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RESEARCH ARTICLE

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Development of SSR loci in *Prosopis tamarugo* Phillipi and assessment of their transferability to species of the Strombocarpa section

Roberto Contreras (Contreras, R.)[1*], Felipe S. Carevic (Carevic, F.S.)[2], Vincenzo Porcile (Porcile, V.)[1] and Mariana Arias (Arias, M.)[1]

[1]*Centro Regional de Investigación y Desarrollo Sustentable de Atacama (CRIDESAT), Universidad de Atacama, Copayapu 485, Copiapó, Chile.*
[2]*Laboratorio de Ecología Vegetal, Facultad de Recursos Naturales Renovables. Universidad Arturo Prat, Campus Huayquique, Iquique, Chile.*

Abstract

Aim of study: Phreatophyte species of the *Prosopis* genus are very important to natural ecosystems in Africa, South America and Asia due to their uses as food and seed sources and in agroforestry. In this research, through next-generation sequencing, we sought to search for and develop SSR markers in *Prosopis tamarugo*, in addition to assessing their transferability to other species in the Strombocarpa section.

Area of study: The study was carried out in species of the Strombocarpa section collected in the "Pampa del Tamarugal", located in the Atacama Desert (Chile); which is considered the driest and oldest desert on Earth.

Material and methods: The next-generation sequencing for the development of simple sequence repeat (SSR) or microsatellite loci for genetic research in *P. tamarugo* and their transferability in *Prosopis burkartii* and *Prosopis strombulifera* was used.

Main results: A total of ~90.000 microsatellite loci in *P. tamarugo* were found, and a set of 43 primer pairs was used for validating SSR locus amplification. We found a large difference in the percentage of amplified SSR markers between species of the Strombocarpa and Algarobia sections.

Research highlights: The present study provides for the first time 24 polymorphic SSR markers for species in the Strombocarpa section, which could be a useful tool for estimating genetic structure, developing breeding programs, quantifying genetic diversity and performing population studies.

Keywords: Strombocarpa section; Prosopis tamarugo; Atacama Desert; microsatellites; NGS.

Authors' contributions: Conceived and designed the experiments, funding acquisition, and coordinating the research project: RC. Performed the experiments: RC, VP, MA. Analyzed the data: RC. Contributed reagents/materials/analysis tools: RC. Wrote the paper and critical revision of the manuscript: RC, FSC.

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Correspondence should be addressed to Roberto Contreras: roberto.contreras@uda.cl

Introduction

The Atacama Desert of northern Chile is the driest and oldest desert on Earth, as revealed by geological and mineralogical evidence (Hartley *et al.*, 2005; Clarke, 2006; Sun *et al.*, 2018), and is characterized by extreme environmental conditions such as extremely low relative humidity, high concentrations of salt in the soil, low average annual rainfall and high UV radiation (Azua-Bustos *et al.*, 2012). Despite this hostile environment, several species survive, such as *Prosopis chilensis* (Molina) Stuntz emend. Burkart, *Prosopis alba* Griseb, *Prosopis flexuosa* DC (Algarobia section species), and species of the Strombocarpa section such as *Prosopis tamarugo* Phillipi, *Prosopis burkartii* Muñoz and *Prosopis strombulifera* (Lam.) Benth (Burkart, 1976; Calderón *et al.*, 2015; McRostie *et al.*, 2017; Garrido *et al.*, 2018). However, the endemic species *P. tamarugo* is one of the most interesting because it lives in the most extreme area of the Atacama Desert, between the parallels 19°33'S and 21°50'S (Pampa del Tamarugal), at an average altitude of 1,100 m above sea level (Burkart, 1976; Altamirano, 2006). *P. tamarugo* is

adapted to high temperatures and solar radiation (Lehner *et al.*, 2001; Chávez *et al.*, 2013). Besides, the species is able to perform osmotic adjustment (Time *et al.*, 2018), access groundwater via its dynamic root system and superficial lateral roots, and tolerate water stress while maintaining high stomatal conductance (Aravena & Acevedo, 1985; Calderón *et al.*, 2015; Carevic *et al.*, 2017). Nevertheless, *P. tamarugo* has been categorized as an endangered species; therefore, additional research is needed concerning the various aspects of the genetic diversity so that it can be used in a more prominent role in future conservation planning and management (Carevic *et al.*, 2012; Decuyper *et al.*, 2016). The fruits and leaves of this species are also important, as they are used as fodder for goats and sheep; and the wood is used for fuel, housing construction and furniture manufacturing; in addition, an anthropogenic context, *P. tamarugo* facilitated the settlement of the indigenous population in the area (Barros, 2010; MMA, 2019). Therefore, *P. tamarugo* is an important forest genetic resource in livestock, anthropogenic and ecosystem contexts; however, to date, few genetic studies have focused on the diversity and genetic variability of this species.

