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Simple sequence repeat (SSR) markers survey of the cassava (*Manihot esculenta* Crantz) genome: towards an SSR-based molecular genetic map of cassava

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Abstract The development of PCR-based, easily automated molecular genetic markers, such as SSR markers, are required for realistic cost-effective marker-assisted selection schemes. This paper describes the development and characterization of 172 new SSR markers for the cassava genome. The placement of 36 of these markers on the existing RFLP framework map of cassava is also reported. Two similar enrichment methods were employed. The first method yielded 35 SSR loci, for which primers could be designed, out of 148 putative DNA clones. A total of 137 primer pairs could be designed from 544 putative clones sequenced for the second enrichment. Most of the SSRs (95%) were di-nucleotide repeats, and 21% were compound repeats. A major drawback of these methods of SSR discovery is the redundancy – 20% duplication; in addition, primers could not be designed for many SSR loci that were too close to the cloning site – 45% of the total. All 172 SSRs amplified the corresponding loci in the parents of the mapping progeny, with 66% of them revealing a unique allele in at least one of the parents, and 26% having unique alleles in both of the parents. Of the 36 SSRs that have been mapped, at least 1 was placed on 16 out of the 18 linkage groups of the framework map, indicating a broad coverage of the cassava genome. This preliminary mapping of the 36 markers has led to the joining of a few small groups and the creation of one new group. The abundance of allelic bridges as shown by these markers will lead to the development of a consensus map of the male- and female-derived linkage groups. In addition, the relatively higher number of these allelic bridges, 30% as against 10% for RFLPs in cassava, underscores SSR as the marker of choice for cassava. The 100% primer amplification obtained for this set of primers also confirms the appropriateness of SSR markers for use in cassava genome analysis and the transferability of the technology as a low-cost approach to increasing the efficiency of cassava breeding. Current efforts are geared towards the generation of more SSR markers to attain a goal of 200 SSR markers, or 1 SSR marker every 10 cM.

Keywords Cassava · Molecular genetic markers · Simple sequence repeats · Enriched libraries · Molecular genetic map

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Introduction

Cassava, *Manihot esculenta* Crantz, is an important starchy staple of the lowland tropics and a mainstay of some of the most hard-pressed populations of the world, food security-wise. The crop accounts for over 60% of the daily calorie intake of some 500 million people in the sub-Saharan region of Africa (FAO 1997) and is irreplaceable in this part of the world as a food-security crop. It is therefore ironical the paucity of genetic studies aimed at improving the efficiency of cassava cultivation. The number of years required for the evaluation of

promising clones, approximately 10 years, is a bottleneck to increased productivity; a quicker means of identifying clones with top-of-the-line performance is clearly required.

A molecular genetic map of cassava was constructed on the basis of the segregation of predominantly restriction fragment length polymorphism (RFLP) markers in a F_1 intra-specific cross (Fregene et. al. 1997), as a first step towards marker-assisted genetic analysis of traits of agronomic importance. To date, the genetics of resistance of two devastating cassava diseases, both major production constraints, the cassava bacterial blight (CBB) and the African cassava mosaic disease (ACMD), have been studied using the mapping population, and a backcross derivative (CIAT, unpublished data). Other traits studied include the inheritance of early bulking and root quality (Fregene et al. 2000). These studies have been exclusively carried out at research centers that can afford the technology required for RFLP markers, thereby limiting the use of marker technology to these centers, which account for a small percentage of the manpower working on breeding of the crop. In an attempt to make marker technology widely available in cassava, an effort was embarked upon to place on the cassava map simple-sequence repeat (SSR) markers, markers that are polymerase chain reaction (PCR)-based and highly polymorphic and best meet the criteria required for the transfer of marker technology to research facilities in developing countries.

SSR markers are found in all eukaryotic genomes. They are short tandem repeat motifs usually consisting of 1–6 bp of nucleotides. They were first referred to as microsatellites by Litt and Lutty (1989) and later as simple sequence repeats (SSRs) by Jacob et al. (1991). Conserved regions flanking the repeats are suitable for designing PCR primer pairs to be used for amplifying the intervening repeat loci. These loci are highly variable on account of the number of repeat units found for each locus in any given population (Morgante and Oliveri 1993). The high levels of heterozygosity and the codominant, and PCR-based nature of these repeat loci have made SSRs the molecular markers of choice for genetic mapping and diversity studies (Wang et al. 1994; Gupta et al. 1996). Many workers have described the use of SSR markers in genetic mapping, usually integrating them onto existing RFLP framework maps (Roder et al. 1998; Liu et al. 1996; Taramino and Tingey 1996; Senior and Heun 1993; Wu and Tanksley 1993; Schmidt and Heslop-Harrison 1996; Bell and Ecker 1994). The discovery, inheritance and variability of fourteen GA repeats have been described for cassava (Chavariaga-Aguirre et. al. 1998). A sub-set of 4 of those SSR markers were used to evaluate the genetic diversity of the core collection, about 600 accessions, of the cassava world germplasm bank at the International Center for Tropical Agriculture (CIAT, the Spanish acronym) (Chavariaga-Aguirre et al. 1999). Results showed high levels of heterozygosity (up to 0.88) of the markers, revealed putative duplicates and indicated the unequal representation of cassava diversity, by country, in the core collection. We describe in this paper the isolation and characterization of 172 SSR markers in cassava for saturating the existing genetic map of cassava and the mapping of 36 of them onto the existing genetic map of cassava.

Materials and methods

Development of SSR-enriched libraries

Two enrichment experiments, "Enrichment A" (after Karagyozov et al. 1993, as modified by Panaud et al. 1996) and "Enrichment B" (after Edwards et al. 1996), differing essentially in the oligonucleotides used for enrichment and the cloning vectors, were conducted with two cassava elite clones. Total genomic DNA used in "Enrichment A" was from TMS 30572, an improved cassava variety developed at the International Institute of Tropical Agriculture, Ibadan, Nigeria. Three sets of filters were prepared by spotting 1 µg of each of three oligonucleotide mixtures – TC and GT; CAA, CAG, ACG and AAT; and CAGA and GATA – in 80 µl of 3× SSC onto 0.5 cm^2 of nylon membrane and air-drying for 1 h. The membranes were then exposed to 245 nm UV for 1 min to covalently bind the oligos to the membranes. Excess oligo was washed off with 10 ml hybridization solution (50% formamide, 5× SSC, 50 m*M* Na-phosphate buffer, pH 7.0, 7% SDS) at 45°C for 2 days followed by extensive washing at room temperature with 100 ml hybridization solution. The membranes were then stored at –20°C until needed.

One microgram of total cassava genomic DNA was digested with *RsaI* in a 20-µl reaction for 1 h at 37°C; DNA linkers were ligated to the digested DNA by the addition of 100 ng, in a 2-µl volume, of *MluI* adaptors (21-mer: 5′CTCTTGCTTACGCGTGGACTA3′; phosphorylated 25-mer: 5′pTAGTCCACGCGTAAGCAAGAGCACA3′), 2 µl of 10 m*M* ATP and 1 µl of ligase to the digested DNA followed by incubation at 37°C for an additional 2 h. About 40 µg of ligated DNA was PCR-amplified for 20 cycles, using a ramp program of 94°C for 40 s, 60°C for 60 s and 72°C for 120 s, in a reaction mixture of 3 µl reaction buffer, 3 µl of 2 m*M* dNTPs, 3 µl of 2 µ*M* 21-mer oligo, 20 µl dH2O and 0.3 µl *Taq* polymerase. The amplification product was visualized by running 5 µl of the PCR on a 1% agarose gel.

