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Development and validation of the first SSR markers for *Mimosa scabrella* Benth.

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ABSTRACT. *Mimosa scabrella* Benth., popularly known as ‘‘bracatinga’’, is a pioneer and endemic species of Brazil, occurring in Mixed Ombrophilous Forest associated with Brazilian Atlantic Rainforest biomes. It is a fast-growing tree of the Fabaceae family that facilitates the dynamics of ecological succession. SSR development, when there is no genome sequence, is time and labor intensive and there are no molecular markers for *M. scabrella.* We developed and validated the first microsatellite markers for this tetraploid species, evaluating mother trees and progenies. Using Illumina sequencing, we identified 290 SSR loci and 211 primer pairs. After 31 SSR loci PCR/agarose electrophoresis selection, a subset of 11 primer pairs was synthetized with fluorescence in the forward primer for PCR and

capillary electrophoresis validation with leaf DNA of 33 adult and 411 progeny individuals. Polymorphic locus percentage was 36, 4 in 11 loci, 3 chloroplast SSRs, and 1 nuclear SSR. Allele number of polymorphic loci ranged from 2 to 11 alleles considering all sampling. All 11 primer pairs were also tested for cross-species amplification for five Fabaceae-Mimosoideae species, ranging from 2 loci transferred to *Calliandra tweedii* Benth. and all 11 loci transferred to *Mimosa taimbensis* Burkart. The assessed and validated SSR markers for *M. scabrella* are suitable and useful for analysis and population genetic studies.

Key words: Bracatinga; Next-generation sequencing; NuclearSSR; cpSSR; Polyploidy; Transferability

INTRODUCTION

Bracatinga (*Mimosa scabrela* Benth.) is a multipurpose forest species, native and endemic of South Brazil. It is a pioneer leguminous tree of rapid growth in open fields, after logging (Reitz et al., 1978) especially when burning the litter that breaks dormancy in seeds (Carneiro et al., 1982). Bracatinga is very common in rural settlements of South Brazil (Moreira et al., 2011) and economically important for timber, charcoal, fuel and energy wood, and honey production due to winter flowering (Pegoraro and Carpanezzi, 1995; Machado et al., 2002). *M. scabrella* is also a valuable resource for reforesting and recovering programs of degraded areas (Carneiro et al., 1982; Urbano et al., 2008). Seeds have high levels of galactomannans with great potential for biotechnological applications (Ganter and Reicher, 1999; Ughini et al., 2004; Vendruscolo et al., 2005).

There are a few articles about *M. scabrella* genetics. Sobierajski et al. (2006) and Moreira et al. (2011) investigated mating system using allozyme loci and found mixed mating system, predominantly outcrossing. Dahmer et al. (2011) determined tetraploid nature of the species, with chromosome number 2n = 4x = 52. A major challenge for molecular characterization is the lack of sufficient DNA markers specific to *M. scabrella*.

Microsatellite markers, or simple sequence repeats (SSRs), unite desirable features for molecular markers: co-dominant, multi-allelic (Varshney et al., 2005) and are widely used because are simple, show high resolution and polymorphism (Squirrell et al., 2003). SSRs, once described and validated, are easily reproducible, abundant and uniformly dispersed in plant genome, so they are effective for population genetic studies, molecular ecology, and for future molecular breeding (Zhao et al., 2013). Chloroplast organelle genome is characterized by uniparental mode of inheritance, so chloroplast SSR (cpSSR) can contribute to check seed and pollen flow by comparing nuclear and chloroplast markers (Provan et al., 2001). Sequence genome knowledge is a barrier to screen SSR loci (Morris et al., 2016). Next-generation sequencing technologies can generate huge sequence data fastly, for genome mining and identification of SSR regions (Abdelkrim et al., 2009) without prior genomic library construction with SSR motif-enriched DNA (Csencsics et al., 2010). It is a promising approach for species with restricted genetic information as many native species like *M. scabrella*. This current study developed the first primers for *M. scabrella* microsatellites identified by next-generation sequencing, validated as a molecular marker evaluating inheritance pattern between mothers and progenies, and also tested cross-species transferability for Fabaceae-Mimosoideae species.

MATERIAL AND METHODS

Plant materials and DNA extraction

Altogether, leaves of 444 individuals were sampled, 33 adults and 411 progenies. These 33 adult trees are in the University of Santa Catarina State, Campus of Lages. They are originated from a mixture of seedlings of three cities of Santa Catarina State: Atalanta, Urupema, and Bocaina do Sul. Five of these 33 adults were selected as mother trees to provide seeds to generate progenies. For each one of the five mother trees, it was sown 108 seeds. After 2 months of growing, some seeds did not germinate and some seedlings did not survive, the leaves of 411 health progenies were collected and analyzed with the 33 adult leaves for microsatellite validation. For cross-species transferability, leaves of five different individuals of Fabaceae-Mimosoideae species were tested: *Calliandra tweedii* Benth., *Mimosa bimucronata* (DC.) Kuntz, *Mimosa taimbensis* Burkart, and *Parapiptadenia rigida* (Benth.) Brenan. For *Mimosa ramosissima* Benth., leaves of two different individuals were tested.