Microsatellites (SSR, short sequence repeats) are codominant markers of short sequences (from 1 to 6 nucleotide bases) repeated in tandem (González, 2003). Compared with other DNA markers, these markers exhibit a high rate of polymorphism, making them a good alternative for diversity studies (Contreras *et al.*, 2019a), bottleneck detection, gene flow, hybridization, and population structure analysis (González, 2003, Porth & El-Kassaby, 2014) and ploidy identification (Contreras *et al.*, 2017). To date, SSR *loci* have been discovered in several *Prosopis* species, such as *P. chilensis*, *P. flexuosa* (Mottura *et al.*, 2005, Bessega *et al.*, 2013), *Prosopis alba* Griseb. (Bessega *et al.*, 2013), *Prosopis rubriflora* E. Hassler and *Prosopis ruscifolia* Griseb. (Alves *et al.*, 2014), on which various studies of diversity and genetic differentiation of populations have been performed (Mottura *et al.*, 2005, Bessega *et al.*, 2013). However, despite the large number of SSRs in species of the Algarobia section, these markers are not sufficiently transferable to species of the Strombocarpa section; moreover, there is not enough genomic information to be able to develop specific SSR primers in these species, including *P. tamarugo*. Next-generation sequencing (NGS) has allowed the efficient identification of large numbers of SSR markers (Bastías *et al.*, 2016). Several studies of Fabaceae species, such as in *Dalbergia odorifera* (Liu *et al.*, 2019), *Acacia koa* (Lawson & Ebrahimi, 2018) and *P. alba* and *P. chilensis* (Bessega *et al.*, 2013), have indicated that NGS is an efficient method for the development of SSR or microsatellite markers. For this reason, we sought to identify neutral markers in *P. tamarugo* that can be used to analyze genetic diversity and population variability in future studies. In the present study, using NGS, we identified and developed SSR markers in *P. tamarugo*, and assessed their transferability to other species within the Strombocarpa section. Furthermore, we expected to obtain a large number of SSR sequences in *Prosopis* species due to the high genetic variability found in the Strombocarpa section. These findings will provide a basis for improving the understanding of the genetic of *Prosopis* Strombocarpa species in northern Chile.

Material and Methods

Material, DNA isolation and Sequencing

In 2019, fresh leaves of six individuals of *P. tamarugo*, six individuals of *P. burkartii* and six individuals of *P. strombulifera* were collected in Tamarugal Province (Tarapacá Region, Chile) and Loa Province. Fresh leaves of species of the Algarobia section, such as *P. flexuosa*, *P. chilensis* and *P. alba*, were also collected (Table 1). Taxonomic identification of the species was carried out according to the descriptions reported by Burkart (1976). During recollection, samples were kept at 4 °C; afterward, they were stored at -80 °C in the laboratory. Table 1 shows the geographical location and registration number of the samples, which were deposited in the Departamento de Silvicultura y Conservación de la Naturaleza herbarium of the Universidad de Chile (EIF, Index Herbariorum Code).

DNA was isolated from the leaves via the modified cetyl-trimethylammonium bromide (CTAB) protocol described by Contreras *et al.* (2019a,b). The quality and concentration of the extracted genomic DNA from samples were verified by the use of a Colibri microvolume spectrophotometer (Titertek-Berthold, Pforzheim, Germany). The ratio of absorbance at 260/280 nm was used to assess the DNA purity, which was ~1.7, and the 260/230 ratio was used as a secondary measure of DNA purity, which ranged from 2.0 and 2.2 (Demeke & Jenkins, 2010; Aleksic *et al.*, 2012). The DNA extracted from *P. tamarugo* was quantified with a Qubit™ 3.0 fluorometer and a Qubit™ dsDNA HS Assay Kit (Life Technologies, San Diego, CA) according to the manual provided by the manufacturer. DNA samples from *P. tamarugo* were stored at -80 °C, and DNA integrity was verified with an Agilent 2100 Bioanalyzer (Agilent Technologies, San Diego, CA) prior to sequencing. Sequencing libraries were generated by a TruSeq Nano DNA LT Kit (Illumina, San Diego, CA). The final libraries were run on an Agilent 2100 Bioanalyzer to verify the fragment size distribution and concentration. Sequencing was performed at Genoma Mayor (Universidad Mayor, Chile) with an Illumina sequencing platform. The sequencing data have been submitted to the National Center for Biotechnology Information (NCBI).

Table 1. Species used in the present study, geographic coordinates, and herbarium where samples are deposited.