Enrichment for sequences with the di-, tri-, and tetra-nucleotides was by incubating the SSR Oligo-bound filters with 25 µl of amplified DNA denatured at 100°C for 5 min, in 500 µl hybridization solution at 37°C for 24 h. The filters were then washed 20 times in 0.5× SSC at 65°C. Bound DNA was then eluted from the individual filters into 200 µl of distilled water by boiling for 5 min. Captured DNA fragments were PCR-amplified as before using 2.5 µl of the eluted DNA as template, and the PCR product was checked by running 5 µl of sample on an agarose gel. The entire process of enrichment was repeated to increase the percentage of sequences containing SSRs.

The amplification product of the final SSR-enriched DNA mixture was cleaned using a PCR cleanup kit (Promega) and eluted into 40 µl distilled H2O. The enriched DNA was digested with *Bg*III in a 50-µl reaction for 1 h. The digestion was ethanol-precipitated and dissolved in 100 µl distilled H2O to give an estimated concentration of 10 ng/µl. Vector (pUC18) DNA was digested with *Bam*HI and phosphorylated using shrimp alkaline phosphatase (Amersham PLC); the phosphatase was then completely removed by a phenol-chloroform extraction and precipitation with ethanol; plamids DNA was re-suspended in 50 µl of distilled H2O to give a final concentration of 100 ng/µl. The equivalent of 100 ng of vector DNA was ligated with 25 ng of insert DNA in 10-µl reactions and incubated at 14°C for 24 h in a PCR machine. Ligation reactions were diluted 1:5 with distilled H2O and 1 µl of each was transformed *into E. coli* DH10 cells (GIBCO BRL) by electroporation according to the manufacturer's protocol. Electroporated cells were plated out on 100 µg/ml ampicillin LB-agar plates and incubated overnight at 37°C.

The DNA for "Enrichment B" was from CMC 40, a cassava accession from CIAT's core collection originally collected from Brazil, and enriched libraries were constructed for only di-nucleotide repeats, $(GA)_{15}$,/$(CA)_{15}$ according to Edwards et al. (1996). Two microlitres from the $(GA)_{15}$/$(CA)_{15}$ enriched library were transformed into E. *coli* DH10 cells (GIBCO BRL) by electroporation according to the manufacturer's protocol. Electroporated cells were plated out on 100 µg/ml ampicillin LB-agar plates and incubated overnight at 37°C.

Enriched library screening and sequencing

Approximately 6,000 clones from each of the di-, tri-, and tetra-enriched libraries of "Enrichment A" were picked out and spotted onto one single 48×48-cm high-density filter using the QBOT robot (Genetix PLC, UK) of the Clemson University Genome Institute (CUGI). A total of 2,300 clones were handpicked from the $(GA)_{15}$/$(CA)_{15}$ enriched library of "Enrichment B" and organized manually onto twelve 18×10-cm filters. The filters from both enrichments were screened with the appropriate di-, tri- or tetra-nucleotide and end-labeled with α–[^{32}P]dATP (Maniatis el al. 1987). Hybridizations were in the Church and Gilbert (1984) hybridization buffer at 65°C or 45°C for 14–16 h. Post-hybridization washes (2) were in 6× SSC at 65°C or 45°C for 5 min each. Autoradiography was for 2–24 h. Plasmid minipreps of overnight 2 ml LB+100 µg/ml ampicillin cultures of positive clones were carried out using the QIAGEN (Gmbh) plasmid miniprep kit or the Promega Wizard prep kit. Forward and reverse strands of all positive clones were sequenced using the M13 universal and reverse primers (New England Biolabs, USA and Microsynth, Switzerland) on an automated sequencer (Perkin Elmer/Applied Biosystems models ABI 373 and 377).

Primer design and SSR analysis

Vector and adaptor sequences were cleaned out of the raw DNA sequence using GCG (University of Wisconsin) or the SEQUENCHER 3.0 (Gene Codes Corp) software. The software packages were also used to align the forward and reverse strands. Duplicate sequences were identified using Local BLAST obtained from http://www.ncbi.nlm.nih.gov. Primers were designed for all unique SSR-containing sequences with at least ten repeats for di-nucleotide repeats and more than six for tri- and tetra-nucleotides. Primer design was with PRIMER3 picking software found at http://waldo.wi.mit.edu/egi-bin/primer/primer3 (Whitehead Institute for Biomedical Research). Oligonucleotide primers were synthesized by Research Genetics (2130 Memorial Parkway SW, Huntsville, AL 35801 USA) and designated Cassava MapPairs. These primers can be obtained directly from Research Genetics. The female parent of the F_1 cassava mapping population, TMS 30572, one of the accessions employed in "Enrichment A", and the male parent, CM2177–2, an improved clone from Colombia, were evaluated with all the 172 SSR markers identified using non-radioactive PCR amplifications and silver-stained (Promega) 6% polyacrylamide sequencing gels. PCR reactions were carried out in 50-µl volumes containing 50–100 ng genomic DNA, 0.2 µ*M* of each forward and reverse primers, 10 m*M* TRIS-HCl (pH 7.2), 50 m*M* KCl, 1.5 or 1 m*M* $MgCl_2$, 200 m*M* of each dNTP and about 1 U *Taq* DNA polymerase. The temperature cycling profile was: an initial denaturation step for 5 min at 94°C, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C or 45°C for 2 min and primer extension at 72°C for 2 min; a final extension cycle of 5 min at 72°C was added. Between 2 µl and 3 µl of the PCR reaction was electrophoresed on 5% ethidium bromide-stained Metaphor agarose gels or on 6% polyacrylamide sequencing gels for 2 h at 100 W, and DNA was visualized by silver staining according to the manufacturer's guide (Promega).

Genetic mapping of SSR markers

SSR markers having a unique allele in either or both parents were analyzed in the entire F_1 progeny of 150 individuals. SSR markers that segregated in the expected ratio of 1:1 presence: absence of the unique parental allele in the F_1 progeny) were placed onto the existing map of cassava using the linkage analysis computer package MAPMAKER 2.0 (Lander et. al.1987), as described earlier (Fregene et. al. 1997). The "group" command, with a LOD threshold of 4.0, and a recombination fraction of 0.3, were used to assign SSR markers to existing linkage groups, and the "try" command was used to find the most likely interval in which to place the new marker on the linkage groups. In a few cases the SSR led to a new linkage group being formed or to 2 smaller groups being joined together. The order was ascertained using the "compare" function. Maximum likelihood orders of linkage groups with newly added markers were verified by the ripple function, and only orders greater or equal to a LOD value of 2 were accepted for the new framework map of cassava. All MAPMAKER analyses were done on a Macintosh G3 computer.

