaaaacctccacctta58CP30 $(AG)_{17}$ 37577349ggaagaagaggcctgtatgggattggatgtegcatggt58CP31 $(AT)_6(GT)_{10}$ 37577372aagggtacgtcatggagcatctgtegccttttatactctig57CP32 $(AT)_8(AG)_8$ 37577444tgcctacaatttatgctccgcaaatgctttttgggtga58CP33 $(ATTAA)_5+(TAA)_9$ 37577568tgacccagtttcgatttggtgtagcgtcatagagg59CP35 $(TA)_{12}$ 37577610gcaagaagcacttggtaatcagacaatgattgttactgc55CP37 $(AAT)_8$ 37577690ttcttacagttgcgtcattagtcagacatggcctatggt59CP38 $(AAT)_{11}$ 37577690ttcttacagttgcgtcatg59CP40 $(AC)_{17}$ 51243481atacgccatccctgagcgaaggaggaggaggggtttagg56CP44 $(AT)_{16}$ 9085717tgacaacgaactactcctaccagaatggcgaggttggg56CP47 $(TA)_{14}$ 90857042gacagagcaccatgagccagaatggccaatttag59CP49 $(AT)_{12}$ 90857060ccatttctgcacgacgatagggccaattcag59CP49 $(AT)_{12}$ 90857042ggacgaagcaaccatttattcgtggagctatagga56CP40 $(AT)_{16}$ 90857042gacagagcaaccattctatcgtggagctatagga56CP44 $(AT)_{16}$ 90857042gacagagcaccattctatcgtggagctatagga56CP49 $(AT)_{16}$ 90857042gacagagcaccattctatcgtggtggcaattcag59CP49 $(AT)_{16}$ 90857060	CP28	(CA) ₉ (TA) ₁₁	33899080	tgtcagttcacactgcaaat	ggacattactcgtttgacacc	56
CP30(AG)1737577349ggaagaagagcctlatgggattggatgtcgcatggt58CP31(AT)6(GT)1037577372aaggtacgtcatggagcatctgtcgcttttatactctg57CP32(AT)6(GT)1037577372aaggtacgtcatggagcatctgtcgccttttatactctg57CP32(AT)6(GT)1037577568tgcccccadtttatgctccgcaatgcttttgggtga58CP33(ATTAA)5+(TAA)937577568tgcccccadtttagctttggtgagctcatatggg59CP36(AT)12+(TA)1037577610gcaagaagcacctggtaatcagacatgacttgttactgc55CP37(AAT)837577685ttccccgctgcattatgtcagacatgacttgtta59CP38(AAT)1137577690ttttaccgttgctgataacaagtcccgttttag59CP30(AG)1237577761ttttggttattccagctaccgttgcagtggggtaggggtagg59CP40(AC)1751243481atacgccattccctgagcgacaggaggttaggt56CP44(AT)1290856112aacagtcttctctctacttgtttgtggggtgatggag56CP44(AT)1290857025catgacggagagttgcaggt56CP47(TA)1490857042ggacgaagttgcacatgcagccagaatgccaatttgg59CP49(AT)1290857082cctgaaggcaccaatgaaggcaatgaagtagtaggag56CP51(AT)1690857104tgaagaagaaccattgtaggcaatgaatgtatttttaccc59CP52(AT)1690857055cagaccaatgaagaggttcatgtggtcattctttaccc59CP54(AT)1690857042ggaagaagtaccaagtag5656CP	CP29	$(AT)_{20} + (AT)_{14}$	33899109	gggtcgcatgttaacgaat	ttcaaaacctcccaccctta	58
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	CP30	(AG) ₁₇	37577349	ggaagaagaggcctgtatgg	gatctggatgtcgcatggt	58
CP32 $(AT)_8(AG)_8$ 37577444tgcctcacatttatgctccgcaatgctttttggtga58CP33 $(ATTAA)_5+(TAA)_9$ 37577568tgaccccagttttcgatttggtgagctcattgg59CP35 $(TA)_{12}$ 37577598ggacgaagctccacaatcaggcaatcaaaccaaatgagg59CP36 $(AT)_{12}+(TA)_{10}$ 37577610gcaagaaagcaacttggtaatcagacaatgacttgttactgc55CP37 $(AAT)_8$ 37577685ttccccgcctgcattatagtcagacatgactgtttactgc57CP38 $(AAT)_{11}$ 37577690ttctttacagttgcctgcataacaagtccccgttttca57CP39 $(AG)_{12}$ 37577761ttttggttattccagctaccgttgcagtggaggtaaggt59CP40 $(AC)_{17}$ 51243481atacgccatctcctgagcgacagagcagggggtttac60CP42 $(AT)_{16}$ 90856112aacagctctcctctaccttgtttgtaggggatgatgagg56CP44 $(AT)_{12}$ 90857025catgaccggaagctagtgcctcacagaatgtgga56CP47 $(TA)_{14}$ 90857042gaccagtcagcaccattctcgatgatgccaatttag59CP49 $(AT)_{12}$ 90857082cctgaagcaccattctcgatgatggccaattcag59CP49 $(AT)_{12}$ 90857104tgaaaaggacccacagtaagcaatgaatgattgttacctc59CP51 $(AT)_{10}(AG)_{12}$ 90857365cagacaccatgaagttggfgggcctttctcttga55CP53 $(AAC)_8$ 90857365cagacaccatgaagttggfgggtgcttctccttga56CP54 $(AT)_{10}+(ATA)_7$ 90857406ttaagactatagggccaagcttagtcaaggatgaacca56	CP31	$(AT)_{6}(GT)_{10}$	37577372	aagggtacgtcatggagca	tctgtcgccttttatactcttg	57