Species	Province	Latitude (S)	Longitude (W)	Herbal[1]
P. tamarugo	El Tamarugal	20°19'46.1"	69°42'27.2"	EIF 13338
P. tamarugo	El Tamarugal	20°20'37.1"	69°39'53.2"	EIF 13337
P. tamarugo	El Tamarugal	20°20'57.5"	69°39'51.1"	EIF 13336
P. tamarugo	El Tamarugal	20°21'03.6"	69°39'48.1"	EIF 13335
P. tamarugo	El Tamarugal	20°21'03.6"	69°39'47.9"	EIF 13334
P. tamarugo	El Tamarugal	20°21'22.5"	69°39'12.9"	EIF 13333
P. burkartii	El Tamarugal	20°23'11.5"	69°35'57.3"	EIF 13344
P. burkartii	El Tamarugal	20°27'59.2"	69°33'23.2"	EIF 13347
P. burkartii	El Loa	22°59'3.29"	68° 9'19.23"	EIF 13824
P. burkartii	El Tamarugal	20°28'00.1"	69°33'23.2"	EIF 13348
P. burkartii	El Tamarugal	20°23'11.2"	69°35'57.7"	EIF 13355
P. burkartii	El Tamarugal	20°24'45.0"	69°41'29.7"	EIF 13343
P. strombulifera	El Tamarugal	20°27'59.8"	69°33'23.5"	EIF 13332
P. strombulifera	El Tamarugal	20°28'00.1"	69°33'23.3"	EIF 13351
P. strombulifera	El Tamarugal	20°27'59.9"	69°33'23.5"	EIF 13350
P. strombulifera	El Tamarugal	20°28'00.2"	69°33'23.3"	EIF 13352
P. strombulifera	El Tamarugal	20°30'09.7"	69°22'54.1"	EIF 13822
P. strombulifera	El Tamarugal	20°30'09.8"	69°22'54.0"	EIF 13823
P. flexuosa	Copiapó	27°21'21.8"	70°39'54.7"	EIF 13330
P. chilensis	Chacabuco	33°05'24.9"	70°39'07.4"	EIF 13328
P. alba	Copiapó	27°21'39.3"	70°20'33.8"	EIF 13329

(1) Code Index Herbariorum = EIF.

SSR discovery

Raw sequencing reads were subjected to a stringent filtering process. Reads with >10% of bases with a quality score of Q<30 (Q30 quality control), reads that represented noncoding RNA, ambiguous sequences represented as “N”, empty reads and adaptor contaminants were removed. To ensure the accuracy and validity of the SSR search, contigs that were shorter than 300 bp were filtered and removed. The forward and reverse reads of raw sequences were merged by the use of PEAR version 0.9.4 (Zhang *et al.*, 2014).

SSR locus search and primer design

SSR markers were searched throughout the assembled genome via MISA software (Thiel *et al.*, 2003). We searched for SSRs whose motifs comprised sequencing ranging from mono- to hexanucleotides. The minimum number of repeat units was set as follows: ten repeat units for mononucleotides, six for dinucleotides and five for tri-, tetra-, penta- and hexanucleotides. Primer pairs were designed for the selected SSR *loci* using Primer3 software (Rozen & Skaletsky, 2000). The parameters for primer design included a preferred amplicon size of 90-230 bp, primer size of 18-27 bp, and primer melting temperature of 58-60 °C; the optimum temperature was 59 °C.

Evaluation of new SSR markers by PCR

In total, 43 primer pairs were randomly selected and synthesized for polymorphism detection among six *P. tamarugo*, five *P. burkartii* and six *P. strombulifera* genotypes, as well as three species of the Algarobia section (Table 1). PCR was carried out in a total volume of 16 µL that consisted of 8 µL of SapphireAmp Fast PCR 2X Master Mix (Takara-Clontech, USA), 3.2 µL of genomic DNA (5 ng/µL), 0.8 µL of each primer (forward and reverse) at 5 µM concentration, and 3.2 µL of nuclease-free water. PCR amplification of the DNA was conducted in a Labnet

MultiGene OptiMax Thermal Cycler according to the following protocol: denaturation at 94 °C for 3 min; 45 cycles of 98 °C for 5 s, 59 °C for 5 s (midpoint temperature [Tm]), and 72 °C for 40 s; and a final extension at 72 °C for 4 min. The PCR products were subsequently analyzed by electrophoresis on 8.0% nondenaturing polyacrylamide gels stained with GelRed DNA stain (10,000X, Biotium). The band sizes were approximated based on 100 bp DNA ladder (Thermo Fisher).

SSR marker validation

The band sizes were subsequently used to determine genotyping data. Statistical analyses of SSR data, including the number of alleles and allele frequency, were performed with GenAlEx v. 6.5 software (Peakall & Smouse, 2012). The polymorphism information content (PIC) for each SSR locus was estimated according to the formula $PIC = 1 - \Sigma pi^2$, where *pi* is the frequency of the different alleles detected at a particular locus. A PIC value of less than 0.25 indicates low polymorphism, a value between 0.25 and 0.5 indicates average polymorphism, and a value greater than 0.5 indicates a highly polymorphic locus (Botstein *et al.*, 1980).