Results

SSR discovery

From "Enrichment A", a total of 148 positive clones, fewer than 1% of the clones picked from the libraries, was obtained from the SSR oligo screen. Plasmid DNA was prepared from the 148 putative clones, and all clones were sequenced. The average size of the clones was 200 bp. Of these, 66 clones, or 45%, contained SSR loci. Primers were designed for 35 unique clones; 4 were duplicates, while the other 17 were clones with the SSR too close to the end of the DNA clone to permit primer design. "Enrichment B" had 1,400 positive clones, or more than 60% enrichment. Plasmid miniprep and DNA sequencing were performed for 544 clones, from which 479 clones had SSR sequences, 30 had no SSR loci, while 35 had sequences that needed repeating. No PCR pre-screen clones was performed. Out of these 479 positive clones, 229 clones had the SSR repeat too close to the end of the sequence, while 113 clones were duplicates. Primers could be designed for 137 clones.

One hundred and sixty-four, or 95%, of the 172 SSR-containing clones for which primers were designed were di-nucleotide repeats, while the balance were tri-nucleotide repeats save for one tetra-nucleotide repeat. Table 1 shows the breakdown of the clones into nucleotide repeat classes. Thirty-seven, or 21%, of the loci were found to contain more than one kind of repeat – compound repeats. Approximately 21% of the SSR clones from both enrichments were duplicated sequences, while 45% had the SSR loci too close to the cloning site to permit primer design from the flanking regions. On the whole, 35% of sequenced positive clones were unique sequences with SSR loci well situated for primer design.

A total of 6,000 cassava inserts, with an average size of 200 bp, were screened for "Enrichment A", and 36 GA-containing clones, or an average of approximately 1 GA marker every 34 kb, were found. Assuming an average of 1 GA repeat every 225 kb which has been found for higher plants (Maroof et. al. 1994), this is a

Table 1 Number, percentage and kind of SSR repeat sequences for which primers were designed

Enrichment A			Enrichment B		
Type of SSR	Number	Percentage (%)	Type of SSR	Number	Percentage (%)
GA/CT	12	34	GA/CT	80	58
CA/GT	5	14	CA/GT	30	22
(CA)(GA)	2	6	(CT)(CA)	15	11
ATT/TAA	5	14	(CA)(GA)	6	4
Others	11	31	Others	6	4
Total	**35**		**Total**	**137**	

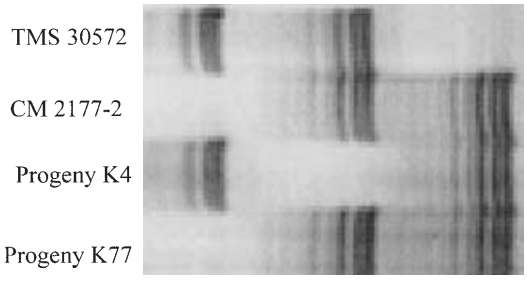


Fig. 1 Silver-stained polyacrylamide gel showing unique alleles in both parents of the mapping progeny, TMS 30572 (female) and CM 277-2 (male). Two progeny, K4 and K77, show the inheritance of these alleles

sixfold enrichment. The "Enrichment B", on the other hand, obtained 875 GA-containing clones from 2,300 clones with an average size of 250 bp, or 1 GA marker every 700 bp more than a 300-fold enrichment.

SSR parental survey

All 172 primer pairs successfully amplified the corresponding SSR loci in the parents of the cassava mapping progeny even though different $MgCl_2$ concentrations and two annealing temperatures, 55°C and 45°C, respectively, were used. The primer pair sequences, annealing temperatures, product sizes, and $MgCl_2$ concentrations are presented as an appendix at the end of this paper. One hundred and thirteen SSR loci, or 66% of all SSR markers tested in the parents, revealed a unique allele in at least one of the parents; 45 SSR markers (26%) showed a unique allele for both parents. SSR polymorphism between the two parents at 12 loci is shown in Fig. 1.

Genome location of SSR markers

Twenty-two SSR markers that were polymorphic in the parent on ethidium bromide-stained 5% Metaphor agarose gels were scored in the 150 F_1 mapping progeny, along with a group of 14 SSR markers polymorphic in the parents only on PAGE gels. Figure 2 shows the map positions of 36 SSR loci from the 172 SSR markers analyzed to date on the male- and female-derived molecular genetic map. Linkage group nomenclature is as described for the molecular genetic map of cassava by Fregene et. al.(1997) except for groups L, O and P, which have now been merged with other groups. The 36 SSR markers reveal a fairly even spread over the cassava genome – 16 of the 18 linkage groups have at least 1 SSR marker, with an exception of 3 SSR markers each clustered on linkage groups C, D and J. A unusual observation is the complete lack of duplication of the SSR markers mapped so far.