CP33(ATTAA) ₅ +(TAA) ₉ 37577568tgaccccagttttegatttggtgagegtecating59CP35(TA) ₁₂ 37577598ggacgaagetecacaateaggeaateaaaccaaatgagg59CP36(AT) ₁₂ +(TA) ₁₀ 37577610gcaagaaageaacttggtaateagacaatgacttgttaetec55CP37(AAT) ₈ 37577685ttecccgcetgeattatagteagacatgetgttgteateggt59CP38(AAT) ₁₁ 37577690ttettacagttgcetgeataacaagteccetgttttea57CP39(AG) ₁₂ 37577761ttttggttattccagetaccgttgcagtggaggtaggt59CP40(AC) ₁₇ 51243481atacgccattectetgagegacaggcagggggtttac60CP42(AT) ₁₆ 90856412aacageteteteteacetetcaagattgtgtgaag56CP44(AT) ₁₂ 90857025catgacggaagetagtagcettcaccagaagtgtgga56CP44(AT) ₁₂ 90857042gacaggaagetagtatgcettcaccagaagtgtgga56CP44(AT) ₁₂ 90857042gagcaagtagetacagt60CP48(TC) ₁₃ 90857060ccatttegtcacgaaccagaatgccaattteg59CP51(AT) ₁₆ 90857104tgaaaaggaccacagtaagcaatgaagtagtagaga56CP51(AT) ₁₀ (AG) ₁₂ 90857365cagacaccatgaagattggtggtgcttetteta55CP53(AAC) ₈ 90857365cagacaccatgaagattg56CP54(AT) ₁₀ +(ATA) ₇ 90857406ttaagactataggaccacgttagtcaaggatgaaacca56CP55(AT) ₁₂ 90857407teaaccactetgtetcacaacccettggetatttg	CP32	$(AT)_8(AG)_8$	37577444	tgcctcacaatttatgctctc	gcaaatgctttttgggtga	58
CP35 $(TA)_{12}$ 37577598ggacgaagctccacaatcaggcaatcaaaccaatgagg59CP36 $(AT)_{12}+(TA)_{10}$ 37577610gcaagaagcacattggtaatcagacatgacttgtactgc55CP37 $(AAT)_8$ 37577685ttccccgcctgcattatgtcagagtttggcctatgttt59CP38 $(AAT)_{11}$ 37577690ttctttacagttgcctgcataacaagtcccgttttca57CP39 $(AG)_{12}$ 3757761ttttggtattccagctaccgttgcagtggaggtaggt59CP40 $(AC)_{17}$ 51243481atacgcatctcctgagcgacagaaggaggtgtttac60CP42 $(AT)_{16}$ 90856412aacagtcctcctaccttgttgtaggggagatggagg56CP44 $(AT)_{12}$ 90856717tgacaacgaactcactccctacctcaccagaagtgtggag56CP44 $(AT)_{12}$ 90857025catgacggagctaggagcctaccagaagtgtggag56CP47 $(TA)_{14}$ 90857042gagcagatgtcacagcaccgagcagatgtgcaaggag56CP49 $(AT)_{12}$ 90857082cctgaagcaccacattctacctggaggctgtaggag56CP51 $(AT)_{16}$ 90857104tgaaaaggaccacagtaggcaagaaggaggtgttccca59CP52 $(AT)_{10}(AG)_{12}$ 90857365cagaacgaaggaccacagtggttctttgtctctca55CP53 $(AAC)_8$ 90857365cagaaccatgaaggttgg5655CP54 $(AT)_{10}+(ATA)_7$ 90857406ttaagactataggacatggg5655CP54 $(AT)_{12}$ 90857406ttaagactataggactatggaggt5656CP55 $(AT)_{12}$ 90857407 <t< td=""><td>CP33</td><td>$(ATTAA)_5 + (TAA)_9$</td><td>37577568</td><td>tgacccccagttttcgatt</td><td>tggtgtagcgtccattgtg</td><td>59</td></t<>	CP33	$(ATTAA)_5 + (TAA)_9$	37577568	tgacccccagttttcgatt	tggtgtagcgtccattgtg	59
CP36 $(AT)_{12}+(TA)_{10}$ 37577610gcaagaagcaacttggtaatcagacaatgacttgtactgc55CP37 $(AAT)_8$ 37577685ttccccgcctgcattaagtcagacaatgacttgtactgc59CP38 $(AAT)_{11}$ 37577690ttctttacagtgcctgcataacaagtccccgttttca57CP39 $(AG)_{12}$ 3757761ttttggttattccagctaccgttgcagtggaggctaaggt59CP40 $(AC)_{17}$ 51243481atacgccatccctgagcgacaggcagggggtttac60CP42 $(AT)_{16}$ 90856412aacagctctcctcatcctgtttgtaggggtgatgaag56CP44 $(AT)_{12}$ 90857077tgacaacgaactacatccctacctcatggttgtgtactcct56CP44 $(AT)_{12}$ 90857025catgacggaggtgttgacettaccagaagtgggg56CP47 $(TA)_{14}$ 90857060ccattctgtaccgaaccagaatgcaattagg59CP49 $(AT)_{12}$ 90857082cctgaaagcaactattatcgtggggtgtagagga56CP51 $(AT)_{16}$ 9085714tgaaaggaccacagtagcaagaagcaattgtgg59CP52 $(AT)_{10}(AG)_{12}$ 9085725cctgaaagcaaccatttctatcgtgggctgtaagga56CP51 $(AT)_{10}(AG)_{12}$ 90857104tgaaaggaccacagtaagcaatggaattgtaggg56CP53 $(AAC)_8$ 90857365cagacaccatgaagattgggtggtggtcctctccttga55CP54 $(AT)_{10}+(ATA)_7$ 90857406ttaagactataggccaagcttaggtcaggatgaaacca56CP55 $(AT)_{10}+(ATA)_7$ 90857407tcaaccacttgtctccacaacccttgggctattg56 </td <td>CP35</td> <td>(TA)₁₂</td> <td>37577598</td> <td>ggacgaagetecacaatea</td> <td>ggcaatcaaaccaaatgagg</td> <td>59</td>	CP35	(TA) ₁₂	37577598	ggacgaagetecacaatea	ggcaatcaaaccaaatgagg	59
CP37 $(AAT)_8$ 37577685ttccccgcctgcattatagtcagagtttggctcatggtt59CP38 $(AAT)_{11}$ 37577690ttctttacagttgcctgcataacaagtcccgttttca57CP39 $(AG)_{12}$ 3757761tttttggtattccagctaccgttgcagtggaggctaaggt59CP40 $(AC)_{17}$ 51243481atacgccattccctgagcgacaggcagggggttttac60CP42 $(AT)_{16}$ 90856412aacagctctctctatccttgtttgtagggatgattgaag56CP44 $(AT)_{12}$ 90856717tgacaacgaactacatccctacctcatggtttgtgtaccct56CP46 $(AT)_{5}(AG)_{8}$ 90857025catgacggaggtgtgagcctacagaagtgtgga56CP47 $(TA)_{14}$ 90857042gagcagattgtcacatgcagaccagaatgccaattcag59CP49 $(AT)_{12}$ 90857082cctgaaagcaaccatttctatcgctggagctgtaagaa56CP51 $(AT)_{16}$ 90857104tgaaaaggaccacagtaagcaatggaatgttgtctctca59CP52 $(AT)_{10}(AG)_{12}$ 90857365cagacaccatgaagttggtgtgttctctca55CP53 $(AAC)_8$ 90857365cagacaccatgaagttgggfggggctattgggagagag56CP54 $(AT)_{10}^+(ATA)_7$ 90857407ttaagccaactatggggggtftagtcaaggaggagagagggggggggggggggggggggg	CP36	$(AT)_{12} + (TA)_{10}$	37577610	gcaagaaagcaacttggtaa	tcagacaatgacttgttactgc	55