Results

A total of 101,336 microsatellite *loci* were found among the assembled contigs by MISA software (Table 2). Mononucleotide repeats were the most abundant, accounting for 75,164 (74.17%) of the total SSRs; followed by dinucleotide repeats (17,577; 17.35%), trinucleotide repeats (7,106; 7.01%), tetranucleotide repeats (1,025; 1.01%), pentanucleotide repeats (318; 0.31%), and hexanucleotide repeats (146; 0.14%). The most frequent SSR length for mononucleotides was 10 bp (22,873), while that for dinucleotides and trinucleotides was 6 bp (4,532) and 5 bp (3,733), respectively; in general, with respect to the six classes of SSR motifs, the quantity of *loci* decreased with an increase in the number of motif repeats (Fig. 1). According to the distribution of microsatellites on the basis of motif type, A/T mononucleotide repeats were highly represented (72,429) in *P. tamarugo* sequences, while the C/G motif were not highly represented according to SSR number (2,735) (Fig. 2). Among the dinucleotide tandem repeats, the highest frequency was observed for AT/AT dimers (6,676), followed by AG/CT (5,451), AC/GT (5,274) and CG/CG (176) dimers; the most common repetition trinucleotide motifs were AAG/CTT (2,174), followed by AAT/ATT (1,584), AAC/GTT (1,011), ATC/ATG (685), CCG/CGG (570) and AGG/CCT (535) (Fig. 2).

A set of 43 primer pairs was randomly selected for validating SSR *locus* amplification. In general, among these primer pairs, a total of 39 (91%) presented successfully

Table 2. Results of microsatellite search from *Prosopis tamarugo* using MISA software

Category	Total number
Total number of sequences examined	1,157,370
Total size examined sequences (bp)	200,836,171
Total number of identified SSRs	101,336
Number of SSR containing sequences	90,643
Number of sequences containing more than 1 SSR	9,638
Number of SSRs present in compound formation	9,820

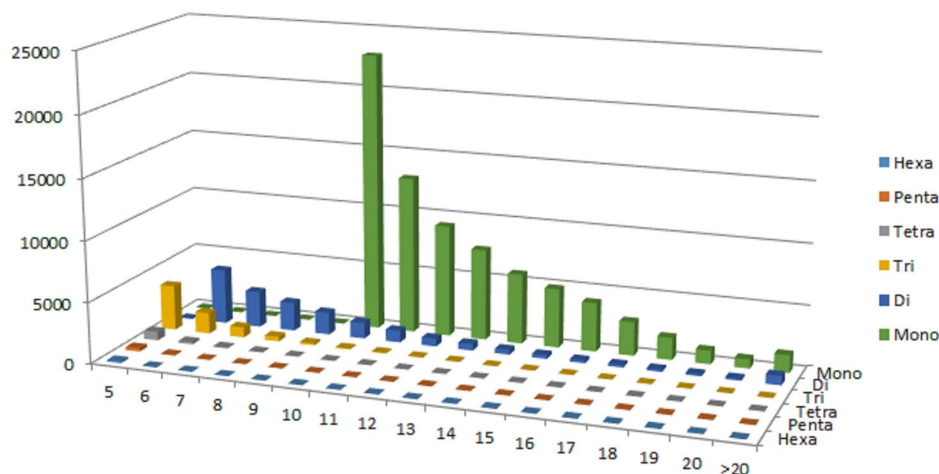

Figure 1. Distribution of six classes of SSR motifs (Mono-to Hexanucleotides) with different numbers of repeats in *Prosopis tamarugo*.