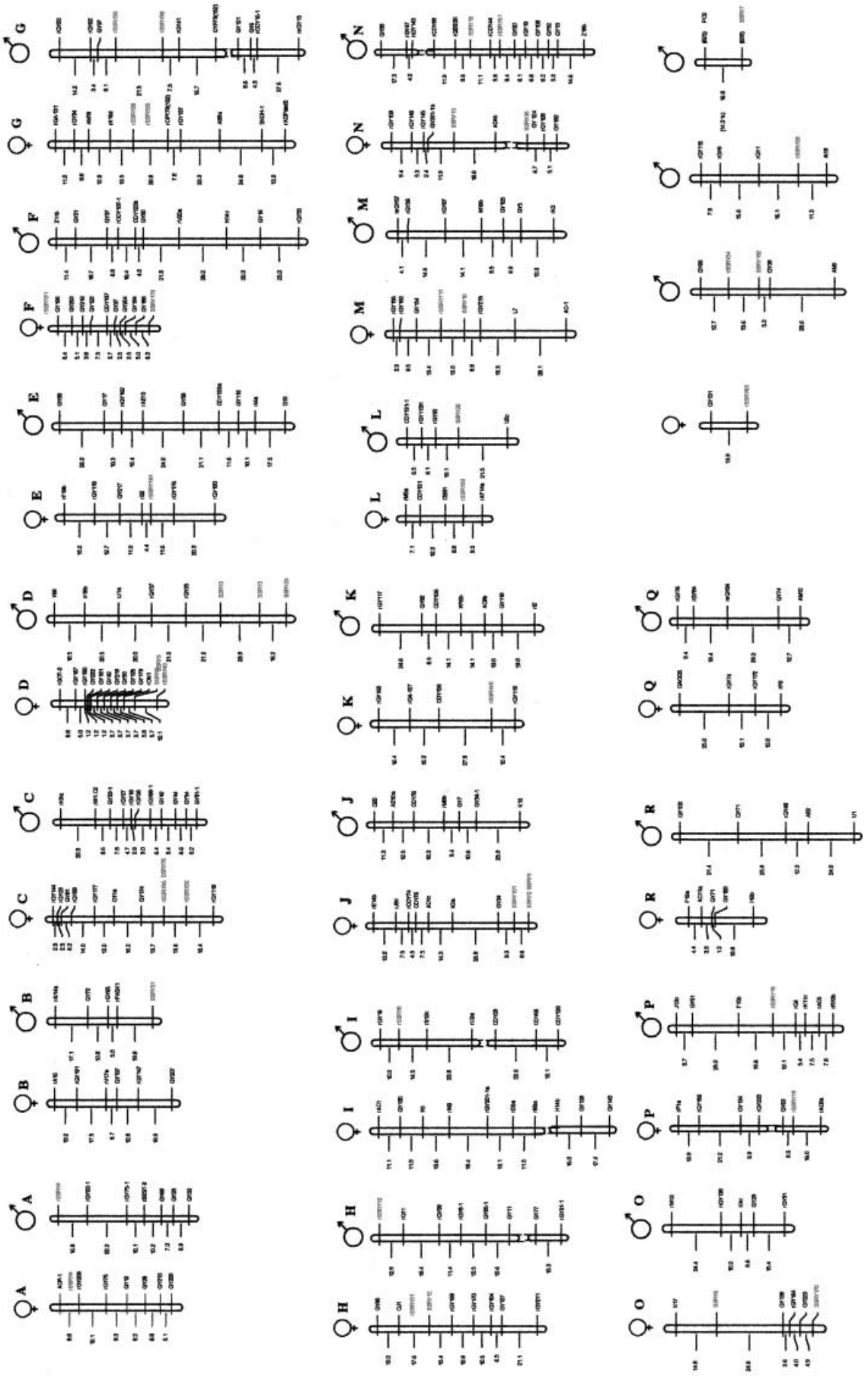
Discussion

The development of SSR markers from enriched libraries using two similar methods showed widely varying results. "Erichment B", which has been successfully used in several crops (Edwards et al. 1996), showed an efficiency of 5000% over "Enrichment A". A particular drawback of "Enrichment A" is the blunt ends that are obtained with the enzyme *Rsa*I; these reduce the efficiency of ligation of the linkers, which in turn might drastically reduce the efficiency of the enrichment process. The unusually long repeat lengths observed with "Enrichment B" supports the assertion of a more thorough sampling of the genome.

The high level of redundancy found in both libraries is presumed to have arisen from the PCR amplification after the affinity capture prior to cloning. The duplication makes sequencing of the positive clones less efficient and increases the cost of SSR marker discovery. To avoid this, Roder et al. (1998) in wheat and Panaud et al. (1996) in rice suggested the use of restriction enzyme-digested, size-fractionated libraries. While their suggestions considerably increase the amount of screening needed, the current availability of high-throughput robots for analyzing genomic libraries makes this less burdensome.

Genetic mapping in allogamous crops, such as cassava, offers the possibility of constructing maps with

Fig. 2 Positions of 36 SSR markers (in *bold print*) on the framework (LOD >2.0) molecular genetic map of cassava. Map distances are in Kosambi map units. Groups in the *lower right-hand corner* have yet to be merged with the analogus male- and female-derived linkage groups ▶



crosses between non-inbred parents; however, genetic mapping is complicated by the separate analysis of gametes segregating from the male and female parent. To create a consensus map of analogous male- and female-derived linkage groups, investigators requise markers that have unique alleles in both parents, or "allelic bridges" (Ritter et. al. 1991). In addition, "allelic bridges" are indispensable for a rigorous marker-assisted quantitative genetic analysis in F_1 progeny from non-inbred parents by permitting an estimation of gene effects from both parents and the evaluation of intra-locus and inter-loci interactions. The higher number of "allelic bridges" obtained using SSR markers, 30%, as against 10% obtained with RFLPs (Fregene et. al. 1997) make SSRs the markers of choice for the genetic mapping of cassava. The level of successful amplification of the primers, 100%, is higher than that found in wheat (36%, Roder et. al. 1999), suggesting the appropriateness of SSR marker systems in cassava compared to complex genomes like wheat.

Current efforts are geared to mapping the 113 SSR markers and continued sequencing of the more than 900 positive clones identified earlier. However, due to the high number of redundant clone in the enriched libraries, more than 40%, sequencing will be done for only one strand and second-strand sequencing performed for only unique clones. Further development of SSR markers will involve the search for 3′- and 5′-un- translated regions of cassava ESTs for SSR repeats.

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Appendix Properties of cassava SSR loci and their primer pairs

SSR locus	Type of repeat	Left primer	Right primer	Product size (bp)	Annealing temperature (°C)	MgCl (m*M*)
SSRY1	$(GCC)_6$	GCAGCTGCCGCTAATAGTTT	CCAAGAGATTGCACTAGCGA	197	45	1.5
SSRY2	$(CT)_{18}CC(CT)_{16}$	CGCCTACCACTGCCATAAAC	TGATGAAATTCAAAGCACCA	167	55	1.5
SSRY3	$(CA)_{17}$	TTAGCCAGGCCACTGTTCTT	GCGAGGTTCAAATATGCGAT	247	55	1.5
SSRY4	$(GA)_{16}TA(GA)_3$	ATAGAGCAGAAGTGCAGGCG	CTAACGCACACGACTACGGA	287	55	1.5
SSRY5	$(GA)_{38}$	TGATGAAATTCAAAGCACCA	CGCCTACCACTGCCATAAAC	173	55	1.5
SSRY6	$(CA)_7(N)_{51}(CA)_{17}(N)_{47}(CA)_{15}$	TTTGTTGCGTTTAGAAAGGTGA	AACAAATCATTACGATCCATTTGA	298	45	1.5
SSRY7	$(CT)_{26}$	TGCCTAAGGAAAATTCATTCAT	TGCTAAGCTGGTCATGCACT	250	45	1.5
SSRY8	$(CA)_{14}CT(CA)_2$	AGTGGTTTGAGAAGACTGGTGA	TTTCCAAAATGGAACTTCAAA	288	45	1.5
SSRY9	$(GT)_{15}$	ACAATTCATCATGAGTCATCAACT	CCGTTATTGTTCCTGGTCCT	278	55	1.5
SSRY10	$(CA)_{17}$	CGTTTGTCCTTTCTGATGTTCT	TGCAATGCAGTGAACCATCT	153	55	1.5
SSRY11	$(GA)_{19}$	TGTAACAAGGCAAATGGCAG	TTCTTGTGTCGTGCAACCAT	265	55	1
SSRY12	$(CA)_{19}$	AACTGTCAAACCATTCTACTTGC	GCCAGCAAGGTTTGCTACAT	266	55	1.5
SSRY13	$(CT)_{29}$	GCAAGAATTCCACCAGGAAG	CAATGATGGTAAGATGGTGCAG	234	55	1.5
SSRY14	$(CA)_6$	TTTGCATCGATTCCATCATC	TTGACCTTAGCACATTTAAGGATTC	300	55	1.5
SSRY15	$(GA)_{24}$	TGAAAGCCTGCATTCAAACA	TGATGCAGGTAGCAAGGATG	215	55	1.5
SSRY16	$(GA)_{13}CA(GA)_3(N)_6(GA)_4$	GCACTGCAAAAAATATCATCTTGA	CTGGAAAGATGGGACGTGTT	218	55	1.5
SSRY17	$(GT)_{13}(N)_{69}(GT)_{15}$	CTTAGAAAAGAAATTGCATGTGAG	TGTCTGATCAAGCTGGTGACA	277	55	1.5
SSRY18	$(CA)_{13}(N)_{90}(GA)_2(N)_8(GA)_2AGA_2(N)_6(GA)_5$	GTGCTGCAAGGCGATTAAGT	GCTACAACTGATAGTTGCATGCTT	198	55	1.5
SSRY19	$(CT)_8(CA)_{18}$	TGTAAGGCATTCCAAGAATTATCA	TCTCCTGTGAAAAGTGCATGA	214	55	1.5

