3 Biotech (2020) 10:273
https://doi.org/10.1007/s13205-020-02268-4

ORIGINAL ARTICLE

Identification and characterization of SSR markers of *Guadua chacoensis* (Rojas) Londoño & P.M. Peterson and transferability to other bamboo species

Márcia D. Rossarolla[1] · Tiago C. Tomazetti[1] · Leila N. Vieira[2] · Miguel P. Guerra[1,3] · Gustavo H. F. Klabunde[4] · Ramon F. Scherer[4] · Rosete Pescador[1] · Rubens O. Nodari[1]

Received: 30 January 2020 / Accepted: 20 May 2020 / Published online: 29 May 2020

Abstract

The aim of this study was to develop simple sequence repeat (SSR) markers for genetic studies on *G. chacoensis*, as well as to evaluate their transferability to other bamboo species. Genomic DNA was isolated from *G. chacoensis* and its partial sequencing was used to find SSR loci. The obtained sequencing data were de novo assembled using the software CLC Genomics Workbench® 8.0v. The SSR loci primers were identified and designed with the software SSRLocator. The selected markers were validated using 56 plants sampled in seven populations from southern Brazil. The markers with potential polymorphism were selected and fluorescently labeled for characterization by capillary electrophoresis. In total, 92 SSR loci were found in *G. chacoensis* contigs. Suitable primers were designed for 70 SSR loci, and the remaining 22 SSR loci did not have sequences for primer development. Out of 35 selected SSR markers, after PCR optimization, 10 with high polymorphism potential were characterized. These loci can be used in genetic analyses of *G. chacoensis* and all of them were successfully transferred to other bamboo species. Non-polymorphic loci require further tests with additional plants, from different populations, to identify possibilities of their use.

Keywords Molecular markers · SSR · Genetic diversity · Primer design · Genetic population · Polymorphism

Introduction

Subfamily Bambusoideae (bamboo) is a major Poaceae group that comprises 115 genera and around 1640 species (Kelchner 2013). Natural bamboo populations are distributed all over the world, except for Europe and Antarctica (Clark et al. 2015). Asia has high diversity of bamboo species, particularly in southern China, where they have economic, ecological and social importance (Yuming et al. 2004). Brazil also has high number of bamboo species, including 18 native and five endemic species in the genus *Guadua* Kunth, which are used in construction, furniture, and handicrafts (Greco et al. 2015; Brazil Flora 2020).

Phylogenetic studies have shown that subfamily Bambusoideae is divided into three main monophyletic lineages that correspond to three tribes: temperate woody (Arundinarieae), tropical woody (Bambuseae) and herbaceous (Olyreae) bamboos (Kelchner 2013, Wysocki et al. 2015). The temperate woody bamboo species diverged from others at the beginning of the evolutionary history of the subfamily (Zang et al. 2011) and are now a distinct group. Among

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s13205-020-02268-4) contains supplementary material, which is available to authorized users.

✉ Rubens O. Nodari
rubens.nodari@ufsc.br

[1] Programa de Pós-graduação em Recursos Genéticos Vegetais, Universidade Federal de Santa Catarina, Rodovia Admar Gonzaga,1346, Florianópolis, Santa Catarina 88040-900, Brazil

[2] Programa de Pós-Graduação em Botânica, Universidade Federal do Paraná, Av. Cel. Francisco H. dos Santos, 100, Curitiba, Paraná 81531-980, Brazil

[3] Programa de Pós-graduação em Ecossistemas Agrícolas e Naturais, Universidade Federal de Santa Catarina, Campus Curitibanos, Rodovia Ulysses Gaboardi 3000, Curitibanos, Santa Catarina 89520-000, Brazil

[4] Estação Experimental de Itajaí, Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina, Rodovia Antônio Heil, n°6800, Itajaí, Santa Catarina 88318-112, Brazil

the described species, woody bamboos can be distinguished from others by their infrequent sexual reproduction, with long flowering intervals that range from 7 to 120 years (Janzen 1976). *Guadua* is a representative woody bamboo genus with many native South America species (Clark 2001), including *G. chacoensis* ($2n = 2x = 46$) (Andrada et al. 2007; Rincón and Castillo 2012). This species is ecologically important in its natural habitat and little is known about its reproductive mechanism (Areta et al. 2009). Further, the genetic structure and diversity of *G. chacoensis* native populations are almost unknown. Reproductive cycle monitoring studies describe that *G. chacoensis* flowers at intervals of approximately 31 years (Guerreiro 2014).

The advancement of scientific and technological knowledge about molecular genetics, such as SSR markers, facilitates the characterization of genetic diversity and structure, and also assists in the selection of descriptors for neglected species, such as *G. chacoensis*. Thus, SSR markers overcome the limitations of previous studies, employing dominant molecular markers, and are expected to yield valuable genetic information (Marulanda et al. 2007; Rugeles-Silva et al. 2012). SSR is one of the most informative molecular markers and its uniqueness and value are intrinsic to its multiallelic nature, co-dominant inheritance, relative abundance, broad coverage of the genome, and simple detection by PCR using oligonucleotide primer pairs (forward and reverse) flanking the SSR locus (Powell et al. 1996; Vieira et al. 2016).

Thus, SSRs can potentially be used in many genetic studies (Varshney et al. 2005). However, they can only be used in two ways, either transferred between species that are usually closely related, like species in the same taxon or genus, or developed (identified and validated) for specific species. Specific SSR development requires sequencing the genome, assembling the sequenced fragments, identifying SSR loci, designing primers that flank the SSR region, validation (Powell et al. 1996), and using the DNA of a certain number of individuals. Accordingly, this study aimed to identify, validate and characterize SSR markers for genetic studies about *G. chacoensis* and examine their transferability among other bamboo species.