CP38(AAT)_{11}37577690ttettacagttgeetgeataacaagteecegttttea57CP39(AG)_{12}37577761ttttggttatteegetaecgttgeegtggaggetaaggt59CP40(AC)_{17}51243481atacgeeateteetgagegaeaggeagggggtttae60CP42(AT)_{16}90856412aacageteteteteactgtttgtaggggatgattgaag56CP44(AT)_{12}9085717tgaeaaggaactaeateectaceteatggtttgtgtateeet56CP46(AT)_5(AG)_890857025catgaeeggaagetagtagcetaaaggatggggag56CP47(TA)_{14}90857042gagcagattgteacatgeagaceagaatgeegaattaeg59CP49(AT)_{12}90857082cettgaaagcaaceattetategetggagetgaaggag56CP51(AT)_{16}90857104tgaaaaggaeecaaegtaageaatggagetgaaggag56CP52(AT)_{10}(AG)_{12}90857241ggaaagateatagaacagtggtgetatettggttgteeteca55CP53(AAC)_890857365cagaacacattaggaggatttgg5656CP54(AT)_{10}+(ATA)_790857406ttaagactatatgggecaage56CP55(AT)_{12}90857407teaacecattegteecattagtcaaggatgaagaacca56CP55(AT)_{12}90857407teaacecattegteecattagtcaaggatgaaacca56CP55(AT)_{12}90857407teaacecattegteecattagtcaaggatgaaacca56CP55(AT)_{12}90857407teaacecattegteecattagtcaaggatgaacca56	CP37	(AAT) ₈	37577685	ttccccgcctgcattatag	tcagagtttggctcatggttt	59
CP39 $(AG)_{12}$ 3757761 ttttggttattccagctaccgttgcagtgaggctaggt 59 CP40 $(AC)_{17}$ 51243481 atacgccatccctgagcgacaggcaggggttttac 60 CP42 $(AT)_{16}$ 90856412 aacagctctctctactcttgtttgtaggggatgattgaag 56 CP44 $(AT)_{12}$ 90856717 tgacaacgaactacatccctacctcatggtttgtgtactcct 56 CP46 $(AT)_5(AG)_8$ 90857025 catgaccggaagctagtatgccttcaccagaagtgtgga 56 CP47 $(TA)_{14}$ 90857042 gagcagattgtcacatgcagaccagaatgccaatttag 59 CP48 $(TC)_{13}$ 90857060 ccatttctgtcacgcatccgatgaggccaaattcag 59 CP49 $(AT)_{12}$ 90857082 cctgaaagcaaccatttctatcgctggagctgtaagaga 56 CP51 $(AT)_{16}$ 90857104 tgaaaaggacccacacgtaagcaatcgaatctttatccc 59 CP52 $(AT)_{10}(AG)_{12}$ 90857365 cagacaccatgaagattggtgctatcttggttgtctctca 55 CP53 $(AAC)_8$ 90857365 cagacaccatgaagatttgggtggtccttctctttga 56 CP54 $(AT)_{10}+(ATA)_7$ 90857407 taagactataggaccaagcttaggcaagatgaaacca 56 CP55 $(AT)_{12}$ 90857407 tcaacccattcgtccacagaccatgagatgaaacca 56	CP38	(AAT) ₁₁	37577690	ttetttacagttgcctgcat	aacaagtccccgtttttca	57
CP40(AC)1751243481atacgccatccctgagegacaggcagggggtttac60CP42(AT)1690856412aacagctctctctactcttgtttgtagggatgttgaag56CP44(AT)1290856717tgacaacgaactacatccctacctcatggtttgtgtactcct56CP46(AT)5(AG)890857025catgaccggaagtagtagacctaagaatgtgga56CP47(TA)1490857042gagcagattgtcacatgcagaccagaatgccaattttgct60CP48(TC)1390857060ccatttctgtcacgcatccgatgatgggccaaattcag59CP49(AT)1290857082cctgaaaggaagcacactttctatcgctggagctgtaaggag56CP51(AT)1690857104tgaaaaggacccacacgtaagcaatcgaatctttcttaccc59CP52(AT)10(AG)1290857241ggaaagatcatagaacagtggtgctacttggttgtctctca55CP53(AAC)890857365cagacaccatgaagatttgggtggtgctcttcctttga56CP54(AT)10+(ATA)790857407tcaacccacttcgtccacagacaccttgggatgaaga56CP55(AT)1290857407tcaacccacttgctccacagacccttgggctattg60	CP39	$(AG)_{12}$	37577761	ttttggttattccagctaccg	ttgcagtggaggctaaggt	59
CP42 $(AT)_{16}$ 90856412aacageteteteteteteteteteCP44 $(AT)_{12}$ 90856717tgacaacgaactacatecetaeeteteteteteCP44 $(AT)_{12}$ 90856717tgacaacgaactacatecetaeetetatgtttgtgtacteet56CP46 $(AT)_5(AG)_8$ 90857025catgaceggaagetagtagcettacacgaagtgtgga56CP47 $(TA)_{14}$ 90857042gagcagattgtacaatgcagaceagaatgccaattttget60CP48 $(TC)_{13}$ 90857060ceatttetgtcacgcategatgatgggccaaattcag59CP49 $(AT)_{12}$ 90857082eetgaaaggaaccaattetategetggagetgtaagaga56CP51 $(AT)_{16}$ 90857104tgaaaggacccacaegtaagcaategaatetttettacee59CP52 $(AT)_{10}(AG)_{12}$ 90857241ggaaagatcatagaaacagtggtgetatettggttgtetete55CP53 $(AAC)_8$ 90857365cagacaccatgaagatttgggtgggteettetetttgaa56CP54 $(AT)_{10}+(ATA)_7$ 90857407teaaccacttegteteeacaaccecttgggctattg56CP55 $(AT)_{12}$ 90857407teaaccacttegteteeacaaccecttgggctatttg60	CP40	(AC) ₁₇	51243481	atacgccatctccctgagc	gacaggcagggggttttac	60
CP44 $(AT)_{12}$ 90856717tgacaacgaactacatecetacetterererererererererererererererererer	CP42	(AT) ₁₆	90856412	aacageteteteteateettg	tttgtaggggatgattgaag	56