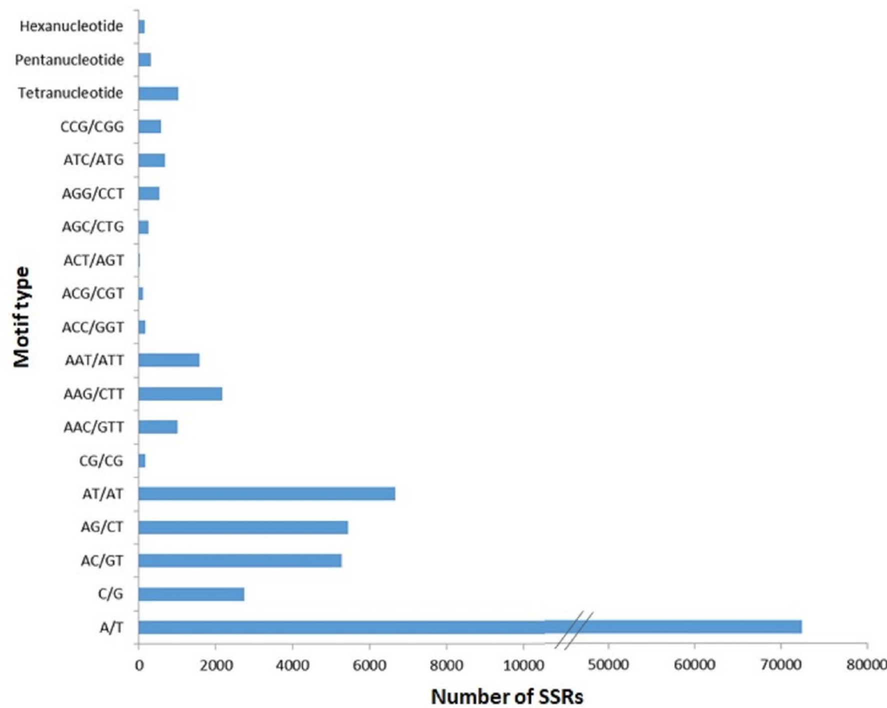

Figure 2. Number of SSRs in *Prosopis tamarugo* based on motif types. The X-axis represents motif types and the Y-axis represents the number of SSRs

amplified products, but four primer pairs (9%; SSRTA8066, SSRTA8081, SSRTA26305 and SSRTA15448) presented no amplified products in any species or presented weak amplification (Table 3). Thirty-nine primer pairs (100%) successfully amplified products in *P. tamarugo* and *P. burkartii*; however, 28 primer pairs (72%) amplified products in *P. strombulifera*, and only seven primer pairs (18%) amplified products in the Algarobia section (Table 3). In *P. tamarugo* and *P. burkartii*, the allele number ranged from 2 to 5, with an average value of 2.38 per *locus*, whereas that in *P. strombulifera* ranged from 1 to 3, with an average value of 1.44 per *locus*. In *P. tamarugo*, the PIC value ranged from 0.18 to 0.74, with an average of 0.36, the PIC value in *P. burkartii* ranged from 0.18 to 0.72, with an average of 0.36, and the PIC value in *P. strombulifera* ranged from 0.15 to 0.61, with an average of 0.16. The PIC value for all species of the Strombocarpa section ranged from 0.17 to 0.86, with an average of 0.55 (Table 3). Twenty-one *loci* in *P. tamarugo*, twenty-four *loci* in *P. burkartii* and seven *loci* in *P. strombulifera* exhibited average polymorphism, while eight *loci* in *P. tamarugo*, nine *loci* in *P. burkartii* and one *locus* in *P. strombulifera* were highly polymorphic.

With the exception of the 43 SSR sequences used in this study, which have been uploaded to the GenBank database (MT136883 - MT136925), all information concerning the SSR sequences of *P. tamarugo* (~90,000 SSR sequences) were deposited in the Sequence Read Archive (SRA) of the NCBI, under BioProject ID PRJNA609952 and BioSample accession SAMN14267073.

Discussion

P. tamarugo has generated much interest because of its capacity to grow and develop in soil under water-deficit and high-salinity conditions (Calderón *et al.*, 2015), both of them considered among the major limiting factors of plant growth and agricultural productivity worldwide (Chaves *et al.*, 2011).

In the present study, SSR markers were developed for *P. tamarugo* based on *de novo* genome assembly on an Illumina sequencing platform, and their transferability to other species of the Strombocarpa section was assessed. The first study of the genetic variability and relationships between populations of species of the Strombocarpa section was performed using isoenzyme markers for the species *Prosopis ferox*, *Prosopis torquata*, *Prosopis pubescens*, *P. strombulifera* and *Prosopis reptans* (Saidman *et al.*, 1996). A previous genetic diversity study reported that four combinations of amplified fragment length polymorphism (AFLP) markers could differentiate *P. strombulifera* populations (Llanes *et al.*, 2011). Moreover, the transferability of six microsatellite markers developed from *P. chilensis* and *P. alba* to species of the Strombocarpa section (such as *P. flerox* and *P. torquata*) was evaluated, but only three SSR markers showed acceptable amplification (Mottura *et al.*, 2005). This number of markers is undoubtedly insufficient for genetic variability studies; in fact, no studies on the development of codominant markers for species in the Strombocarpa section have been performed thus far. In this study, approximately 90,000

Table 3. Characteristics of 43 SSR loci validated on *Prosopis tamarugo* and Strombocarpa section species