Appendix (Continued)

SSR locus	Type of repeat	Left primer	Right primer	Product size (bp)	Annealing temperature (°C)	MgCl (m*M*)
SSRY20	$(GT)_{14}$	CATTGGACTTCCTACAAATATGAAT	TGATGGAAAGTGGTTATGTCCTT	143	55	1.5
SSRY21	$(GA)_{26}$	CCTGCCACAATATTGAAATGG	CAACAATTGGACTAAGCAGCA	192	55	1.5
SSRY22	$(GT)_{13}$	CTTGCCACTAGAACAGCCAC	GGCGTGGACTAACCTGTTCT	299	45	1.5
SSRY23	$(CA)_{17}$	GCGAGGTTCAAATATGCGAT	TTAGCCAGGCCACTGTTCTT	247	45	1.5
SSRY24	$(GA)_{16}$	CTTTCACATGATTGCAGCGT	GGATTATCCACTTCTCCAAATGTT	100	45	1.5
SSRY25	$(GA)_{27}$	TGGCTACATGATAGCAACATCAA	CGCATGGTTTGTCTCGTTTA	296	55	1
SSRY26	$(GA)_{18}$	TGCTAATTGCAGGAAATAGGAT	GCAGCTTTTTAGCATAACAATCAA	121	55	1.5
SSRY27	CA14	CCATGATTGTTTAAGTGGCG	CCATTGGAGAACTTGGCAAC	277	55	1.5
SSRY28	$(CT)_{26}(AT)_3AC(AT)_2$	TTGACATGAGTGATATTTTCTTGAG	GCTGCGTGCAAAACTAAAAT	180	55	1.5
SSRY29	$(CT)_{18}$	TGGTAGCTTTTGAATATCTGATGG	TGCCAACCAAACCATTATAGAC	281	55	1.5
SSRY30	$(CT)_{22}$	CCATCCACTAGAAACTTTAAAAGCA	CAACTCAGCGGAGCTTTTTC	220	55	1.5
SSRY31	$(GA)_{21}$	CTTCATCACGTGTTAATACCAATC	ATTGTTGTGGTTGCAGGACA	188	55	1.5
SSRY32	$(CA)_{11}$	CAAATTTGCAACAATAGAGAACA	TCCACAAAGTCGTCCATTACA	298	55	1.5
SSRY33	$(CT)_{18}$	AACTCTTTTGACTGAAGATGCTGA	CATGATTACCGCCAAGGCT	273	55	1.5
SSRY34	$(GGC)_5GGTGGC\ (GGT)_2$	TTCCAGACCTGTTCCACCAT	ATTGCAGGGATTATTGCTCG	279	55	1.5
SSRY35	$(GT)_3GC(GT)_{11}(GA)_{19}$	GCAGTAAAACCATTCCTCCAA	CTGATCAGCAGGATGCATGT	282	55	1.5
SSRY36	$(CT)_{15}$	CAACTGTTTCAACCAACAGACA	ATTCTCGTGAACTGCTTGGC	134	55	1.5
SSRY37	$(CT)_{33}$	ATGGCAAAAGATCGAGCAAC	GGCCAGTAATTCCTCAAGGC	187	55	1.5
SSRY38	$(CA)_{17}$	GGCTGTTCGTGATCCTTATTAAC	GTAGTTGAGAAAACTTTGCATGAG	122	55	1.5
SSRY39	$(CT)_{24}AT(CT)_3(AT)_3$	TCAATGCATAGGATTTTGAAAGTA	AATGAAATGTCAGCTCATGCT	293	55	1.5
SSRY40	$(GA)_{16}$	TGCATCATGGTCCACTCACT	CATTCTTTTCGGCATTCCAT	231	55	1.5
SSRY41	$(CT)_4TC(CT)_3(N)_4(CT)_{20}$	TATCACAATCGAAACCGACG	TTTTCCAACAATCTGATACTCGT	271	55	1.5
SSRY42	$(CACACG)_3(N)_6(CACACG)_3$ CA $(CG)_2(CA)_4(CG)_2$ $(CA)_{12}\ (GA)_3\ (G)_{12}(GA)_9$	TTCCTCCAAAGTTATCTAGAACCA	CAATCCTTGTAGTAGCCAGTCTCA	221	55	1.5
SSRY43	$(CT)_{25}$	TCAGACGTTGATACCTCACTTCA	CCAGAGCATGGTCTTTCTGA	255	55	1
SSRY44	$(GA)_{28}$	GGTTCAAGCATTCACCTTGC	GACTATTTGTGATGAAGGCTTGC	194	55	1.5
SSRY45	$(CT)_{27}$	TGAAACTGTTTGCAAATTACGA	TCCAGTTCACATGTAGTTGGCT	228	55	1.5
SSRY46	$(CT)_{19}$	TCAGGAACAATACTCCATCGAA	CGCTAAAGAAGCTGTCGAGC	268	55	1.5
SSRY47	$(CA)_{17}$	GGAGCACCTTTTGCTGAGTT	TTGGAACAAAGCAGCATCAC	244	55	1
SSRY48	$(CA)_{11}$	AGCTGCCATGTCAATTGTTG	TCATAAAGCTCGTGATTTCCA	178	55	1.5
SSRY49	$(GA)_{25}$	TGAAAATCTCACTGGCATTATTT	TGCAACCATAGTGCCAAGC	300	55	1.5
SSRY50	$(CA)_6(N)_6(GA)_{31}$	CCGCTTAACTCCTTGCTGTC	CAAGTGGATGAGCTACGCAA	271	55	1.5
SSRY51	$(CT)_{11}CG(CT)_{11}(CA)_{18}$	AGGTTGGATGCTTGAAGGAA	GGATGCAGGAGTGCTCAACT	298	55	1.5
SSRY52	$(GT)_{19}$	GCCAGCAAGGTTTGCTACAT	AACTGTCAAACCATTCTACTTGC	266	55	1.5
SSRY53	$(CT)_8CC(CT)_8$	CCATGCAGTAGTGCCATCTTT	ATTTTCACCAACCGCAACTC	138	55	1.5
SSRY54	$(GT)_{18}(GA)_{18}$	GCGACTTTCTGGATGGATTC	TGCAAATGACAAATAACCATCTC	151	55	1.5
SSRY55	$(GA)_{16}$	GCAATTTGCAAAGACATACCA	TGTGGAGCTTGATTTTGCAG	145	55	1.5
SSRY56	$(GA)_{21}G(GA)_5$	AACTCTTAATGGCTAAAATTATTGATG	TTTTAGTTTAGTTTAGTTAGTTGCGCT	137	55	1.5
SSRY57	$(GA)_{21}$	TGTCATTGTCTGTTGACCATTT	TAACCTGCCAAGAACAAGGC	293	55	1.5
SSRY58	$(GA)_6AA(GA)_{31}$	GAAGGACAAGCAAAGAAGCAA	TGGAATCCAATATTGATGACTAAGA	217	55	1
SSRY59	$(CA)_{20}$	GCAATGCAGTGAACCATCTTT	CGTTTGTCCTTTCTGATGTTC	158	55	1.5
SSRY60	$(CT)_{20}$	CGGCCACCAACTCAAATAAC	TTGCAATGATATCAACGGCT	137	55	1.5
SSRY61	$(CA)_{12}$	GGCTGCTTTACCTTCTACTCAGA	CAAGAACGCCAATATGCTGA	233	55	1.5
SSRY62	$(GT)_{11}GA(GT)_4GA\ (GT)_5(GA)_9$ AA$(GA)_5$	CATTCTCCAGGAAAGTCATTTTG	AGCTCATGCCATACAAGCAA	250	55	1.5
SSRY63	$(GA)_{16}$	TCAGAATCATCTACCTTGGCA	AAGACAATCATTTTGTGCTCCA	290	55	1.5