CP46 $(AT)_5(AG)_8$ 90857025catgaccggaagctagtagccttcaccagaagtgtgga56CP47 $(TA)_{14}$ 90857042gagcagattgtcacatgcagaccagaatgccaattttgct60CP48 $(TC)_{13}$ 90857060ccatttctgtcacgcatccgatgatgggccaaattcag59CP49 $(AT)_{12}$ 90857082cctgaaagcaaccatttctatcgctggagctgtaagaga56CP51 $(AT)_{16}$ 90857104tgaaaaggacccacacgtaagcaatcgaatctttctttaccc59CP52 $(AT)_{10}(AG)_{12}$ 90857241ggaaagatcatagaaacagtggtgctatcttggttgtctctca55CP53 $(AAC)_8$ 90857365cagacaccatgaagatttgggtgggtccttctcctttga56CP54 $(AT)_{10}^+(ATA)_7$ 90857406ttaagactatagggccaagcttaggtcaaggatgaaacca56CP55 $(AT)_{12}$ 90857407tcaacccattgctccacaaccccttgggctattg60	CP44	$(AT)_{12}$	90856717	tgacaacgaactacatcccta	cctcatggtttgtgtactcct	56
CP47(TA) ₁₄ 90857042gagcagattgtcacatgcagaccagaatgccaattttgct60CP48(TC) ₁₃ 90857060ccatttctgtcacgcatccgatgatgggccaatttag59CP49(AT) ₁₂ 90857082cctgaaagcaaccatttctatcgctggagctgtaagaga56CP51(AT) ₁₆ 90857104tgaaaaggaccacacgtaagcaatcgaatctttcttaccc59CP52(AT) ₁₀ (AG) ₁₂ 90857241ggaaagatcatagaaacagtggtgctatcttggttgtctctca55CP53(AAC) ₈ 90857365cagaccactagaagtttgggtgggtccttctcctttga56CP54(AT) ₁₀ +(ATA) ₇ 90857406ttaagactatagggccaagcttaggtcaaggatgaaacca56CP55(AT) ₁₂ 90857407tcaacccacttgctccacaaccccttgggctattg60	CP46	$(AT)_5(AG)_8$	90857025	catgaccggaagctagtatg	ccttcaccagaagtgtgga	56
CP48 $(TC)_{13}$ 90857060ccattlegtcacgcatccgatgatgggccaatteag59CP49 $(AT)_{12}$ 90857082cctgaaagcaccattletategetggagetgtaagaga56CP51 $(AT)_{16}$ 90857104tgaaaaggaccacaegtaagcaategaatetttetttaece59CP52 $(AT)_{10}(AG)_{12}$ 90857241ggaaagateatagaaacagtggtgetatettggttgtetetca55CP53 $(AAC)_8$ 90857365cagacaecatgaagatttgggtgggteetteetttga56CP54 $(AT)_{10}+(ATA)_7$ 90857406ttaagaetatagggceaagettaggteaaggatgaaacca56CP55 $(AT)_{12}$ 90857407teaaeceaettegteecacaaeceetteggetatttg60	CP47	$(TA)_{14}$	90857042	gagcagattgtcacatgcaga	ccagaatgccaatttttgct	60
CP49 $(AT)_{12}$ 90857082cctgaaagcaaccatttctatcgctggagctgaaagaa56CP51 $(AT)_{16}$ 90857104tgaaaaggaccacacgtaagcaatcgaatctttctttaccc59CP52 $(AT)_{10}(AG)_{12}$ 90857241ggaaagatcatagaaacagtggtgctatcttggttgtctctca55CP53 $(AAC)_8$ 90857365cagacaccatgaagatttgggtgggtccttctcctttga56CP54 $(AT)_{10}+(ATA)_7$ 90857406ttaagactatagggccaagcttaggtcaaggatgaaacca56CP55 $(AT)_{12}$ 90857407tcaaccacttcgtctccacaaccccttgggctatttg60	CP48	$(TC)_{13}$	90857060	ccatttctgtcacgcatcc	gatgatgggccaaattcag	59
CP51 $(AT)_{16}$ 90857104tgaaaaggaccacacgtaagcaatcgaatctttctttaccc59CP52 $(AT)_{10}(AG)_{12}$ 90857241ggaaagatcatagaaacagtggtgctatcttggttgtctctca55CP53 $(AAC)_8$ 90857365cagacaccatgaagatttgggtgggtccttctcctttga56CP54 $(AT)_{10}+(ATA)_7$ 90857406ttaagactatatgggccaagcttaggtcaaggatgaaacca56CP55 $(AT)_{12}$ 90857407tcaaccacttcgtctccacaaccccttgggctatttg60	CP49	$(AT)_{12}$	90857082	cctgaaagcaaccatttcta	tcgctggagctgtaagaga	56
CP52 $(AT)_{10}(AG)_{12}$ 90857241ggaaagatcatagaaacagtggtgctatcttggttgtctctca55CP53 $(AAC)_8$ 90857365cagacaccatgaagatttgggtggtccttctcctttga56CP54 $(AT)_{10}+(ATA)_7$ 90857406ttaagactatatgggccaagcttaggtcaaggatgaaacca56CP55 $(AT)_{12}$ 90857407tcaaccacttcgtctccacaaccccttgggctatttg60	CP51	$(AT)_{16}$	90857104	tgaaaaggacccacacgtaa	gcaatcgaatctttctttaccc	59
CP53 $(AAC)_8$ 90857365cagacaccatgaagattigggtagtcettecettiga56CP54 $(AT)_{10}+(ATA)_7$ 90857406ttaagactatatgggceaagettaggteaaggatgaaacca56CP55 $(AT)_{12}$ 90857407teaaccacttegeteceacaacceettgggetattig60	CP52	$(AT)_{10}(AG)_{12}$	90857241	ggaaagatcatagaaacagtgg	tgctatcttggttgtctctca	55
CP54 $(AT)_{10}+(ATA)_7$ 90857406ttaagactatatgggccaagcttaggtcaaggatgaaacca56CP55 $(AT)_{12}$ 90857407tcaaccacttcgtctccacaaccacttgggctatttg60	CP53	$(AAC)_8$	90857365	cagacaccatgaagatttgg	gtgggtccttctcctttga	56
$CP55 (AT)_{12} 90857407 teaaccacttegteteea caaccettgggetatttg 60$	CP54	$(AT)_{10} + (ATA)_7$	90857406	ttaagactatatgggccaagc	ttaggtcaaggatgaaacca	56
· ··· ·	CP55	(AT) ₁₂	90857407	tcaacccacttcgtctcca	caaccccttgggctatttg	60