Materials and methods

Plant material

Guadua chacoensis leaf samples were collected from natural populations. Collection was authorized (ICMbio-SISBIO nº 45390 and 48802-1) by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio). The collected leaf samples were dehydrated and stored in silica gel for subsequent genomic DNA extraction. The DNA from a fresh leaf of an individual (voucher FLOR_0058621) grown in Florianópolis, Brazil (27° 35′ 49″ S 48° 32′ 56″ W), was used for sequencing and SSR loci identification. Leaf samples of other bamboo species were also collected from the germplasm maintained at the Federal University of Santa Catarina, namely: *Bambusa oldhamii*, *Chusquea tenella*, *Dendrocalamus latiflorus, Dendrocalamus yunnanensis*, *Farguesia gaolinensis*, *Farguesia yunnanensis*, *Guadua angustifolia*, *Guadua paniculata*, *Guadua paraguaiyana*, *Merostachys scandens*, *Merostachys speciosa*, *Merostachys glauca*, *Oxytenanthera abyssinica*, *Phyllostachys aurea, Phyllostachys edulis*, *Phyllostachys pubescens*, *Pseudosasa mirabilis* and *Shibatea kumasasa*. Eight plants of *G. chacoensis* were sampled from six natural populations (1.3 km–12 km apart) in Parque Nacional do Iguaçu, Foz do Iguaçu, Brazil, and one cultivated population from Rancho Queimado, Brazil, which is 570.43 km from the natural populations and was used to validate the SSR markers.

DNA isolation

Genomic DNA was extracted from 100 mg of dried leaves, previously ground using a Precellys® homogenizer (Bertin Technologies, Montigny-le-Bretonneux, France), with NucleoSpin Plant II kit (Macherey–Nagel) according to the manufacturer's instructions. The 100 µl of extracted DNA was immediately frozen at − 20 °C until analysis. DNA quality and quantity were determined with NanoDrop ND-1000 Spectrophotometer (Thermo Fischer Scientific, Waltham, MA, USA) and 0.8% agarose gel electrophoresis performed at 6 V cm^{-1} for 60 min and stained with GelRed™ (Biotium Inc., Hayward, CA, USA). For comparison and quantification, Lambda DNA was loaded into gel at concentrations of 12.5 ng μl^{-1}, 25 ng μl^{-1}, 50 ng μl^{-1}, and 100 ng μl^{-1}. For the NanoDrop analysis, 1 µL of DNA was used at the wavelengths 230, 260, and 280 nm, and both the 260/280 and 230/280 ratios were examined for every sample.

DNA sequencing and SSR identification

A total of 1 ng of genomic DNA was used to prepare sequencing libraries with a Nextera XT DNA Sample Prep Kit (Illumina Inc., San Diego, California, USA) according to the manufacturer's instructions. The libraries were sequenced using a MiSeq Reagent Kit v3 (600 cycles) and an Illumina MiSeq Sequencer (Illumina Inc., San Diego, California, USA). The obtained paired-end reads (2 × 300 bp) were used for de novo assembly with CLC Genomics Workbench 8.0v. Paired-end sequence reads were trimmed of low-quality data with a quality score limit of 0.01 using CLC Genomics Workbench 8.0v and reads of less than 50 bp in length were discarded. Organellar reads were excluded by mapping against two bamboo mitochondrion genomes (gb|JN120789.1, gb|EU365401.1)

and the *G. chacoensis* chloroplast genome (gb|KT373814.1; Vieira et al. 2015) using the Basic Local Alignment Search Tool (BLAST, Altschul et al. 1990). Trimmed read sequences were de novo assembled. The obtained nuclear contigs with depth coverage (higher than 5X) were selected and analyzed with SSRLocator (Maia et al., 2008) for SSR identification, with a threshold of twelve repeat units for mononucleotide SSR, six repeat units for dinucleotide SSR, four repeat units for trinucleotide SSRs, three repeat units for tetra- and pentanucleotide SSR, and two repeat units for hexanucleotide SSR. Primers were designed with SSR Locator using the PRIMER3 algorithm by setting product size ranges from 100 to 350 bp, primer size from 18 to 22 bp, GC content from 40 to 60%, 1 °C as the maximum difference between the melting temperatures of the left and right primers, and a melting temperature (TM) of 57 °C (minimum) and 63 °C (maximum). Primers were analyzed with the software Gene Runner® to ensure the absence of secondary structure formation.

SSR validation by gel electrophoresis

Among the identified SSRs for primer design, 35 with a higher potential for use in population genetic studies were selected. PCR amplifications were carried out to test annealing temperatures (52–62 °C) and reagent concentrations for each of the selected primers. The following thermal cycle conditions were initially used for PCR reactions: 0.2 µM of each primer, 1 unit of *Taq* DNA polymerase (Invitrogen), 0.2 mM of each dNTP, 2.0 mM $MgCl_2$, 15 ng of template DNA, and 1 × PCR buffer (Invitrogen); the final volume was adjusted to 10 µl. The PCR profile was the following: 95 °C for 3 min; 35 cycles of denaturation at 95 °C for 30 s, annealing temperature (52–62 °C) for 30 s, and 72 °C for 1 min; followed by a final extension at 72 °C for 30 min in a Veriti® 96-Well Thermal Cycler (Applied Biosystems, California, USA). PCR products were submitted to 3% agarose gel electrophoresis at 6 V cm^{-1} for 120 min and stained with GelRed™. Product sizes were determined by comparison with a 1-kb ladder (Invitrogen). The PCR reactions with dubious results were submitted to new PCR conditions for optimization. After optimization, polymorphism was evaluated in 4% denaturing polyacrylamide gels stained with silver nitrate. Gels were run with 1 × TBE buffer on a vertical electrophoresis apparatus for 90 min at 75 V. Product sizes were determined by comparison with a 100-bp DNA ladder (Invitrogen) (Figure S1).

