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

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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.

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 co-dominant 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 (Bastias 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 the 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 cetil-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 (Titertrack-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, Santa Clara, 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).
Development of SSR loci in *Prosopis tamarugo* and species of the Strombocarpa section

**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 $\text{PIC} = 1 - \sum p_i^2$, where $p_i$ 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 *Prosopis 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

![Figure 1. Distribution of six classes of SSR motifs (Mono-to Hexanucleotides) with different numbers of repeats in *Prosopis tamarugo*.](image-url)
Development of SSR loci in *Prosopis tamarugo* and species of the Strombocarpa section

amplified products, but four primer pairs (9%; SSR- TA8066, 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 0.15 to 0.61, 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. ferox* 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

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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.
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 Pt | PIC Pb | PIC Ps | PIC Total Species of Algarobia section (Allele No.) |
|------------|-------|-------------------------|-----------|-------------------|------------|--------|--------|--------|--------------------------------------------------|
| SSRTA20966 | (GAA)8 | F: TTGCCCTGTGCTGCTGCTG<br>R: GCCAAGAAGATTTAGTGGTT | MT136883  | 158               | 2 3 2       | 0.50   | 0.64   | 0.15   | 0.70 -                                           |
| SSRTA23727 | (GAA)8 | F: GCGGAGAACCTGACATC<br>R: TTACCTCTCTGCCGTTGCGAG | MT136883  | 137               | 2 2 2       | 0.18   | 0.32   | 0.15   | 0.57 -                                           |
| SSRTA24650 | (GAA)8 | F: TGCCCTACTCAAAAATAGGAGGC<br>R: TCCACCTGTGACGAGG | MT136885  | 177               | 2 4 2       | 0.48   | 0.58   | 0.15   | 0.70 -                                           |
| SSRTA28961 | (GAA)8 | F: AAATATGATTTGGGATGATTAG<br>R: ATGATTACAGAAACACTAT | MT136886  | 125               | 3 2        | 0.54   | 0.32   | -      | 0.76 -                                           |
| SSRTA9000  | (AAAG)7 | F: TAAAGCAGGAGGATGATCATC<br>R: GTTTAACGTCAGTTAGCTC | MT136887  | 113               | 4 2        | 0.66   | 0.32   | -      | 0.79 -                                           |
| SSRTA21497 | (AAAG)7 | F: AATATGCTGAGAATCAATCATC<br>R: AGTGCCATTTTGTCCGATTTA | MT136888  | 148               | 2 -        | 0.32   | 0.18   | -      | 0.67 -                                           |
| SSRTA12887 | (TTC)5 | F: TCTGTGATACACATCAATAC<br>R: CCGGACAAAGAAATGAAAC | MT136889  | 125               | 2 2        | 0.18   | 0.18   | -      | 0.64 -                                           |
| SSRTA24192 | (TTC)5 | F: CGTCCCTCTTCATCCTCAC<br>R: GAAAGAAGAAAGCAACACCA | MT136890  | 147               | 2 3 2       | 0.18   | 0.34   | 0.44   | 0.47 -                                           |