Table 2 (continued)

Marker	Repeat motif	Genbank accession (gi)	Pimer Sequence (5'-3')	<i>Ta</i> (°C)	
			Forward	Reverse	
CP56	(ATG) ₆	90857410	gaacggcctagcgaaagac	cctgcgctaacacttgatga	59
CP57	$(CA)_6(TA)_7$	90857692	ttgagtcttggtttcaactcc	ttcccactatcttctgtttgg	56
CP58	(TTG)7	90857748	ttccacgagacaaactgtacg	ctcccaccgctgtacttga	58
CP59	$(TA)_7 + (AT)_{12}$	90857930	gttgtttgcatcccactgc	ctcgccattccatctggt	60
CP61	$(AT)_{12} + (AC)_8$	90858177	ggagtgatgtaagtgcctcat	catgagccctactccgaac	57
CP62	$(AT)_8 + (AAG)_{10}$	90858365	accttggcccaccactatt	ttgattctgcttgtgggaga	59
CP63	$(AT)_{14}$	90858411	aaccagcaggetttcataa	ggcttcaagagaggcgtat	56
CP64	(TC) ₁₇	90858444	gggaggacaaagctccaaa	gcatgatccaagggaggag	60
CP66	(TG) ₉ cgc(GA) ₁₂	90858501	agteccatcaggetteteg	cctttttgtgcgcatatggt	60
CP67	(TTC) ₁₀	90858512	tggcaaaagatggtggtgt	cttgctttgcacttttcagg	59
CP68	(AG) ₁₅	90858681	agttcctgcatgtgctgct	cccccattctgaaaatctcc	60
CP69	(TA) ₁₂	90858696	gctgcatcgacatttacgaa	catgtgattccaacaaggcta	59
CP70	(TTC) ₉	90858729	cggagcttgacgtgactga	gcctgaggagccattaagc	60
CP71	(CT) ₁₄	90858774	tccccaacctcaagaagataa	ttacaccaccatcgccatc	59
CP72	$(ATAC)_7(AT)_6$	90858906	cccaaatcacctttttctctc	aacgtgaactgagggtgga	58
CP73	$(AT)_5 gt(AT)_9$	90858959	gaaaccgctcatttgcatt	ttgattactcccctccatctc	58
CP74	$(TTA)_8$	90859162	caatgaggggttgataattgg	gctcccccaatgagaatttta	59
CP75	$(GA)_{13} + (AG)_7$	90859250	gcgtattgagagggtgcaa	tttggtagccgtcattcgt	59
CP78	$(TTA)_7 + (TTTA)_5$	90859390	ggagttggttgtgtatgttacc	attttcaccccctttttcc	57
CP79	(TA) ₁₇	90859529	gcctcagcatccaaacaaa	caaccatgtgaatggagaacc	60
CP80	(TC) ₁₂	90859560	gtggcaatgaactcggcta	atectecetecaceaacag	59
CP81	(CT) ₁₅	90859561	gaaaaaccggcaaaatcac	ttcaccagatttcaccacttg	58
CP82	$(AAT)_7$	90859590	ccctccccaggaagataca	gaagagtgtttggcggatg	59
CP83	$(TA)_5(CT)_9$	90859697	ttcgcaccttgaatccact	ggcagcctttagggttctc	59
CP86	$(AT)_{12}$	90859959	tttcacacggattctacacga	tgggggatatgagaagtttcc	59
CP89	$(AT)_{12} + (AT)_{11}$	90860069	agatgattcaccgctccag	ctcaagcaagaaatatgcctct	58
CP90	$(T)_{21}(CTT)_5$	90860101	cttgggctttcgacctga	agcaatctaatggaggcaaag	59
CP92	$(ATT)_{11}$	90860241	ctttttggtcctggccttc	gaacataataccgtgcgttca	59
CP94	(AG) ₁₂ at(AG) ₇	90860608	tcgcagagaaagagaaagca	tcctctcaccagttattggaa	58
CP95	(AG) ₆ tg(AG) ₇	90860628	cagtccttgcaggcgatta	ctcaaccatttttcctcacca	60
CP97	(TA) ₁₁	90860894	tttctgttacctctctcggatt	gagatgacaaccatacagcaa	56
CP98	(TC) ₁₃	90860935	gattggcgggaaaaactc	agagagagtgtaggaccatgaa	56
СР99	(AG) ₂₄	90861034	cgaggacaaaacggcacta	tgcacgcactctcaaatctc	60
CP100	$(GA)_{11} + (AG)_{22}$	90861159	tgatcgctttcgcttcact	gatttcactgccacggact	58