SSR characterization by multiplex-ready PCR for fluorescence-based genotyping

SSR characterization was made using plant DNA from six natural populations (24 plants of each population) from Paraná State, Brazil, and 12 plants from a population grown in Santa Catarina State, Brazil. The loci with higher polymorphism potential, validated by gel electrophoresis, were selected and primers were fluorescent dye labeled. Genotyping was performed on the ABI Genetic Analyzer 3500xL platform (Applied Biosystems, Forster City, CA). The different fluorescent dye-labeled primers, with different spectra, allowed for multiplex reactions (3–4 primers per reaction). The polymorphism level of each locus was estimated by the polymorphism information content (PIC). Where $PIC = 1 - \sum p_i^2$ and p_i is the frequency of the i-th allele (Maroof et al. 1994; Anderson et al. 1993).

SSR transferability to other species

SSR transferability was tested using DNA from 18 different bamboo species. Regarding the polymorphic loci, the primers were considered transferred when the amplification resulted in one or two alleles near the expected size. The PCR condition and electrophoresis protocols were the same used for *G. chacoensis*.

Results

Sequencing the *G. chacoensis* genomic DNA resulted in 296,699 high-quality paired-end reads. After trimming (0.01 quality threshold) and excluding organellar reads, we obtained 239,621 paired-end reads (average length = 238.79). These reads were submitted to de novo assembly and 6013 contigs were obtained (N50 = 685).

We analyzed the occurrence, type, and distribution of SSRs in *G. chacoensis* contigs. In total, 92 SSRs were identified. Among them, trinucleotide repeats were the most common with 40 occurrences; whereas, di- (17), tetra- (18), penta- (8), and hexanucleotide repeats (9) occurred with lower frequency (Fig. 1). The most frequent motifs were AT (7.6%), TA (6.5%), TTC (5.4%) and TTA (4.3%). However, only 70 loci were in possible regions to design primers free of secondary structures (Table S1). Among them, 35 loci were selected for validation (Table 1).

PCR reactions with *Gcha17*, *Gcha19*, *Gcha22*, *Gcha23,* and *Gcha24* revealed no amplification products for any of the tested temperatures (52–62 °C) or plants. Different results were obtained for PCR reactions with primers designed for the *Gcha25*, *Gcha30* and *Gcha35* loci, since they showed an abundance of nonspecific bands. These characteristics were used as criteria for selecting the markers to be characterized, as well as the amplicons sharpness and quality revealed after gel electrophoresis. The 12 markers selected for characterization and fluorescent labeling are listed in Table 2.

The SSR characterization showed that the *Gcha33* and *Gcha18* loci had nonspecific amplification products for all plants, possibly due to instability during amplification

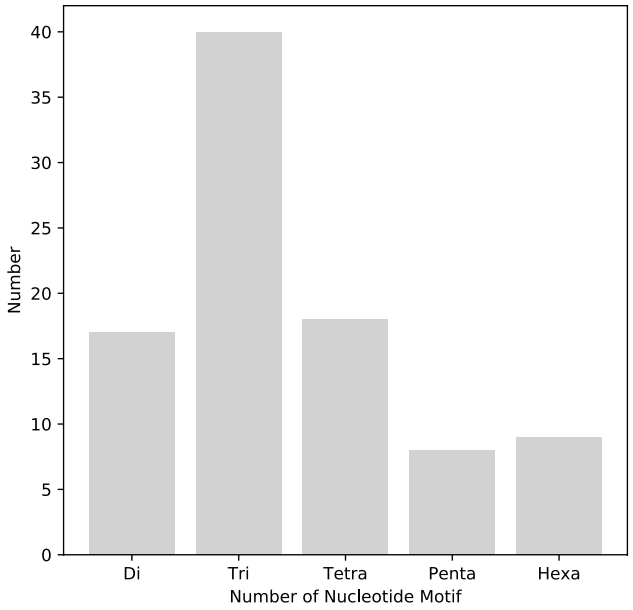

Fig. 1 Number of distinct types of genomic simple sequence repeats (SSRs) identified in low-depth sequences of the bamboo *Guadua chacoensis*, grown in Florianópolis, SC (Brazil), using the Illumina MiSeq Sequencer platform (Illumina Inc., San Diego, California, USA). Di-, tri-, tetra-, penta- and hexanucleotides represent 2, 3, 4, 5 and 6 nucleotides that are the length of an SSR repeat

reactions or annealing inconsistencies and hence, these loci were discarded. The *Gcha01, Gcha02, Gcha04, Gcha05, Gcha07, Gcha10, Gcha11, Gcha12, Gcha13* and *Gcha21* loci were polymorphic, of which the *Gcha02* locus had the highest allele number (4) and the highest PIC value (0.507). The *Gcha04* locus, with only 2 alleles, of which one is rare (frequency < 5%), had the lowest PIC value (0.039) (Table 3).

To further characterize the new SSR markers, five multiplexes were formed. Multiplex "A" was composed of the *Gcha04* and *Gcha18* loci (Figure S2), multiplex "B" of the *Gcha08*, *Gcha10*, *Gcha21* and *Gcha33* loci (Figure S3), multiplex "C" of the *Gcha02, Gcha05* and *Gcha06* loci, multiplex "D" of *Gcha01* and *Gcha07* loci (Figure S4), and multiplex "E" of the *Gcha11*, *Gcha12* and *Gcha13* loci (Figure S5). All of these multiplexes showed good and unambiguous amplification results.