Appendix (Continued)

SSR locus	Type of repeat	Left primer	Right primer	Product size (bp)	Annealing temperature (°C)	MgCl (m*M*)
SSRY64	$(CT)_{13}CG(CT)_6$	CGACAAGTCGTATATGTAGTATTCACG	GCAGAGGTGGCTAACGAGAC	194	55	1.5
SSRY65	$(CT)_{13}(CA)_{13}CCA$	CATCGCCAAATCGTCAAGTA	TGATGCCATGCATTTCACTT	299	55	1.5
SSRY66	$(GA)_{19}AAGA$	ATCTCAGCTTCCAACTCTTTCAGT	CGAAATGCTTGGAGACAGGTATAG	261	55	1.5
SSRY67	$(GA)_{20}TGA$	AGTTTGCACCACCTTTTTCC	TGTCAAGTGATGAGCTGCTG	278	55	1
SSRY68	$(CT)_{12}CC(CT)_{17}$	GCTGCAGAATTTGAAAGATGG	CAGCTGGAGGACCAAAAATG	287	55	1.5
SSRY69	$(CT)_{18}ATT(AT)_2\ (N)_7\ (CTTT)_2$	CGATCTCAGTCGATACCCAAG	CACTCCGTTGCAGGCATTA	239	55	1.5
SSRY70	$(GT)_{18}$	CGCTATTAGAATTGCCAGCAC	CGCTTGTTGTATCCATTGGC	249	55	1.5
SSRY71	$(CTT)_5(N)_{10}(CT)_9TT\ (CT)_{15}CCT$	TGATGCAGGTAGCAAGGATG	TGAAAGCCTGCATTCAAACA	217	55	1.5
SSRY72	$(CA)_{10}$	AAGCATCAGTGGCTATCAACA	TTTTGCTGTGCTATTTCTGAGC	141	55	1.5
SSRY73	$(CT)_{30}CCT$	AAGTTGATGGTTCTGAATCTGGA	ACAGTGATTGAGCGAGGCTT	265	55	1.5
SSRY74	$(CT)_{21}$	TTGCTCGAATTCCACACAAT	GGTCAGGTGAGTAATAAAGAACAGTG	114	55	1.5
SSRY75	$(GA)_{23}$	TCTGGTAAACCTACTAGTGCTCCA	TTCATGCACGTCCTGATACA	284	55	1.5
SSRY76	$(GT)_{17}(GA)_{21}$	AAAGGAAGCAACCTTCAGCA	CATGATTTGGATTTTGGAATGA	273	55	1.5
SSRY77	$(GT)_{20}CT(GT)_2$	CAGGAGGTGGCAGATTTTGT	GCATGTTCCACCTGCATAAG	275	55	1.5
SSRY78	$(CT)_{22}$	TGCACACGTTCTGTTTCCAT	ATGCCTCCACGTCCAGATAC	248	55	1.5
SSRY79	$(CT)_{19}T(CT)_7$	CAAACCAATGGTCATGCTGT	CAGCATCAGAAAGACAAAAACAA	210	55	1.5
SSRY80	$(GA)_{25}$	TTCCTGGAAATGTCCTTAGATG	TGGCACATGCAACAATTAGC	299	55	1.5
SSRY81	$(GA)_{22}$	GGCGATTTCATGTCATGCTT	TGATTTTCTGCGTGATGAGC	204	55	1.5
SSRY82	$(GA)_{24}$	TGTGACAATTTTCAGATAGCTTCA	CACCATCGGCATTAAACTTTG	211	55	1.5
SSRY83	$(GT)_{22}CT(GT)_2$	TGGCTAGATGGTGATTATTGCTT	TGCTTACTCTTTGATTCCACG	239	55	1.5
SSRY84	$(GA)_{24}$	TTCCTTTCATTCATCCTGGC	AGAACTTCATGCACACAAGTTAAT	203	55	1.5
SSRY85	$(CT)_{27}$	AAGGTGGCAGCACTTTTCTG	AAGAATACTATACGGACTACATGCCA	292	55	1.5
SSRY86	$(GT)_4(N)_6\ (GA)_{15}GG\ (GA)_2$	GACACCTGCTGATTCCGAG	TTGCCACATAGCAGAATCCTT	296	55	1.5
SSRY87	$(GA)_{18}$	CTCATCTCATGAAGAACTTGTGC	AGAGCACGCATTGTGCATTT	102	55	1.5
SSRY88	$(CA)_7(N)_6(GA)_{22}$	CCAAGTCCTCACCTCCAAAG	CCTTGATGTGGCCAAAGTG	243	55	1.5
SSRY89	$(GT)_{19}$	AGTTGAGAAAACCTTGCATGAG	GGCTGTTCGTGATCCTTATTAAC	120	55	1.5
SSRY90	$(GGA)_5(N)_3(GGA)_2$	AGGTTATGGCGGTGGCAG	GCGATTTTGCGAATTACCAC	193	55	1
SSRY91	$(GA)_{16}$	GTCTGCATGGCTCGATGAT	TGCCTGCTTCATATGTTTTTG	300	55	1
SSRY92	$(GT)_{14}$	CCAATGCTCAGTTTGACAACTC	TCGGCTTAAGGTATGAACGC	171	55	1.5
SSRY93	$(CT)_{25}$	TTTGTTGCTCACATGAAAACG	CAGATTTCTTGTGGTGCGTG	289	55	1.5
SSRY94	$(CA)_{23}TC\ (CA)_3(CT)_3\ (N)_9\ (CCT)_2\ (CT)_2$	AGGATGGACTTGGAGATGGA	GGTGGAAGTAAGGCTGTTAGTG	268	55	1
SSRY95	$(CT)_{19}(CA)_{16}CC\ (CA)_2CC(CA)_3$	CATGATTTGGATTTTGGAATGA	CAAAAGAAGCAACCTTCAGCA	282	55	1
SSRY96	$(GT)_{12}$	CTTTACCTGCATGCCATTGA	CTCCATGTTATCCAAGGTTGC	149	55	1.5
SSRY97	$(GT)_{12}$	GAGCAATCAAATTCAACAGCA	AAGCCGAAGCTTATGAAGGA	194	55	1.5
SSRY98	$(GT)_{11}$	ACCAATCCAAGCTGCAAATC	GTGATTGGTAGTGGTGGCCT	209	55	1.5
SSRY99	$(GT)_{15}(GA)_{13}AA(GA)_3$	ATCAAGGCGCAAAAGTCAAT	CTTGCTTTGGTTCCAATTATTTA	192	55	1.5
SSRY100	$(CT)_{17}TT(CT)_7$	ATCCTTGCCTGACATTTTGC	TTCGCAGAGTCCAATTGTTG	210	55	1.5
SSRY101	$(GCT)_{13}$	GGAGAATACCACCGACAGGA	ACAGCAGCAATCACCATTTC	213	55	1.5
SSRY102	$(GT)_{11}$	TTGGCTGCTTTCACTAATGC	TTGAACACGTTGAACAACCA	179	55	1.5
SSRY103	$(GA)_{22}$	TGAGAAGGAAACTGCTTGCAC	CAGCAAGACCATCACCAGTTT	272	55	1.5
SSRY104	$(CA)_4GA(CA)_2C\ (CA)_4\ (N)_4\ (CA)_{16}(GA)_2\ (GATA)_3(GA)_{21}$	AGGCCATGGCAATTACTGAA	TTCTTGATATGCGCAACAGC	258	55	1.5
SSRY105	$(GT)_6GC(GT)_2(GA)_{16}$	CAAACATCTGCACTTTTGGC	TCGAGTGGCTTCTGGTCTTC	225	55	1
SSRY106	$(CT)_{24}$	GGAAACTGCTTGCACAAAGA	CAGCAAGACCATCACCAGTTT	270	55	1.5
SSRY107	$(CT)_{23}$	CCATTTTCTCTTGCTTCTGTCA	TGGTTTGAAGTCCTATAAAATCCTT	120	45	1.5
SSRY108	$(CT)_{24}CCT$	ACGCTATGATGTCCAAAGGC	CATGCCACATAGTTCGTGCT	203	55	1.5