Repeat motif is derived from a sequenced clone deposited on Genbank *Ta* annealing temperature

banding patterns, and successfully amplified PCR product of high quality, besides the polymorphic amplification product in the expected size. Characteristics of the 81 primer pairs and optimal conditions for their amplification are given in Table 2. Subsequently, all primer pairs that amplified a specific band were used for the genotyping of 30 germplasm accessions and 18 landraces. In total, 59 primer pairs amplified a polymorphic and easily scorable PCR product, while 22 pairs amplified a monomorphic one. Considering the 59 SSR loci analyzed in the present study and a total of 48 genotyped individuals obtained from partial outcrossing and selfing germplasm, the SSR markers detected a total of 237 alleles. The least and the most variable loci displayed 2 (CP06, CP09, CP11, CP20, CP22, CP23, CP24, CP27, CP28, CP29, CP33, CP36 and CP38) and 11 (CP16) alleles, respectively (Table 3). The average allele number per locus was 4.02 (monomorphic loci excluded). Table 3 summarizes the locus specific descriptive statistics for the 59 SSR markers.

Table 3 Allelic composition, allele size variation (bp), expected and observed heterozygosity, and polymorphic information content (PIC) of the 59 polymorphic SSR loci in 48 individuals of *Carica papaya*

Marker	Allele			Unique	alleles	Size variation (bp)	H_E	H_O	PIC	
	Number	Rare	Common	Most frequent	Germ	Land				
CP02	3	0	1	2			144–158	0.62	0.33	0.54
CP03	4	0	2	2	1		238-276	0.66	0.32	0.61
CP05	4	0	1	3			290-320	0.69	0.04	0.63
CP06	2	0	0	2			240-320	0.49	0.04	0.37
CP07	4	0	1	3	1		175-208	0.71	0.50	0.66
CP09	2	0	1	1	1		395-411	0.30	0.00	0.26
CP10	6	0	4	2	3		180-202	0.73	0.28	0.69
CP11	2	0	0	2			568-610	0.41	0.25	0.33
CP14	5	0	3	2	3		217-232	0.52	0.00	0.46
CP16	11	1	9	1	1	2	202-250	0.82	0.67	0.81
CP18	4	0	2	2	1		238-260	0.54	0.23	0.47
CP19	3	0	1	2			156-166	0.51	0.21	0.43
CP20	2	0	0	2			248-252	0.35	0.25	0.29
CP21	4	0	2	2			142-158	0.64	0.46	0.57
CP22	2	0	- 1	- 1	1		246-254	0.08	0.00	0.08
CP23	2	0	0	2			218-236	0.44	0.21	0.35
CP24	2	0	0	2			236-246	0.35	0.25	0.29
CP26	3	0	1	2	1		142-152	0.33	0.04	0.41
CP27	2	0	0	2			347-353	0.38	0.00	0.31
CP28	2	0	0	2			186-190	0.38	0.08	0.36
CP29	2	0	0	2			550-556	0.40	0.00	0.30
CP30	5	1	2	2	2		222 200	0.41	0.15	0.55
CP21	1	1	2	2	2		160 174	0.58	0.58	0.55
CP22	+	0	2	2			100-174	0.75	0.85	0.08
CP35	5	0	1	1			401-411	0.29	0.25	0.23
CP35	3 2	0	3	2			102-100	0.75	0.23	0.09
CF 30	2	0	0	2			452-458	0.30	0.27	0.37
CP30	2 4	0	0	2		1	496-310	0.59	0.33	0.51
CP40 CP42	4	0	3	1	1	1	134-170	0.55	0.23	0.51
CP42	5	0	3	2	1		260-280	0.00	0.22	0.00
CP44 CP47	4	0	1	3			228-236	0.75	0.30	0.70
CP4/	4	0	2	2			248-262	0.68	0.00	0.63
CP48	3	0	1	2		2	210-250	0.51	0.62	0.43
CP49	6	0	4	2		2	196-216	0.73	0.63	0.68
CP51	6	0	4	2		1	290-310	0.77	0.38	0.73
CP52	3	0	0	3			186–205	0.66	0.00	0.59
CP53	6	1	2	3		1	305-320	0.70	0.08	0.65
CP54	7	0	5	2	3		270–290	0.76	0.43	0.73
CP55	5	0	2	3	2		250-270	0.73	0.00	0.68
CP57	4	0	1	3	1		280-290	0.70	0.00	0.63
CP58	3	0	0	3			357–367	0.66	0.11	0.58
CP59	5	0	3	2	3		367-378	0.59	0.00	0.51
CP61	8	0	7	1		2	207-220	0.81	0.21	0.78
CP62	5	0	3	2		1	240-260	0.65	0.35	0.58
CP63	3	0	1	3	1		235–249	0.51	0.00	0.40
CP66	5	0	3	2	1	1	244-270	0.59	0.18	0.50
CP68	4	0	2	2		1	141-155	0.59	0.00	0.51

Table 3 (continued)

Marker	Allele			Unique	alleles	Size variation (bp)	H_E	H_O	PIC	
	Number	Rare	Common	Most frequent	Germ	Land				
CP69	4	0	2	2	2		350-377	0.52	0.00	0.47
CP71	6	0	4	2		1	240-260	0.79	0.39	0.76
CP72	4	0	2	2	3		190-205	0.65	0.00	0.60
CP73	5	0	3	2	3		225-242	0.73	0.00	0.68
CP80	3	0	0	3			207-220	0.64	0.00	0.56
CP83	4	0	1	3			240-245	0.73	0.00	0.68
CP86	3	0	2	1	2		447-451	0.27	0.08	0.24
CP89	3	0	2	1	1		423-450	0.34	0.00	0.30
CP94	6	0	4	2	2		170-180	0.79	0.00	0.76
CP95	6	0	5	1	5		180-192	0.73	0.00	0.70
CP97	4	0	2	2		1	248-265	0.70	0.00	0.65
CP98	4	0	2	2			194-200	0.53	0.14	0.48
CP100	4	0	2	2	1		282–305	0.67	0.00	0.61

Rare allele, common allele and most frequent allele, present frequency <1%, $\ge1\% \ge 20\%$, and >20%, respectively; unique alleles present in germplasm accessions (Germ) and landraces (Land)

Ta annealing temperature, H_E expected heterozygosity, H_O observed heterozygosity

Expected heterozygosity was nominally larger than the observed heterozygosity under HWE for all loci, except on CP31 and CP48. Only loci CP21, CP33, and CP38 were found to be in HWE. The observed heterozygosity ranged from 0.00 (CP09, CP14, CP22, CP27, CP47, CP52, CP55, CP57, CP59, CP63, CP68, CP69, CP72, CP73, CP80, CP83, CP89, CP94, CP95, CP97, and CP100) to 0.85 (CP31), and gene diversity (H_E) from 0.08 (CP22) to 0.82 (CP16). The CP16 locus showed the highest polymorphism information content (PIC=0.81) and the CP22 locus the lowest (PIC=0.08), average PIC being 0.53.