| SSRTA15719 | (TTC)3 | F: CACCCGCTGATACAGTACGG<br>R: CATTAAAGAAGGACACACCA | MT136891  | 111               | 2 2 2       | 0.18   | 0.18   | 0.15   | 0.17 -                                           |
| SSRTA10814 | (TTC)3 | F: TGGGGCCGCATTTTGGAC<br>R: GGCTCAGGTTTGGTTGTC | MT136892  | 144               | 2 2 3       | 0.36   | 0.36   | 0.50   | 0.37 2                                           |
| SSRTA14343 | (AAAT)5 | F: GTTCCTAGAGTCTCCTACA<br>R: TTTAAGAAGCAAGGCTT | MT136893  | 148               | 2 -        | 0.18   | 0.18   | -      | 0.64 -                                           |
| SSRTA3506  | (AAAT)5 | F: AAGCGAGGTGATGTCATGGA<br>R: GCTAAGGTTTTTTTATT | MT136894  | 147               | 2 2 2       | 0.18   | 0.18   | 0.28   | 0.45 -                                           |
| SSRTA10919 | (AAAT)5 | F: TTCGAGTGCTCTAGTAACT<br>R: GGGTTGATGCTATGAAACC | MT136895  | 135               | 2 2 2       | 0.18   | 0.18   | 0.15   | 0.17 1                                           |
| SSRTA25408 | (AAAT)5 | F: TTTGATAGGGCTTGGT<br>R: AGGGTTGTTTTGAAAGTTTGA | MT136896  | 150               | 2 2 2       | 0.18   | 0.18   | 0.15   | 0.17 1                                           |
| SSRTA9179  | (TTTC)5 | F: TGAATCATGGAAATAGACTCTG<br>R: TCATTGGGTCCTTGGTTTA | MT136897  | 124               | 2 2 2       | 0.18   | 0.50   | 0.15   | 0.56 -                                           |
| SSRTA23450 | (ATAC)5 | F: TCTGCAACAGATGAAATAG<br>R: AAGGCGAAGCTACGTTCAATG | MT136898  | 124               | 2 2 2       | 0.32   | 0.48   | 0.15   | 0.42 1                                           |
| SSRTA11003 | (ATAC)5 | F: AACACCGTCTGAACTGAC<br>R: TGTTAGCTGAAACCATCCCA | MT136899  | 149               | 2 2       | 0.18   | -      | 0.18   | 0.64 -                                           |
| SSRTA13846 | (ATAC)5 | F: TCCAAGCAAATAAAATGTT<br>R: GAAATTCTGCTTCTCTTCA | MT136900  | 141               | 2 2       | 0.18   | 0.18   | -      | 0.64 -                                           |
| SSRTA19679 | (TA)13 | F: TGATTTGTTTTGGAGTTTATT<br>R: TCTCAACTGATCAAATCCCAA | MT136901  | 143               | 2 3 2       | 0.18   | 0.34   | 0.15   | 0.51 -                                           |
| SSRTA8066  | (TA)13 | F: TTTAAAAAGAGGCTACATTTAACCA<br>R: CAGTTGGTCAAAATACACTACAA | MT136902  | 136               | - - -       | -      | -      | -      | -                                                |
| SSRTA8081  | (TA)13 | F: TTGGGATAGACTAGTGTTGA<br>R: CCGTTGCAAAATGTTCAACACA | MT136903  | 138               | - - -       | -      | -      | -      | -                                                |
| SSRTA26305 | (TA)13 | F: TCGGCAGACACTTTTGAG<br>R: TTCACGTTTCTCTCACGTTGA | MT136904  | 146               | - - -       | -      | -      | -      | -                                                |
| SSRTA23535 | (TG)13 | F: TGAAAGCTAGTACCTTGCACC<br>R: GATCAGCAGTGCAGAGCTA | MT136905  | 111               | 3 2 2       | 0.46   | 0.56   | 0.15   | 0.70 2                                           |
| SSRTA12501 | (TG)13 | F: TCGGCTCATCACCACACTAGA<br>R: TCGAGTATTTTTATGATGTTTCAA | MT136906  | 150               | 2 2 2       | 0.18   | 0.18   | 0.15   | 0.17 -                                           |