Appendix (Continued)

SSR locus	Type of repeat	Left primer	Right primer	Product size (bp)	Annealing temperature (°C)	MgCl (m*M*)
SSRY109	$(CT)_{20}CCT$	TGCTAATTGCAGGAAATAGGAT	GCAGCTTTTTAGCATAACAATCAA	125	55	1.5
SSRY110	$(GT)_{12}$	TTGAGTGGTGAATGCGAAAG	AGTGCCACCTTGAAAGAGCA	247	55	1.5
SSRY111	$(GA)_{29}$	GCATCTTACATCCAGAATACTGCT	GAAGGAATGCCTGGCTTAAA	235	55	1.5
SSRY112	$(CT)_{15}C(CT)_3$	CGCAAGGTAAATCGGAGCTA	ACAATCAAAGGAGTCGTGTAATC	117	55	1.5
SSRY113	$(GA)_{19}$	TTTGCTGACCTGCCACAATA	TCAACAATTGGACTAAGCAGC	187	45	1.5
SSRY114	$(GA)_9CAGA$	AACAGGAAGGAAAATCAAGCC	TCAACTGCAGATTCATTCAAGA	167	55	1.5
SSRY115	$(GA)_8$	CAACCGCTTTCGATGGTATT	TGCCATCACAATTTTGCCTA	296	55	1
SSRY116	$(GC)_5A(CA)_7$	CGTTTTCCTGTTAAATCTTGCAT	TAGAGCAGCTGCAAAGCAAA	167	55	1.5
SSRY117	$(GA)_{12}GTCA\ (GA)_3(N)_{32}(GA)_5\ (GT)_2\ (N)_6(GT)_3(N)_{123}(GA)_5$	TAAAGTTTGGCATGCCTGTG	GCAAATGTGTTTTCAATATAAGGC	142	55	1.5
SSRY118	$(GT)_8(GC)_5$	TAGAGCAGCTGCAAAGCAAA	TCGTTTTCCTGTTGAAATCTTG	169	55	1.5
SSRY119	$(GA)_8(G)_3(GA)_3(N)_4(GA)_3(N)_{32}\ (A)_5(GT)_2(N)_6(GT)_3$	AACATAGGCATTAAAGTTTGGCA	GCAAATGTGTTTTCAATATAAGGC	155	55	1.5
SSRY120	$(CA)_7AA(GA)_8$	TCACCGTTAATTGTAGTCTGCG	GCGAGGTTCAAATATGCGAT	139	55	1.5
SSRY121	$(GA)_{17}$	CCAGAAACTGAAATGCATCG	TGGAATTGTTGTCTGGATCG	168	45	1.5
SSRY122	$(GA)_{12}GT(GA)_3GT(GA)_3GT\ (GA)_6\ (N)_{41}(GA)_{14}$	AAGCCAATTGTTGTGAGTTGC	GGTGCTTGGTTTATGCCTGT	273	45	1.5
SSRY123	$(CT)_{14}$	AGCAGATCCAAATCACTGAAA	TTCAACAATAAAGCTCAGAAAGAG	136	55	1.5
SSRY124	$(GA)_9A(GA)_2AGA$	CTGCTGGACGGAGGATTCTA	TGGCATCAATTTTTGCTTCA	146	55	1.5
SSRY125	$(GT)_2(T)_8(GT)_6$	CAGGACATGACGCAATTCTG	GCATGTTAGAAGTTTTTGCAATTT	247	55	1.5
SSRY126	$(GT)_2T(GT)_5(GC)_4$	AATGGATCATGTTCAATGTCTTC	TTGAAATACGGCTCAAGCTC	245	55	1.5
SSRY127	$(GT)_8(GC)_5$	CTTCGGCCTCTACAAAAGGA	GCTGAACTGCTTTGCCAACT	130	45	1.5
SSRY128	$(GT)_2(T)_7(GT)_6$	CAGGACATGACGCAATTCTG	GCATGTAGAAGTCTTTGCAATTATG	243	45	1.5
SSRY129	$(GC)_3G(CA)_6A(CA)_3$	CTTTTTGCCAGTCTTCCTGC	AATGGATCATGTTCAATGTCTTC	205	55	1.5
SSRY130	$(GT)_2T(GT)_5(GC)_3$	GGTCCCTGATAGTTGATAATGGAT	CTTTTTGCCAGTCTTCCTGC	223	55	1.5
SSRY131	$TAAA(TAA)_4$	CATTGTTCAGCAAACACTGGA	GCGAGAGATGTTGCTATTGCT	111	45	1.5
SSRY132	$(CA)_6A(CA)_2$	CTTTTTGCCAGTCTTCCTGC	TGTCCAATGTCTTCCTTTCCTT	196	55	1.5
SSRY133	$(GTTT)_2(GT)_5AT\ (GT)_2$	AGCATGTCATTGCACCAAAC	CGACTGCATCAGAACAATGC	295	45	1.5
SSRY134	$(CA)_4(GA)_4$	TCCACAAAGATAAGCTAAGCG	GCAAGTTCAAAAGGAGCAGC	213	55	1.5
SSRY135	$(CT)_{16}$	CCAGAAACTGAAATGCATCG	AACATGTGCGACAGTGATTG	253	45	1.5
SSRY136	$(CA)_2TA\ (CA)_4\ (CAAA)_2CAA$	CGACTGCATCAGAACAATGC	AGCATGTCATTGCACCAAAC	296	55	1.5
SSRY137	$(ATT)_3ATTT\ (ATT)_2(T)_9(N)_9AT\ (AAT)_4ATAAAT$	TAGTTAGCTCGGTTCGTCCG	TTTTGATAGATCAAGAGAGTTTTTGAA	157	55	1.5
SSRY138	$TA(TTA)_8(CT)_5$	AGAATGTCTCTTTATTCTTGACAATTT	TTCAGGAAACATGCACAAACA	129	55	1.5
SSRY139	$(GT)_2(GC)_4AT(GT)_{23}\ T\ (GT)_{10}$	AAAAAGTGACAGAGTTCCGCTC	CAGATTCTTCAAGCCAAATGTC	129	55	1.5
SSRY140	$(ATT)_4(N)_{15}(ATTTT)_3ATTT$	CAGTGAGCAGAAACTAAAAACATTG	GGCACTTTGGAAAGGAAGAG	212	55	1
SSRY141	$(CT)_8CG(CT)_2$	TCCAAAATCTTGGTCATTTTGA	TGCTGTGATTAAGGAACCAACTT	262	55	1.5
SSRY142	$(GC)_3A(CA)_5A(CA)_2$	CTTTTTGCCAGTCTTCCTGC	AATGGATCATGTTCAATGTCTTC	206	55	1.5
SSRY143	$(GA)_{12}$	GCTCATGAACTGAGCCTTCA	AGCAGATCCAAATCACTGAAA	153	55	1.5
SSRY158	$(CT)_{23}(N)_6(GT)_7$	CCTTACTTGTGTTTCTTACTGACAAG	CCAAGTCCTCACCTCCAAAG	224	55	1.5
SSRY159	$(CT)_4TT(CT)_{16}$	CTTATCCTGTCCCCTCCACC	GACAATTGCATAGGAAGCACA	159	45	1.5
SSRY160	$(CT)_{30}$	CTGGCTCTTCCAGACACCTT	GGCAAGAGAAGCCATAAAGC	151	55	1.5
SSRY161	$(CT)_{11}TT(CT)_{21}(CA)_{19}$	AAGGAACACCTCTCCTAGAATCA	CCAGCTGTATGTTGAGTGAGC	220	55	1.5
SSRY162	$(CT)_{28}$	TTTAGTTAGTTGCGCTAGCTTCC	AACTCTTAATGGCTAAAATTATTGATG	126	55	1.5
SSRY163	$(CT)_{21}(CA)_{19}$	TCATGATGCTATTCCAAGTGTG	AGGCCTCCAACAATTAGCCT	231	55	1.5
SSRY164	$(GA)_{29}$	TCAAACAAGAATTAGCAGAACTGG	TGAGATTTCGTAATATTCATTTCACTT	187	45	1.5