The transferability to other bamboo species was successfully achieved for all tested SSR loci in 15 (i.e., all but *Phyllostachys pubescens*, *Phyllostachys edulis* and *M. glauca*) of the 15 species (Table 4, Figs. 6S and 7S).

Discussion

AT and GC content of the genome has been used in evolutionary ecology studies about monocots (Smarda et al. 2014). The higher AT content than GC content in the *G. chacoensis* genome, observed in the present work, is in accordance with previous studies about other bamboos (Liu et al. 2012), as well as other plant species, such as *Zea mays*, *Oryza sativa*, *Beta vulgaris,* and *Arabidopsis thaliana*. On the contrary, in animal genomes, GC content is higher (Beven et al. 1998; Kubo et al. 2000; Tóth et al. 2000; Barow and Meister 2002; McCouch et al. 2002; Jaillon et al. 2004; Yu et al. 2012).

High di- and trinucleotide repeat frequency was already described for the bamboos *D. latiflorus* (Bhandawat et al. 2015) and *Phyllostachys violascens* (Cai et al. 2019), as well as for conifer species, such as *Pinus taeda* and *Picea glauca* (Bérubé et al. 2007). These SSR motifs are reported as more informative, due to their greater stability compared to mononucleotides, and higher polymorphism compared to tetra-, penta- and hexanucleotide repeats (Samadi et al. 1998; Bérubé et al. 2007).

The annealing temperature is important to develop protocols with accurate results in molecular genetic analyses. An incorrect annealing temperature can cause unspecific amplifications or a lack of amplifications, resulting in a false positive or false negative, respectively, making it impossible to use (Ishii and Fukui 2001; Sipos et al. 2007). During the annealing temperature test, which ranged from 52 to 62 °C, we were able to discard the primers designed for the *Gcha17*, *Gcha19*, *Gcha22*, *Gcha23* and *Gcha24* loci, due to the absence of amplification at all tested temperatures. We also discarded the *Gcha25*, *Gcha30* and *Gcha35* loci, due to the presence of many unspecific amplicons at all tested temperatures. Further studies are necessary to optimize the protocol of these loci and to develop additional primers without these problems. For all other primer loci, the annealing temperature was 58 °C, except for *Gcha11*, *Gcha12*, and *Gcha13*, where 55 °C resulted in the best amplification (Figure S1). Thus, all the multiplex mixes were standardized with this temperature.

Among the polymorphic loci characterized in this study, only the *Gcha05* (dinucleotide + tetranucleotide) and *Gcha21* (tetranucleotide) loci were not trinucleotide repeats, which demonstrated that trinucleotide repeats were also informative for *G. chacoensis*. The allele number and PIC values showed reduced diversity for the characterized loci compared to other species (Hammami et al. 2014; Cubry et al. 2014). However, this seems to be an intrinsic feature of bamboo populations, since similar results were found for *Dendrocalamus giganteus* (Tian et al. 2012). It is worth mentioning that these estimators and values may be evaluated again for further characterization with additional and distant populations to confirm this feature of *G. chacoensis*.

The phenological cycle and reproductive biology of *G. chacoensis* are possible causes of the reduced polymorphism found in this study. Flowering and, consequently, allelic recombination in bamboos are governed by poorly

Table 1 Features of 35 simple sequence repeat (SSR) markers of *Guadua chacoensis* selected for validation

Locus	GenBank accession		Primer sequence (5′–3′)	TM (°C)	Product size (bp)	(Motif) repeat number
Gcha01	MN644912	F	CCAGTTGATATGCAGAACCT	55	238	(GCC)4
		R	TTCTCTCTATCGTCCTCAGC			
Gcha02	MN644913	F	TAGCACGTACCATCAAACAA	55	249	(GCG)4
		R	CCTACCGTAAGCTCTCTGAA			
Gcha03	MN644914	F	TTCACCTAGACTTCTGGCAT	55	289	(TTC)4
		R	TGAGGAGAGAAAGGTTGAGA			
Gcha04	MN644915	F	TTGTGCATGACTTGTTGAGT	55	239	(AGA)4
		R	TCACCTTACAATAATTCGCC			
Gcha05	MN644916	F	TAGTCGGGTGATCATGAAAT	55	291	(AT)6-(AATT)3
		R	GTCGATATATTCTTGGCTGC			
Gcha06	MN644917	F	AGGGGAATACAGATATGCAA	55	312	(GGA)4
		R	CGTTCTCATGATACTCCCAT			
Gcha07	MN644918	F	AACTTCTCCCTCGAAACCT	55	250	(GGA)4-(GGA)5
		R	GTGTCACAGATGACGCAATA			
Gcha08	MN644919	F	AGCTTCACAGATGACGAACT	55	285	(AATA)3
		R	AAGATGACAACGACGATGAT			
Gcha09	MN644920	F	GCGGGTTGTAGAATTAACAG	55	265	(TGC)4
		R	GAGAATGTTGCACTGGATTT			
Gcha10	MN644921	F	CTGTGGACATGAACAATCTG	55	244	(AAC)4
		R	ATGTGCAGCTTTCTCTCAAT			
Gcha11	MN644922	F	GATTGAGGAATGAGATGGAA	55	214	(CAAG)3
		R	GAACTTGGCTTGAAAGATTG			
Gcha12	MN644923	F	CAATCACATCACTGTTCAGC	55	166	(TA)6
		R	CTGCACCTGGAGAGTTTTAC			
Gcha13	MN644924	F	ATGTTGAGGATGAGATCGAC	55	177	(AGC)4
		R	CGAAGAAAATCTGGAACAAG			
Gcha14	MN644925	F	GAGTACTTCGCCTTGCTAGA	55	243	(GAA)4
		R	GTGCTGGTGTTTCTTCTTCT			
Gcha15	MN644926	F	CTCTTCGTTCACACTTCTCC	55	159	(TCT)4
		R	AAGGAAATACCGAAGAGGAC			
Gcha16	MN644927	F	GGTCTGTTCTTCTTCCTTCC	55	246	(TTC)4
		R	CTCCAAAATTGAAGATGAGG			
Gcha17	MN644928	F	GGACCTAAACGCAATTTCTA	55	226	(TA)18
		R	CACCAAAGTTGGAGATGTTT			
Gcha18	MN644929	F	GAACTACGGCAAGAACAAAG	55	305	(AAG)4
		R	AACACACATGAACGTTAGCA			
Gcha19	MN644930	F	GTATTGGGCCGAGTACTGT	55	254	(CCG)5
		R	GGCATAAAAGGTAGCAAATG			
Gcha20	MN644931	F	AATGTCTTTGTTCTGGTTGG	55	194	(AT)16
		R	AGACAGGTTTGCAATAGAGC			
Gcha21	MN644932	F	AACTAGGGAATTGGAAGCTC	55	310	(TAGC)3
		R	TATGAAGTTGACACCCCTTT			
Gcha22	MN644933	F	CGAAGAAAGGAGATCAACTG	55	210	(CGG)4
		R	GGGACGTTACAGCAATAGAG			
Gcha23	MN644934	F	TCCGCCTATATTGTTGAGTT	55	244	(TTTA)3
		R	CTAGTGCTTCTTCCTCATGG			
Gcha24	MN644935	F	GGTGAAGAAGGATGTATGGA	55	304	(AC)6
		R	TATTGCGTGATGTAGTTTGC			