Locus	Motif	Primer Sequence (5'–3')	Accession	Fragment Size (bp)	Allele No.			PIC			PIC Total	*Species of Algarobia section* (Allele No.)
					Pt	Pb	Ps	Pt	Pb	Ps		
SSRTA20966	$(GAA)_9$	F: TTGCCTCTGTTCTGTCGT R:GCCAAGAGATTTAGGTTCT	MT136883	158	2	3	2	0.50	0.64	0.15	0.70	-
SSRTA23727	$(GAA)_9$	F: GCCGAAGATCCTAGCATC R: TTACCCTTTTCCGTGCAG	MT136884	137	2	2	2	0.18	0.32	0.15	0.57	-
SSRTA24650	$(GAA)_7$	F: TGCCTACTCAAAATAATGGAGC R: TCACCTTCTGAGATGACGG	MT136885	177	2	4	2	0.48	0.58	0.15	0.70	-
SSRTA28961	$(GAA)_8$	F: AATTAGTGATTTTGGATTAG R: ATGATTACAACGAAACACTAT	MT136886	125	3	2	-	0.54	0.32	-	0.76	-
SSRTA9000	$(AAAG)_7$	F: TAAGCACGGATGGCATAC R: GTTTACTCTGTGTTATGCCTT	MT136887	113	4	2	-	0.66	0.32	-	0.79	-
SSRTA21497	$(AAAG)_9$	F: AAATATTGGCGTCAGTAACTA R: AGTTGCTTTTGTTGCTCGATT	MT136888	148	2	2	-	0.32	0.18	-	0.67	-
SSRTA12887	$(TTC)_5$	F: TCGTGATATGCACATACCATAAT R: GCGGACAAGAAAATGAAAGC	MT136889	125	2	2	-	0.18	0.18	-	0.64	-
SSRTA24192	$(TTC)_8$	F: GCGTCCGCTACTTCTTCAAC R: CGAAGAAGAAAGCACAAGCA	MT136890	147	2	3	2	0.18	0.34	0.44	0.47	-
SSRTA15719	$(TTC)_5$	F: CCACCGTCGAGTACAATGTC R: CATTAACACCACGAAAACAACC	MT136891	111	2	2	2	0.18	0.18	0.15	0.17	-
SSRTA10814	$(TTC)_5$	F: TGGGTTCAGACCTTTTGACA R: GGCTTCAGGTTTTCTGTTGC	MT136892	144	2	2	3	0.18	0.36	0.50	0.37	2
SSRTA14343	$(AAAT)_5$	F: GCTTCCAGAAGCTGACGAAG R: TTTTAAGACACAAGGGGCTTTT	MT136893	148	2	2	-	0.18	0.18	-	0.64	-
SSRTA3506	$(AAAT)_5$	F: AAACGAGAGTCAATGTCAATGG R: GCTAAAGGGTGGTTTAAATCGTT	MT136894	147	2	2	2	0.18	0.18	0.28	0.45	-
SSRTA10919	$(AAAT)_5$	F: TTCCAGGTGCCTGAAATACC R: GGGTTCGCTAATGTAAGATCC	MT136895	135	2	2	2	0.18	0.18	0.15	0.17	1
SSRTA25408	$(AAAT)_5$	F: TTTGATTAAGGCCCTTGGTG R: AGGGGTGTTTTGAAGGTTGA	MT136896	150	2	2	2	0.18	0.18	0.15	0.17	1
SSRTA9179	$(TTTC)_6$	F:TGAATTGTATGGAAATACGACTCTG R: TCATTGGCCCTTGTAGTTGA	MT136897	124	2	2	2	0.18	0.50	0.15	0.56	-
SSRTA23450	$(ATAC)_5$	F: TCATGAACAACATGTAAAATTGC R: AAGGCAAGTAGACCAAGTCAATG	MT136898	124	2	2	2	0.32	0.48	0.15	0.42	1
SSRTA11003	$(ATAC)_5$	F: AACACCGCTAGGAATCGAAC R: TGATTAGCCTGAAAACCACCA	MT136899	149	2	2	-	0.18	0.18	-	0.64	-
SSRTA13846	$(ATAC)_5$	F: TCCAAGACCAAATAAAATGGTT R: GGAATTGTCTCGCCTTTTCA	MT136900	141	2	2	-	0.18	0.18	-	0.64	-
SSRTA19679	$(TA)_{13}$	F: TCGATTGTGTTTTTGAAGTTTATT R: TCTCAACTGATCAACATCCTCAA	MT136901	143	2	3	2	0.18	0.34	0.15	0.51	-
SSRTA8066	$(TA)_{23}$	F: TTTTAAAAAGAAGTGACATTTAACCAA R: CATGTTTCAATCAAAATAACACTACA	MT136902	136	-	-	-	-	-	-	-	-
SSRTA8081	$(TA)_{28}$	F: TTGGAGTAAAGGCTACGTGTGA R: CCTACATAAGCCGTTGCACA	MT136903	138	-	-	-	-	-	-	-	-
SSRTA26305	$(TA)_{33}$	F: TCTGGCAAGACACTTTGGAA R: TTTGCGTTGCTTCTTTGAGA	MT136904	146	-	-	-	-	-	-	-	-
SSRTA23355	$(TG)_{18}$	F: TGGAAAGCTAGAGTCCTTGACC R: GATGCCAGCATGCCAAGTA	MT136905	111	3	3	2	0.46	0.56	0.15	0.70	2
SSRTA12501	$(TG)_{13}$	F: TGTGCGTATCAACCACATTAGA R: TTCAGTAATTTTAAATGATGGTCAAA	MT136906	150	2	2	2	0.18	0.18	0.15	0.17	-