When looking at SSR classes and motifs, the compound SSRs showed higher allele numbers (average 4.27 per locus) and PIC values (average 0.55 per marker) followed by dinucleotide (average alleles, 4.08 per locus; PIC value average 0.52 per marker) and trinucleotide SSRs (average alleles, 3.2 per locus; PIC value, average 0.47 per marker). Among dinucleotide SSRs, AG/TC or GA/CT repeat motifs exhibited more informativeness (average alleles, 4.56 per locus and PIC value, average 0.59 per marker) as compared with TA/AT repeat motifs (average alleles, 4.0 per locus and PIC value, average 0.50 per marker) and to GT/CA or TG/AC repeat motifs (average alleles, 3.5 per locus and PIC value, average 0.52 per marker) (Table 3).

Allelic Composition

The allelic composition revealed that rare alleles were represented by < 1.3% of the total number of alleles detected. Of the 237 alleles detected, three were rare, 115

common, and 119 most frequent alleles. Rare alleles were detected from CP16, CP30, and CP53. Common alleles were detected at 46 SSR loci, with an average of 1.95 alleles. In contrast, all SSR loci detected 1 to 3 most frequent alleles in the individuals (Table 3).

This study detected unique alleles within groups (Table 3). The germplasm accessions contained the largest number of unique alleles (46), while 14 unique alleles differentiated landraces from the papaya germplasm. Of the total alleles detected, papaya germplasm and landraces shared 74.7% of alleles (177).

Genetic Diversity

Based on the unique DNA fingerprint profiles of each genotype obtained by the polymorphic markers, a dendrogram was constructed to understand the relationships among the germplasm and landraces surveyed. The neighbor-joining cluster analysis based on shared-allele distance could successfully differentiate all the papaya accessions (group A) and landraces (group B) included in this study (Fig. 1) with the high bootstrap value of 95%. Clearly, four subgroups could be seen in germplasm accessions while landraces presented just two.

Subgroup A1 comprised all gynodioecious accessions, being one cultivar (CM128), eight improved germplasm (CMF068, CMF082, CMF108, CMF115, CMF125, CMF138, CMF142 and CMF143) and five unimproved germplasm (CMF054, CMF058, CMF101, CMF102 and CMF129). Subgroup A2 had two improved (CMF008 and



Fig. 1 Neighbor-joining tree based on the shared allele distance of 59 SSR markers using 30 germplasm accessions at PGM-CNPMF (code CMF) and 18 landraces (code M). A1, A2, A3, and A4 are subgroups

of the A group clustered by germplasm accessions; B1 and B2 are subgroups of B group that contained only papaya landraces. Distance bootstrap values are given together with subgroups in parenthesis

CMF038) and three unimproved germplasm (CMF011, CMF017 and CMF188), whereas subgroup A3 was formed by one improved (CMF123) and six unimproved germplasm (CMF134, CMF135, CMF157, CMF165, CMF189 and CMF191). The other cultivars analyzed (CMF024) were clustered with three germplasm accessions in subgroup A4. Subgroups A1, A2, A3 and A4 were clustered with a relatively low bootstrap support value (36%, 25%, 28%, and 30%, respectively), but subgroups B1 and B2, had a moderate bootstrap support value (48% and 38%, respectively).

The genotypes belonging to landraces collected in the State of Bahia (Brazil) were grouped in clusters (B1and B2), with 14 and four individuals, respectively. No specific grouping was observed for the improved and unimproved cultivars, which dispersed in all subgroups.

Discussion

Microsatellite Informativeness

SSR markers have been extensively used for DNA fingerprinting and elucidating genetic relationships within plant species (Paniego et al. 2002; Stajner et al. 2005). The ability to distinguish between closely related genotypes is a function of the high heterozygosity values of SSR markers. The set of SSR markers characterized in this study proved to be useful for a broad genetic analysis of *C. papaya*.

Previous studies have shown that papaya contains abundant SSRs (Santos et al. 2003; Ocampo Pérez et al. 2006; Eustice et al. 2008; Oliveira et al. 2008a), but the characterization of SSR markers in *C. papaya* germplasm is not yet well studied. Here, we observed relatively high levels of multiallelism at all 59 SSR loci analyzed. Mean number of alleles per locus (4.02, for 59 loci) and expected heterozygosity (mean of 0.59) were higher than those reported by Ocampo Pérez et al. (2006) who used 26 polymorphic markers, and observed 3.8 alleles per locus and H_O and H_E values of 0.42 and 0.57, respectively, but intermediate for samples collected in Guadeloupe, Venezuela, Colombia, Barbados and Costa Rica, that showed 6.6 alleles per locus for 15 loci, and H_E values between 0.37 and 0.69 (Ocampo Pérez et al. 2007).

The number of alleles per locus reported in our study is most likely a minimum value. The lower number of alleles can be due to the relatively few samples analyzed and to the different mating systems of these genotypes. Half of the accessions were gynodioecious, which is almost exclusively inbreeding and tends to decrease the allele number per locus. The number of alleles should increase when all germplasm accessions of the PGM of CNPMF were sampled.

Except CP11 and CP40, all SSR described here are derived from a genomic sequence and showed considerable polymorphism. This is in agreement with Eustice et al. (2008), who found a relatively high level of polymorphism in the genomic rather than the genic region, using seven papaya accessions (SunUp, Kapoho, 2H94, UH918, Kaek Dum, UH928, and AU9) to screen SSR polymorphisms. As in other crops, the selective pressures from breeding can significantly reduce genetic diversity in the target genes or genic regions, while levels of genetic diversity in the genomic region remain high.

The broad range of observed heterozygosity (0.00 to 0.85) and expected heterozygosity (0.08 to 0.82) result from the broad variation in number of alleles per locus and allele frequency distribution within genotypes. The obtained zero values of H_O at twenty-one loci could be explained by the low number of alleles at these loci (2 to 6 alleles) as well as their combination in homozygous state. Loci with smaller numbers of alleles or with a skewed frequency distribution such as CP06, CP09, CP11, CP20, CP22, CP23, CP24, CP27, CP28, CP29, CP33, CP36, and CP38, tend to have lower heterozygosity values and, consequently, lower probability of paternity exclusion when studying natural population.

Ninety-five percent of the SSR markers surveyed for heterozygosity (56 of 59) deviated significantly from Hardy–Weinberg equilibrium. In all cases, the deviation was in the direction of reduced heterozygosity due to inbreeding, presence of null alleles, natural or artificial selection favoring homozygosity for particular loci and population bottlenecks. Such result has been commonly observed in surveys of other species (Carrasco et al. 2009). A possible reason for high levels of inbreeding in *C. papaya* is the elimination of the male plants in trioic accessions (male, female, and hermaphrodite plants) and self-compatibility that stimulates crossing among related individuals and increases the degree of selfing.