Table 1 (continued)

Locus	GenBank accession		Primer sequence (5′–3′)	TM (°C)	Product size (bp)	(Motif) repeat number
Gcha25	MN644936	F	GAGCCCATATGTCATTGTCT	55	328	(TTCT)3
		R	GATCTTCAATCTCTGCTTGC			
Gcha26	MN644937	F	GAGGGCTGTAAGCAACTAAA	55	309	(TTC)4
		R	ACATGAAGAACAGGGATGAG			
Gcha27	MN644938	F	AACGTATTTTCGACCGC	55	315	(TCA)4
		R	GTAGATGGATCGAAGATGGA			
Gcha28	MN644939	F	TCTATTGTCCTTAGCCAGTCA	55	280	(GGC)4
		R	CTGGAAACATCAATGAGGAC			
Gcha29	MN644940	F	GAGCACAAAAACCTCAAAAC	55	297	(ACTC)3
		R	AAGGAATGGATGAGATGCTA			
Gcha30	MN644941	F	GGGACTACGAGGTAGGACTT	55	318	(CAAT)3
		R	GAGCTTGGGTTAAATGAGTG			
Gcha31	MN644942	F	AGTCCAGTCGCACTCTTCTA	55	167	(CTTC)3
		R	TGTGTAATATAACCCGGAGG			
Gcha32	MN644943	F	ATACCGCCTGGAGAAGTT	55	284	(GAC)5
		R	GACAATCATCCTTGGAGAAA			
Gcha33	MN644944	F	GATCTCAGAAGATGGATTCG	55	208	(CAAC)3
		R	ATACATAATGAATGGGTGGC			
Gcha34	MN644945	F	TTGAGTACAAGGGATGCTCT	55	283	(GT)7
		R	GGATCTAGGTCGAACATTCA			
Gcha35	MN644946	F	AACTCAAGACCCTGGACC	55	187	(GAG)4
		R	GATTTCCAACTACGAAGTGC			

understood environmental factors (Campanello et al. 2007). In *G. chacoensis* species, flowering occurs at intervals of approximately 31 years (Areta et al. 2009), pollen is predominantly dispersed by wind, seeds are predominantly dispersed by associated fauna, and reproductive behavior is similar to other woody bamboo species (Areta and Bodrati 2008; Montti et al., 2011a). These features do not favor the quick establishment or spread of new alleles (Eriksson 1997) or allelic transgressive combinations, yet little is known about the effects of this reproductive behavior on the ecology and genetic structure of bamboo populations (Budke et al. 2010; Montti et al. 2011b).

This is the first report that characterizes SSR loci for *G. chacoensis* genetic studies. The informative value of each characterized locus obtained in the present study may vary from the analysis of other populations. Therefore, genetic studies with geographically more distant populations can be based on the molecular markers developed here. With additional data, more accurate estimates of the polymorphic potential and allele number of each locus could be made. The polymorphic loci described here represent an advance in phylogenetic and population genetic studies in *G. chacoensis* and closely related species, in which primer transferability may be possible.

Conclusion

The shotgun genome sequencing of *G. chacoensis* with the Illumina platform allowed the identification, validation and characterization of 12 SSR markers for this species. Among them, 10 were polymorphic and can be used in *G. chacoensis* population genetic studies. These markers could be used in analyses about *G. chacoensis* genetic diversity, relationships between natural populations and phylogenetics, as well as populations of the 12 bamboo species that were found to be transferable.