Pt: *Prosopis tamarugo*; Pb: *Prosopis burkartii*; Ps: *Prosopis strombulifera*; Species of Algarobia section: *Prosopis flexuosa*, *Prosopis alba*, *Prosopis chilensis*."-"indicates no amplification or weak banding pattern.

Continue Table 3

Locus	Motif	Primer Sequence (5'–3')	Accession	Fragment Size (bp)	Allele No.			PIC			PIC Total	*Species of Algarobia section* (Allele No.)
					Pt	Pb	Ps	Pt	Pb	Ps		
SSRTA13112	$(TG)_{14}$	F: TGACCCTCCTTTCTCACAACTT R: GGATCAATGGCTTGTTGGTT	MT136907	150	5	4	3	0.74	0.72	0.61	0.80	-
SSRTA23157	$(TG)_{15}$	F: CGTTCTACCCATTAAAATTAGAAAA R: TGACTGACAGTGCACATTGAT	MT136908	150	2	2	-	0.18	0.18	-	0.64	-
SSRTA21110	$(TG)_{12}$	F: TGGTTGGCTCAAAAGTGAAA R: TGTGAGAAGCAAGTCCTCGTT	MT136909	145	3	2	2	0.62	0.50	0.15	0.58	1
SSRTA24919	$(GA)_{12}$	F: TCCTTTTTCAGTGGGTTTGG R: TCTGTGATTTCATCGCTCCA	MT136910	101	2	2	2	0.18	0.18	0.15	0.17	1
SSRTA6566	$(GT)_{10}$ $(GA)_{10}$	F: GCTTTGAGGAATCACAGCAA R: CGAGCTCTTTGCCTGAATGT	MT136911	226	2	2	-	0.42	0.18	-	0.70	-
SSRTA16923	$(CA)_{13}$	F: CGATGACAAGCATGGAAATG R: TGTGGAAGACCTTTATGTCCCTA	MT136912	108	2	3	2	0.32	0.56	0.15	0.39	-
SSRTA6611	$(TG)_{15}$	F: TGACAATTGCGATCAACTCA R: TTTTTAAGTGGCAGGGTGGT	MT136913	101	2	2	2	0.48	0.18	0.15	0.31	-
SSRTA23382	$(TG)_{12}$	F: AAGGTACAAAATTAGATAGCTTGCAT R: ACTGCCGTCTTACCATGCTT	MT136914	130	2	2	2	0.18	0.18	0.44	0.57	-
SSRTA14008	$(TG)_{13}$	F: CCTCCTCCTTCAACATGTGC R: GGCTGTGCCTGGTTTAGAGA	MT136915	127	2	2	-	0.18	0.32	-	0.67	-
SSRTA7980	$(AAG)_{14}$	F: TGTCCCATTTCCAATCACTAAA R: AATTGGAATTGTTTCGGTGAA	MT136916	106	2	2	2	0.50	0.50	0.50	0.61	-
SSRTA10222	$(AAG)_{11}$	F: CATGCAAATCCTGAAGGTCA R: TGAGCATTTGAGCAGATTGG	MT136917	119	2	2	2	0.48	0.48	0.15	0.67	1
SSRTA29655	$(AAG)_{10}$	F: TTCTGTAAGGTTGGTTTGAGGA R: CTTTGGTTCTTGGCCATTGT	MT136918	126	2	2	2	0.48	0.32	0.15	0.38	1
SSRTA21072	$(ATG)_{8}$	F: GCTAACGGAAACTGCTGTTCA R: GGGCTATGGTAGTCATCATTGTG	MT136919	200	3	2	2	0.62	0.48	0.15	0.68	-
SSRTA15448	$(CT)_{3}$ $(ATT)_{15}$	F: TTTTATGCCACCAGTTGTTTG R: TCTCACAGCATCAATTTTATCCA	MT136920	137	-	-	-	-	-	-	-	-
SSRTA6832	$(ATT)_{13}$	F: GAAATGAGCGGGGCAGTT R: GGGAGTTATGTGCGCTGAAT	MT136921	147	4	4	2	0.58	0.66	0.15	0.68	-
SSRTA22018	$(ATT)_{13}$	F: CATGTGTGGCACAAAATTTAAGA R: AACGGATAGGTGACAATGCAG	MT136922	191	2	3	2	0.32	0.54	0.38	0.71	2
SSRTA8169	$(GTT)_{14}$	F: CGTTGGACTTTCATCATCAATC R: AGATTGCTCGTTGCCAAAAT	MT136923	126	4	3	-	0.72	0.58	-	0.86	-
SSRTA22468	$(GTT)_{12}$	F: CACTGCTGAGCGTTAGTTGC R: TTCACGTTGCTCCGTTATCA	MT136924	104	3	2	1	0.64	0.42	0.00	0.46	-
SSRTA11047	$(GTT)_{10}$	F: AAAGCGCTCGAAGATAACGA R: CACTTTGGGGACCTCCTTTA	MT136925	144	3	4	1	0.46	0.58	0.00	0.60	-
Average					2.38	2.38	1.44	0.36	0.36	0.16	0.55	0.30