Pt: *Prosopis tamarugo*; Pb: *Prosopis burkartii*; Ps: *Prosopis strombocarpa*; Species of Algarobia section: *Prosopis flexuosa*, *Prosopis alba*, *Prosopis chilensis*. "-" indicates no amplification or weak banding pattern.
Development of SSR loci in *Prosopis tamarugo* and species of the Strombocarpa section

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).

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### Continue Table 3

| Locus       | Motif       | Primer Sequence (5’–3’) | Accession | Fragment Size (bp) | Allele No. | PIC Total |
|-------------|-------------|----------------------|-----------|--------------------|------------|-----------|
| SSRTA13112  | (TG)4       | F: TGGACCCCTCCTTTCTCACAACTT R: GGATCAATGGCTTGTTGTT | MT136907  | 150                | 5 4 3     | 0.74 0.72 0.61 0.80     |
| SSRTA23157  | (TG)3       | F: CTTCTACCACCTTTGATTAAATTAGAAA R: TGGACGACATGGCAACATTGT | MT136908  | 150                | 2 2 -     | 0.18 0.18 - 0.64     |
| SSRTA21110  | (TG)2       | F: TGGTGCGCTAAGGTAAAAGR: TGGGAAACGGACGTTCGGT | MT136909  | 145                | 3 2 2     | 0.62 0.50 0.15 0.58 1  |
| SSRTA24919  | (GA)2       | F: TCTCCTTCACTGGGTTGTT R: TCGTGATTCTTAGCTGTTCA | MT136910  | 101                | 2 2 2     | 0.18 0.18 0.15 0.17 1  |
| SSRTA6566   | (GT)3       | F: GCTTGGAGGAATCACAGGCA R: CGACTCTTTGCGTGAAGTG | MT136911  | 226                | 2 -       | 0.42 0.18 - 0.70     |
| SSRTA16923  | (CA)3       | F: CAGTGCAAGCACTGGAATTTG R: TGGGAAACGTTCTGGGCTCT | MT136912  | 108                | 2 3 2     | 0.32 0.56 0.15 0.39  |
| SSRTA6611   | (TG)3       | F: TGGACCGTGACACATTTGCGTT R: TGTAGGCTGTTACTGTT | MT136913  | 101                | 2 2 2     | 0.48 0.18 0.15 0.31  |
| SSRTA23382  | (TG)2       | F: AAGTGCTATAATTAGACCTTGCGAT R: ACTGCTGCTTACATGCTT | MT136914  | 130                | 2 2 2     | 0.18 0.18 0.44 0.57  |
| SSRTA14008  | (TG)3       | F: CCTCCTCCTTCAACATGTCG R: GGCTCTGCTGTTAGGAGA | MT136915  | 127                | 2 2 -     | 0.18 0.32 - 0.67     |
| SSRTA7980   | (AAG)4      | F: TGGCATCCTTAATCACTAAA R: AATGGGAATTTGTTGCTGGGA | MT136916  | 106                | 2 2 2     | 0.50 0.50 0.50 0.61  |
| SSRTA10222  | (AAG)4      | F: CAGGAAATCTGAAGGATTTG R: TGGACATTGGAAGCTTGTG | MT136917  | 119                | 2 2 2     | 0.48 0.48 0.15 0.67 |
| SSRTA29655  | (AAG)4      | F: TTCCTGCTATGTTGTTGAGGA R: CTCTTTGCTTTGCTCACCT | MT136918  | 126                | 2 2 2     | 0.48 0.32 0.15 0.38 |
| SSRTA21072  | (ATG)3      | F: GCTAATGGAAACATGCTGTTCA R: GGGCTATGTAATGCTTAGTG | MT136919  | 200                | 3 2 2     | 0.62 0.48 0.15 0.68 |
| SSRTA15448  | (CT)3       | F: TTTATGACCAGCATTGTTG R: TTCACAGCATCAATTTACCA | MT136920  | 137                | - - -     | - - - - - - - - |
| SSRTA6832   | (ATT)3      | F: GAAATGCGGGGGCCAGTT R: GGGATTTATGTTGCGCTGAAT | MT136921  | 147                | 4 2 2     | 0.58 0.66 0.15 0.68 |
| SSRTA22018  | (ATT)3      | F: CATGTTGGGACAAAAATTTAGA R: AAGCGATAGGTTGCAATGCA | MT136922  | 191                | 2 3 2     | 0.32 0.54 0.38 0.71 |
| SSRTA8169   | (GTT)4      | F: CTTGGACCTTTCATCATAACCT R: AGATGCTGCTTGCCAAATT | MT136923  | 126                | 4 3 -     | 0.72 0.58 - 0.86 |
| SSRTA22468  | (GTT)2      | F: CACTGCTGAGTTATTGTTGC R: TTCAGTTCTCCTGGIATCA | MT136924  | 104                | 3 2 1     | 0.64 0.42 0.00 0.46 |
| SSRTA11047  | (GTT)3      | F: AAAGCGCTGAGAAATGCAAAGCA R: CACTTGGGAGCCCTTCTTIA | 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 strombolifera*; Species of Algarobia section: *Prosopis flexuosa, Prosopis alba, Prosopis chilensis.* "-" indicates no amplification or weak banding pattern.
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) (Burtart, 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.

**Acknowledgements**

We sincerely thank the Corporación Nacional Forestal (CONAF), Tarapacá Region, for the sampling authorization (N°00024/08-11-2019 (JBH/FAP/JVO)).

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