Table 2 Multiplex sets of loci used for characterization 12 SSR markers of *Guadua chacoensis*

Locus	Motif	Sequence (5′–3′)	AT[a]	Product size	Fluorescent dye
Multiplex A					
Gcha04	(AGA)4	F: TTGTGCATGACTTGTTGAGT	58	235–238	NED
		R: TCACCTTACAATAATTCGCC			
Gcha18	(AAG)4	F: GAACTACGGCAAGAACAAAG		–	PET
		R: AACACACATGAACGTTAGCA			
Multiplex B					
Gcha10	(AAC)4	F: CTGTGGACATGAACAATCTG	58	241–247	NED
		R: ATGTGCAGCTTTCTCTCAAT			
Gcha21	(TAGC)3	F: AACTAGGGAATTGGAAGCTC		300–308	VIC
		R: TATGAAGTTGACACCCCTTT			
Gcha33	(CAAC)3	F: GATCTCAGAAGATGGATTCG		–	PET
		R: ATACATAATGAATGGGTGGC			
Multiplex C					
Gcha02	(GCG)4	F: TAGCACGTACCATCAAACAA	58	225–249	FAM
		R: CCTACCGTAAGCTCTCTGAA			
Gcha05	(AT)6-(AATT)3	F: TAGTCGGGTGATCATGAAAT		287–291	NED
		R: GTCGATATATTCTTGGCTGC			
Multiplex D					
Gcha01	(GCC)4	F: CCAGTTGATATGCAGAACCT	58	235–238	FAM
		R: TTCTCTCTATCGTCCTCAGC			
Gcha07	(GGA)4-(GGA)5	F: AACTTCTCCCTCGAAACCT		249–252	NED
		R: GTGTCACAGATGACGCAATA			
Multiplex E					
Gcha11	(CAAG)3	F: GATTGAGGAATGAGATGGAA	55	202–214	FAM
		R: GAACTTGGCTTGAAAGATTG			
Gcha12	(TA)6	F: CAATCACATCACTGTTCAGC		153–165	VIC
		R: CTGCACCTGGAGAGTTTTAC			
Gcha13	(AGC)4	F: ATGTTGAGGATGAGATCGAC		174–180	NED
		R: CGAAGAAAATCTGGAACAAG			

[a]Annealing temperature (°C)

Table 3 Allele frequency and polymorphism information content (PIC) for 10 of the characterized SSR markers of *Guadua chacoensis*

Locus	GenBank access	Allele frequency (%)				PIC
		Allele A	Allele B	Allele C	Allele D	
Gcha01	MN644912	0.50	0.50			0.500
Gcha02	MN644913	0.57	0.41	0.01	0.01	0.507
Gcha04	MN644915	0.98	0.02			0.039
Gcha05	MN644916	0.51	0.49			0.500
Gcha07	MN644918	0.50	0.50			0.500
Gcha10	MN644921	0.51	0.47	0.02		0.519
Gcha11	MN644922	0.50	0.43	0.07		0.560
Gcha12	MN644923	0.50	0.50			0.500
Gcha13	MN644924	0.85	0.15			0.255
Gcha21	MN644932	0.51	0.49			0.500

Table 4 Transferability of SSR loci from *Guadua chacoensis* to other bamboo species

Species	SSR loci transferable	Species	SSR loci transferable
Bambusa oldhamii	Yes[a]	*Merostachys glauca*	Not[b]
Chusquea tenella	Yes	*Merostachys scandens*	Yes
Dendrocalamus latiflorus	Yes	*Merostachys speciosa*	Yes
Dendrocalamus yunnanensis	Yes	*Oxytenanthera abyssinica*	Yes
Farguesia gaolinensis	Yes	*Phyllostachys aurea*	Yes
Farguesia yunnanensis	Yes	*Phyllostachys edulis*	Not
Guadua angustifolia	Yes	*Phyllostachys pubescens*	Not
Guadua paniculata	Yes	*Pseudosasa mirabilis*	Yes
Guadua paraguaiyana	Yes	*Shibatea kumasasa*	Yes

[a]Yes—all tested loci were successfully transferred, [2]Not—none SSR loci amplified in these bamboo species

Acknowledgements The authors thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) (Grant no. Finance Code 001) for the scholarship awarded to MDR and LNV, and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the financial support (Proc. 457726/2013-0) and scholarships awarded to MPG, RON, TCT, GHFK, and RP. The authors would also like to thank to Núcleo de Fixação Biológica de Nitrogênio/UFPR for sequencing. We are also grateful to the Parque Nacional do Iguaçu (ICMBio/Brazil), and to the farms Sarakura and Vagalume, for supporting and allowing the collection of plant material.

Author contributions MDR, MPG, RP and RON conceived the research. MDR and RON designed the experiments. MDR, TCT, GHFK and RFS conducted the lab and statistical analyses. MDR and RON wrote the manuscript. RP, RFS, GHFK, TCT, and LNV revised the draft of the manuscript. MPG coordinated and supported the research and revised the manuscript. All authors read and approved the final manuscript version.

Compliance with ethical standards

Conflict of interest All authors hereby declare that there is no conflict of interest.

Ethical approval This article does not include any studies with human participants or animals performed by any of the authors.

Informed consent This article does not involve any informed consent.

References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Anderson JA, Churchill GA, Autrique JE, Tanksley SD, Sorrels ME (1993) Optimizing parental selection for genetic linkage maps. Genome. 36:181–186. https://doi.org/10.1139/g93-024

Andrada AR, Lozzia ME, Cristóbal ME (2007) Contribution to cytological knowledge of *Guadua chacoensis*. Lilloa 44:3–6

Areta JL, Bodrati A (2008) Behaviour, identification and relationship with bamboos of the sooty grassquit (*Tiaris fuliginosa*) in Misiones, Argentina. Hornero 23:77–86

Areta JI, Bodrati A, Cockle K (2009) Specialization on Guadua bamboo seeds by three bird species in the Atlantic forest of Argentina. Biotropica 41:66–73. https://doi.org/10.1111/j.1744-7429.2008.00458.x

Barow M, Meister A (2002) Lack of correlation between AT frequency and genome size in higher plants and the effect of non-randomness of base sequences on dye binding. Cytometry 47:1–7. https://doi.org/10.1002/cyto.10030