Pt: *Prosopis tamarugo*; Pb: *Prosopis burkartii*; Ps: *Prosopis strombulifera*; Species of Algarobia section: *Prosopis flexuosa*, *Prosopis alba*, *Prosopis chilensis*."-"indicates no amplification or weak banding pattern.

SSR sequences were obtained (approximately 10% of which were imperfect) for *P. tamarugo*, which was high with regard to the number of SSR sequences obtained in other species by the NGS method (Bessega *et al.*, 2013; Liu *et al.*, 2019). The number of SSR sequences obtained by NGS varies among species; for example, 760 sequences have been obtained in *Prosopis sp*. (Bessega *et al.*, 2013), 35,774, in *Dalbergia odorifera* (Liu *et al.*, 2019); and 130,931, in *Acacia koa* (Lawson & Ebrahimi, 2018). The DNA extraction method is critical for obtaining a large quantity of high-quality NGS reads (Healy *et al.*, 2014; Psifidi *et al.*, 2015); the DNA extraction method used in

this study may have been crucial to obtain a large number of SSR sequences in *P. tamarugo*.

According to Saidman *et al.* (1996) and Hunziker *et al.* (1986), there is an important difference in genetic variability between species of the Strombocarpa and Algarobia sections. Consistently, a large difference in the transferability of amplified SSR markers between species of the Strombocarpa (100% for *P. burkartii* and 72% for *P. strombulifera*) and species of the Algarobia section (18%) was detected in this study. Transferability of the new SSR markers described in this work to other *Prosopis* species, such as *P. ferox*, *P. torquata*, *P. pubescens*, *P. palmeri*, *P. abbreviata* or *P. reptans* should be checked in the future.

The microsatellites developed in *P. tamarugo* may be useful for studying the diversity and genetic variability of populations of species within the Strombocarpa section, which encompass eight species distributed in America. Our results presented acceptable amplification of the SSR markers (>70%) in three species studied. Moreover, these SSR markers could also be used to identify possible hybrids between species of the Strombocarpa section, such as *P. burkartii* (*P. tamarugo* x *P. strombulifera*, which is endemic to Chile) (Burkart, 1976) and *Prosopis abbreviata* (*P. strombulifera* x *P. torquata*, which is endemic to Argentina) (Mollard *et al.*, 2000; Burghardt *et al.*, 2004). Moreover, genetic studies have confirmed the occurrence of hybridization and introgression within species of the Algarobia section (Vega & Hernández, 2005; Ferreyera *et al.*, 2013), but not within species of the Strombocarpa section.

Prosopis species are considered as invasive woody tree species that affect native prairie grassland in Africa, while in America, they are valuable species associated with afforestation and rehabilitation of arid grassland ecosystems (Mworia *et al.*, 2011). On a global scale, the degree of adaptability of *P. tamarugo* to saline and alkaline soils is high, as this species has even been introduced with great success in India as a legume species for the soil recovery of degraded grasslands (Nandwani & Ramawat, 1992). From this perspective, developing reliable tools involving codominant markers such as SSRs is key to population genetics studies, which can provide support for forest tree breeding program of species of the Strombocarpa section. On the other hand, the conservation of species in danger of extinction, such as *P. tamarugo*, requires prior and deep knowledge of their dynamics and population structure, which involves the determination of genetic variability both within and between populations. According to Felker (2009), there may not be another species of *Prosopis* with the potential to generate development in very poor, desolate and inhospitable areas. Its adaptation mechanisms to survive one of the most hostile areas on the planet are exceptional. Together, these qualities confer a very high genetic value to *P. tamarugo*.

In conclusion, a new set of SSR markers was developed for the endemic species *P. tamarugo* for the first time, and their transferability to species of the Strombocarpa section was assessed. The present study provides 24 polymorphic SSR markers for species within the Strombocarpa section, which could be a useful tool for estimating genetic structure, developing breeding programs, quantifying genetic diversity and performing population studies.

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