In relation to polymorphism, microsatellites of papaya showed enrichment for di and trinucleotide SSR repeat motifs (Eustice et al. 2008; Oliveira et al. 2008a). Overall, AT/TA is the predominant dinucleotide motif, and AAT/TTA is the predominant trinucleotide motif. The previous surveys carried out on microsatellite abundance in plant genomes have shown AT as the most frequently occurring dinucleotide repeat motif followed by AG/TC and GT/CA (Condit and Hubbel 1991; Powell et al. 1996; Yonemaru et al. 2009). Compound SSRs, mainly from different dinucleotide repeats, and dinucleotide repeats were more informative (more average alleles per locus and PIC). Among dinucleotides, although AT/TA-rich motif is prominent, AG/TC or GA/CT repeat motifs exhibited more informativeness. The same pattern was observed in other species (Ferguson et al. 2004; Moretzsohn et al. 2005).

Diversity Structuring

Our study based on SSR analysis clearly revealed genetic diversity in *C. papaya* germplasm samples and some landraces cultivated by farmers in the State of Bahia. Neighbor-joining tree broadly separated germplasm accessions from landraces with high bootstrap support (95%). However, clusters from each subgroup were poorly supported by bootstrap values (Fig. 1). A low bootstrap value means that a grouping is sensitive to the combinations of genotypes that are evaluated, implying that more data may alter the grouping.

The main cluster (group A) is composed of germplasm accessions of *C. papaya* subdivided in four smaller clusters. The smaller one (group B) is clustered only by landraces, which have two subgroups. The grouping of genotypes representing landraces in different clusters is in agreement with their origin, and is important as a first indicative of the genetic background of most of the germplasm of PGM at CNPMF.

The genetic variability in landraces grown by small farmers in the region of Recôncavo of Bahia, where the landraces were obtained, was maintained over generations. This variability is quite different from the one in the PGM, because papaya accessions from the Recôncavo of Bahia has not been deposited in the germplasm bank. The additional genetic variability will be used in breeding programmes to enhance the diversity of breeding populations for selection gains in the future.

Ocampo Pérez et al. (2007) analysed genotypes from Costa Rica, Colombia, Venezuela, Guadeloupe and Antillean islands using SSR markers and PCO analysis, and found that, with few exceptions, they were clustered according to their geographic origin. In our study, although there are few accessions belonging to different countries, no high correlation between the clustering pattern and the geographical location was observed. The Brazilian accessions were clustered in all subgroups. The accessions from South Africa and Hawaii grouped into subgroup A1, Thailand into subgroup A3; whereas accessions from Costa Rica and Malaysia grouped into A2 and A4 subgroup; and Taiwan into A1 and A2 subgroup (Fig. 1).

The 30 germplasm accessions and 18 landraces used for characterization of SSR polymorphism allowed the detection of considerable genetic variability, as showed on six diversity subgroups (Fig. 1). The average similarity based on shared allele distance between accessions was 0.44, which is very similar to the value of 0.48 obtained by Ocampo Pérez et al. (2007), using Dice distance. Previous reports using dominant markers, as RAPD (Stiles et al. 1993) and AFLP (Kim et al. 2002), showed values of 0.78 (Jaccard distance) and 0.88 (Dice distance), respectively. So, the SSRs markers revealed a more important polymorphism than the other nuclear DNA markers used so far in papaya (Ocampo Pérez et al. 2007).

Most of the observed variation occurred among accessions. All genotypes present in A1 and A4 subgroup are gynodioecious, whereas B1 and B2 are dioecious. In addition, the A2 and A3 subgroups are composed of gynodioecious and dioecious genotypes. The gynodioecious tends to show lower genetic variability within accessions than the dioecious, because self-compatibility increases the inbreeding.

Many aspects, such as the breeding system, seed and pollen dispersal, plant longevity and agricultural practices influence the genetic diversity, including the proportion of variation distributed within and between populations (Hamrick and Godt 1996). Kim et al. (2002) analysed the genetic relationships among C. papaya cultivars, breeding lines, unimproved germplasm, and related species using AFLP markers and suggested limited genetic variation in papaya, with smaller genetic diversity within the same gene pools, such as gynodioecious and dioecious cultivars. According to Carrasco et al. (2009), the genetic diversity of Vasconcellea pubescens, a species of the Caricaceae family, was remarkably low using ISSR (Inter Single Simple Repeat). The major genetic diversity was found within groups (65%) when South and the North samples from Chile were analyzed together. In addition, samples from South and the North analyzed separately showed that 82% and 60% of genetic diversity were within groups, respectively.

Efforts are made to maintain the genetic diversity of papaya breeding through the stocks in germplasm banks to study natural variation. Although artificial by their nature and submitted to several forces, including genetic drift and man driven artificial selection, germplasm collections play an important role in the way the banks are structured. In this study, we found a great depth of allelic diversity among landraces and germplasm, because this diversity is not distributed randomly among the genotypes, but rather structured into two groups. Thus, many landraces will be incorporated to PGM at CNPMF, to increase the stored variability for future use in conservation and breeding programs.

SSR in Genetic Studies of C. papaya

SSRs are powerful molecular biology tools that have been used for a wide variety of applications. Although good estimators of population genetic parameters have been presented for dominant markers (Lynch and Milligan 1994; Zhivotovsky 1999), the codominant nature of SSR allows more precise calculations of heterozygosity, Hardy-Weinberg equilibrium, differentiation and gene flow (Lowe et al. 2004). Polymorphisms based on SSR are more powerful to estimate genetic parameters of populations and understand detailed patterns of gene flow and parentage composition (Dow and Ashley 1996; Collevatti et al. 1999; Ren et al. 2008). Besides, SSRs markers are more useful for genetic mapping (Oliveira et al. 2008b; Wang et al. 2009), and ancestry studies (Guilford et al. 1997; Gianfranceschi et al. 1998).

The papaya microsatellite loci published here provide an abundant set of genetic markers for detailed studies of population genetic structure, hybridization among populations, paternity, migration, phylogeography of the genus *Carica*, creation of linkage and physical maps, and location of genes of interest, particularly those functioning as QTL, encoding important agricultural traits such as disease resistance, yield, fruit type, and fruit size. Specifically, we are now using these markers to understand the genetic variability of 160 papaya germplasm accessions collected from different countries, and that are part of the PGM at CNPMF.

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