Bérubé Y, Zhuang J, Rungis D, Ralph S, Bohlmann J, Ritland K (2007) Characterization of EST-SSRs in loblolly pine and spruce. Tree Genet Genomes 3:251–259. https://doi.org/10.1007/s11295-006-0061-1

Beven M, Bancroft I, Bent E, Love K, Goodman H, Dean C et al (1998) Analysis of 1.9 Mb of contiguous sequence from chromosome 4 of *Arabidopsis thaliana*. Nature 391:485–493. https://doi.org/10.1038/35140

Bhandawat A, Singh G, Raina AS, Kaur J, Sharma RK (2015) Development of genic SSR marker resource from RNA-Seq data in *Dendrocalamus latiflorus*. J Plant Biochem Biotechnol. https://doi.org/10.1007/s13562-015-0323-9

Brazil Flora G (2020) Brazilian Flora 2020 project—Projeto Flora do Brasil 2020. v393.237. Instituto de Pesquisas Jardim Botânico do Rio de Janeiro. Dataset/Checklist. https://doi.org/10.15468/1mtkaw

Budke JC, Alberti MS, Zanardi C, Baratto C, Zanin EM (2010) Bamboo dieback and tree regeneration responses in a subtropical forest of South America. For Ecol Manag 260:1345–1349. https://doi.org/10.1016/j.foreco.2010.07.028

Cai K, Zhu L, Zhang K, Li L, Zhao Z, Zeng W, Lin X (2019) Development and characterization of EST-SSR markers from RNA-Seq data in *Phyllostachys violascens*. Front Plant Sci 10:50. https://doi.org/10.3389/fpls.2019.00050

Campanello PI, Gatti MG, Ares A, Montti L, Goldstein G (2007) Tree regeneration and microclimate in a liana and bamboo-dominated semideciduous Atlantic Forest. For Ecol Manag 252:108–117. https://doi.org/10.1016/j.foreco.2007.06.032

Clark LG (2001) Diversification and endemism in and woody bamboos (Poaceae: Bambusoideae). Bamboo Sci Cult 15:14–19

Clark LG, Londoño X, Ruiz-Sanchez E (2015) Bamboo taxonomy and habitat. In: Liese W, Kohl M (eds) Bamboo, tropical forestry, vol 10. Springer International Publishing, Berlin, pp 1–30

Cubry P, Pujade-Renaud V, Garcia D, Espeout S, Guen VL, Granet F, Seguin M (2014) Development and characterization of a new set

of 164 polymorphic EST-SSR markers for diversity and breeding studies in rubber tree (*Hevea brasiliensis* Müll. Arg.). Plant Breed 133:419–426. https://doi.org/10.1111/pbr.12158

Eriksson G (1997) Sampling of genetic resources populations in the absence of genetic knowledge. In: Turok J, Collin E, Demesure B, Erikkson G, Kleinschmit J, Rusanen M, Stephan R (eds) Noble hardwoods network: second meeting. International Plant Genetic Resources Institute, Rome, pp 61–75

Greco TM, Pinto MM, Tombolato FC, Xia N (2015) Diversity of bamboo in Brazil. J Trop Subtrop Bot 23:1–16

Guerreiro C (2014) Flowering cycles of woody bamboos native to southern South America. J Plant Res 127:307–3013. https://doi.org/10.1007/s10265-013-0593-z

Hammami R, Jouve N, Soler C, Frieiro E, González JM (2014) Genetic diversity of SSR and ISSR markers in wild populations of *Brachypodium distachyon* and its close relatives *B. stacei* and *B. hybridum* (Poaceae). Plant Syst Evol 300:2029–2040. https://doi.org/10.1007/s00606-014-1021-0

Ishii K, Fukui M (2001) Optimization of annealing temperature to reduce bias caused by a primer mismatch in multitemplate PCR. Appl Environ Microbiol 67(8):3753–3755. https://doi.org/10.1128/AEM.67.8.3753-3755.2001

Jaillon O, Aury JM, Brunet F, Petit JL, Thomann NS, Maucell E et al (2004) Genome duplication in the teleost fish *Tetraodon nigroviridis* reveals the early vertebrate proto-karyotype. Nature 431:21. https://doi.org/10.1038/nature03025

Janzen DH (1976) Why bamboos wait so long to flower. Annu Rev Ecol Syst 7:347–391

Kelchner SA (2013) Bamboo Phylogenetic Group. Higher level phylogenetic relationships within the bamboos (Poaceae: Bambusoideae) based on five plastid markers. Mol Phylogenet Evol 67:404–413. https://doi.org/10.1016/j.ympev.2013.02.005

Kubo T, Nishizawa S, Sugawara A, Itchoda N, Estiati A, Mikami T (2000) The complete nucleotide sequence of the mitochondrial genome of sugar beet (*Beta vulgaris* L.) reveals a novel gene for tRNA Cys (GCA). Nucl Acids Res 28:2571–2576. https://doi.org/10.1093/nar/28.13.2571

Liu M, Qiao G, Jiang J, Yang H, Xie L, Xie J, Zhuo R (2012) Transcriptome sequencing and de novo analysis for Ma bamboo (*Dendrocalamus latiflorus* Munro) using the Illumina platform. Plos One 7:10. https://doi.org/10.1371/journal.pone.0046766

Maia LC, Palmieri DA, Souza VQ, Kopp MM, Carvalho FIF, Oliveira AC (2008) SSR locator: tool for simple sequence repeat discovery integrated with primer design and PCR simulation. Int J Plant Genom 1:5. https://doi.org/10.1155/2008/412696