Appendix (Continued)

SSR locus	Type of repeat	Left primer	Right primer	Product size (bp)	Annealing temperature (°C)	MgCl (m*M*)
SSRY165	$(GT)_{17}(GA)_{18}$	AAATGAGTTGCAAAGGCCAA	GGTAAACAAATGATGTGGTGTTC	243	55	1.5
SSRY166	$(GA)_{16}(GGGA)_2(GA)_2$	AATAACAACAAGAGTTGTGGAAAAA	TATCCATGACTGTGATGCGG	244	55	1.5
SSRY167	$(CT)_{27}$	AAAATTGGATGGGACCGTTT	AAGGAAAGGGAGAAATCAAAGA	183	55	1.5
SSRY168	$(GA)_8(N)_4(GA)_2$AGA $(N)_{11}$ $(GA)_{16}$GGA	ACAGCCACACTTGTTCTCCA	CTGCAATCTCCAACAGCAAC	277	45	1.5
SSRY169	GA19A3GAA2	ACAGCTCTAAAAACTGCAGCC	AACGTAGGCCCTAACTAACCC	100	55	1
SSRY170	$(TA)_5(N)_{71}(CT)_{24}$	TCTCGATTTGGTTTGGTTCA	TCATCCTTGTTGCAGCGTTA	299	55	1.5
SSRY171	$(TA)_5$CATA$(GATA)_8$ GC$(GA)_{23}$ $(GTGA)_2$	ACTGTGCCAAAATAGCCAAATAGT	TCATGAGTGTGGGATGTTTTTATG	291	55	1.5
SSRY172	$(CT)_{17}$	TCCAACTGGCTTAACTTGAGG	TTTAGTTTTTGAAACAATGATGAAA	201	55	1.5
SSRY173	$(GT)_3$GC$(GT)_2$ $(GA)_{20}$ GG$(GA)_2$	TGTAAATATGCAAAGAAGCACGA	TACCTTTGGTGGAGTTTGCC	281	55	1.5
SSRY174	$(GA)_{16}$	AACAAAACCATTTTCATGTTGA	TTGCATACTCATCTCCATCTTCA	136	55	1.5
SSRY175	$(GA)_{38}$	TGACTAGCAGACACGGTTTCA	GCTAACAGTCCAATAACGATAAGG	136	55	1.5
SSRY176	$(GA)_{19}$	TGGCTAAATTATTGATGTTTTAGTGT	TTTTTCAAAATAGAGGGACCAA	112	55	1.5
SSRY177	$(CCT)_6$CT$(N)_{65}(CT)_4$ AT$(CT)_{18}$	ACCACAAACATAGGCACGAG	CACCCAATTCACCAATTACCA	268	45	1.5
SSRY178	$(GA)_{20}(N)_{123}(GA)_6$	GGCCCGTAAGGTTTACAGAG	CTGCAAAAACACGATCCCTT	104	55	1.5
SSRY179	$(GA)_{28}$	CAGGCTCAGGTGAAGTAAAGG	GCGAAAGTAAGTCTACAACTTTTCTAA	226	55	1.5
SSRY180	$(GA)_{16}(G)_4(GA)_5$	CCTTGGCAGAGATGAATTAGAG	GGGGCATTCTACATGATCAATAA	163	55	1.5
SSRY181	$(GA)_{22}(G)_3C(GA)_3$GGAA$(GA)_4$	GGTAGATCTGGATCGAGGAGG	CAATCGAAACCGACGATACA	199	55	1.5
SSRY182	$(CA)_{17}(N)_{31}$GAGG $(GA)_8$	GGAATTCTTTGCTTATGATGCC	TTCCTTTACAATTCTGGACGC	253	55	1.5
SSRY183	$GAGC(GA)_8$	TGCTGTGATTAAGGAACCAACTT	TTAACTTTTTCCAGTTCTACCCA	221	55	1.5
SSRY184	$(ATT)_4T(ATT)_3(T)_7$	TCATCCCAAAAATACCTCTAACA	CTCCGACAAGCATGTGAATG	163	55	1.5
SSRY185	$(GC)_3AC(GC)_2A$ $(CA)_2(N)_3(CA)_{10}(GA)_8$	GAAGAAGACGGTTAAAGCAAGTT	ATGCCAGTTTGCTATCCAGG	243	55	1.5
SSRY186	$(CA)_{13}$	GCTTTGTGTAAACAACCTCGC	AATGACCATGCCAACACAAG	297	55	1.5

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