Maroof MAS, Biyashev RM, Yang GP, Zhang Q, Allard W (1994) Extraordinarily polymorphic microsatellite DNA in barley: Species diversity, chromosomal locations, and population dynamics. Proc Natl Acad Sci USA 91:5466–5470. https://doi.org/10.1073/pnas.91.12.5466

Marulanda ML, López AM, Claroz JL (2007) Analyzing the genetic diversity of *Guadua* spp. in Colombia using rice and sugarcane microsatellites. Crop Breed Appl Biotechnol 7:43–51

McCouch SR, Teytelman L, Xu Y, Katarzyna B, Lobos KB, Clare K et al (2002) Development and mapping of 2240 new SSR Markers for rice (*Oriza sativa* L.). DNA Res 9:199–207. https://doi.org/10.1093/dnares/9.6.199

Montti L, Campanello PI, Goldstein G (2011a) Flowering, die-back and recovery of a semelparous woody bamboo in the Atlantic Forest. Acta Oecol 37:361–368. https://doi.org/10.1016/j.actao.2011.04.004

Montti L, Campanello PI, Gatti MG, Blundo C, Austin AT, Sala OE, Goldstein G (2011b) Understory bamboo flowering provides a very narrow light window of opportunity for canopy-tree recruitment in a neotropical forest of Misiones, Argentina. For Ecol Manag 262:1360–1369. https://doi.org/10.1016/j.foreco.2011.06.029

Powell W, Machray GC, Provan J (1996) Polymorphism revealed by simple sequence repeats. Trends Plant Sci 1:215–222. https://doi.org/10.1016/1360-1385(96)86898-1

Rincón JCV, Castillo NR (2012) Estimation of cell cycle duration and standardization of cytogenetic protocol *Guadua angustifolia* Kunth var. angustifolia (Bambusoideae, Poaceae). Rev Invest Univ Quindío (Col) 23:81–91

Rugeles-Silva PA, Posso-Terranova AM, Lodoño X, Barrera-Marín N, Muños-Flórez JE (2012) Molecular characterization of *Guadua angustifolia* Kunth using RAM's. Acta Agron 61:325–330

Samadi S, Artiguebielle E, Estoup A, Pointier JP, Silvain JF, Heller J, Cariou ML, Jarne P (1998) Density and variability of dinucleotide microsatellites in the parthenogenetic polyploid snail *Melanoides tuberculata*. Mol Ecol 7:1233–1236. https://doi.org/10.1046/j.1365-294x.1998.00405.x

Sipos R, Székely AJ, Palatinszky M, Révész S, Márialigeti K, Nikolausz M (2007) Effect of primer mismatch, annealing temperature and PCR cycle number on 16S rRNA gene-targeting bacterial community analysis. FEMS Microbiol Ecol 60(2):341–350. https://doi.org/10.1111/j.1574-6941.2007.00283.x

Smarda P, Bures P, Horová L, Leitch IJ, Mucina L, Pacini E, Tichy L, Grulich V, Rotreklová O (2014) Ecological and evolutionary significance of genomic GC content diversity in monocots. Proc Natl Acad Sci USA 111:E4096–E4102. https://doi.org/10.1073/pnas.1321152111

Tian B, Yang H-Q, Wong K-M, Liu A-Z, Ruan Z-Y (2012) ISSR analysis shows low genetic diversity versus high genetic differentiation for giant bamboo, *Dendrocalamus giganteus* (Poaceae: Bambusoideae), in China populations. Genet Resour Crop Evol 59:901–908. https://doi.org/10.1007/s10722-011-9732-3

Tóth G, Gáspári Z, Jurka J (2000) Microsatellites in different eukaryotic genomes: survey and analysis. Genome Res 10:967–981. https://doi.org/10.1101/gr.10.7.967

Varshney RK, Graner A, Sorrells ME (2005) Genic microsatellite markers in plants: features and applications. Trends Biotechnol 23:48–55. https://doi.org/10.1016/j.tibtech.2004.11.005

Vieira LN, Anjos KG, Faoro H, Fraga HPF, Greco TM, Pedrosa FO, Souza EM, Rogalski M, Souza RF, Guerra MP (2015) Phylogenetic inference and SSR characterization of tropical woody bamboos tribe Bambuseae (Poaceae: Bambusoideae) based on complete plastid genome sequences. Curr Genet. https://doi.org/10.1007/s00294-015-0549-z

Vieira MLC, Santini L, Diniz AL, Munhoz CF (2016) Microsatellite markers: what they mean and why they are so useful. Genet Mol Biol 39(3):312–328. https://doi.org/10.1590/1678-4685-GMB-2016-0027

Wysocki WP, Clark LG, Attigala L, Ruiz-Sanchez E, Duvall MR (2015) Evolution of the bamboos (Bambusoideae; Poaceae): a full plastome phylogenomic analysis. BMC Evol Biol 15:50. https://doi.org/10.1186/s12862-015-0321-5

Yu M, Hon GC, Szulwach KE, Song CX, Zhang L, Kim A, Li X, Dai Q, Shen Y, Park B, Min JH, Jin P, Ren B, He C (2012) Base-revolution analysis of 5-hydroxymethylcytosine in the mammalian genome. Cell 149:1368–1380. https://doi.org/10.1016/j.cell.2012.04.027

Yuming Y, Kanglin W, Shengji P, Jiming H (2004) Bamboo diversity and traditional uses in Yunnan, China. BioOne 24:157–165. https://doi.org/10.1659/0276-4741(2004)024%5b0157:bdatui%5d2.0.co;2

Zang YJ, Ma PF, Li DZ (2011) High-throughput sequencing of six bamboo chloroplast genomes: phylogenetic implications for temperature woody bamboos (Poaceae: Bambusoideae). Plos One 6:5. https://doi.org/10.1371/journal.pone.0020596