All samples were extracted as follows: grinding and homogenizing leaves were performed with Precellys Evolution (Bertin, France) and total DNA was isolated from 30 mg of grinded leaves with CTAB method according to Doyle and Doyle (1990). Just for proceeding with sequencing, a single sample of *Mimosa scabrella* DNA was purified with the Zymo™ Purification Kit (ZYMO Research, USA). The voucher specimen of the sequenced sample was included in the Herbarium Lages of Universidade do Estado de Santa Catarina, with the number LUSC 8690.

Sequencing and *de novo* partial assembly

Mimosa scabrella Benth. paired read sequencing was performed from genomic DNA using the MiSeq Illumina platform. Raw high throughput sequencing data and trimmed data were evaluated with FastQC to quality control check (quality value ³33). CLC Genomics Workbench 7.5 (CLC Bio, Denmark) was used to trim data and to form contigs.

SSR identification and primer development

Contigs were screened with SSR Locator (da Maia et al., 2008) to find microsatellite loci and design primers. Microsatellite criteria were at least three repetitions of each motif and minimum of 12 bp (Castoe et al., 2012). Primer criteria were from 18 to 22 bp in size, amplicon size 100-550 bp, average annealing temperature of 55°C, at least 100 bp of gap between microsatellites and 50% of CG. Primer quality was assessed *in silico* with the OligoAnalyzer 3.1 of IDT DNA website (IDT DNA Tech., Coralville, IA, USA; http://www.idtdna.com/site) and Gene Runner (Hastings Software Inc., Hastings, NY, USA; www.generunner.net). Primer pair approval consisted in Tm difference of less than 1°C, no compound, imperfect and monomer SSR, ΔG higher than -5 kcal/mol and no palindrome (Table 1).

Validation of SSR markers

PCR selection was performed to test 31 primer pairs from 65 *in silico* approved primers (Table 1). Genomic DNA was isolated from 10 adult samples. PCR was carried out separately for each locus in a 12.5-μL volume reaction with 1.5 ng template DNA, 1X PCR buffer Tris-HCl, 2.5 mM $MgCl_2$, 0.2 mM dNTP mix, 0.1 μM of each primer, and 0.5 U Platinum Taq

Table 1. Sequences of *in silico* approved primer pairs, synthetized unlabeled primers for PCR selection and synthetized primers for polymorphism analysis.

Loci	Forward primer (5'-3')	Reverse primer (5'-3')	Expected size (bp)	Repeat motif
Msc001[a]	GGCATGTAACGAATTTCTTC	AATCCTTTGCAGACGACTTA	519	$(CCCG)_3$
Msc003[b]	CAATCCGTGTTTATCTAGGC	CTAGACCCTTGCTTCGTATG	220	$(GCC)_6$
Msc009[b]	TGAGTAAAGGGCCTGATAAA	TACGTTTGGTGTTTGTGAGA	521	$(ATATAG)_3$
Msc017	GCAGTTAGCTCTATCGGAGA	GACCCAGTAGATCCAGTTGA	147	$(TA)_8$
Msc020[a]	TTGCCAACAGAACTTAGGAT	AAATGATGTCATGAAGAGGC	420	$(CAAT)_3$
Msc022	AACTTGGTCCAGAGCATCTA	TATCTTGTGAGCAAACATGC	391	$(TA)_7$
Msc023[a]	GCATGTTTGCTCACAAGATA	AGAATGATGACTTTGGGTTG	505	$(AAAT)_3$
Msc030	ACAGAAACGTCGCTATCAAT	TTAACTGGGCTAGCATCTTC	504	$(AAG)_4$
Msc032	AACTTAGCCTGAACCTCTCC	TAGGCTGGCAGAGACTAGAG	415	$(TA)_6$
Msc041[b]	CTTAGATCAATCTCAACCCG	GAGAATTTATCATGGAAGCG	426	$(ATTA)_3$
Msc045[b]	ATGATACGTAAGCAAGGCAT	AGTTCAAGTCACACACTCCC	286	$(ATTAT)_3$
Msc063[a]	ATGACACTGATTTCCTCCAG	CCAATATGGGAGATCAAAGA	269	$(GAA)_6$
Msc064	AAATGACTATGGCTTCCTGA	CAAGCTTCTCCTAATCCAAG	288	$(AC)_6$
Msc066[a]	CAGGAATTCAACAACCATCT	TATTTGAGAATCCTTGAGGG	319	$(AAC)_5$
Msc069	GGAATGATGACTATCGAGGA	GTAAGCTCCAAGAACCACTG	239	$(TA)_6$
Msc072[b]	CGCTCAAGCATCTTCTCTAT	TGTTCGTTGTGTCTTTGTGT	436	$(AACT)_4$
Msc075	TACCCATCCTGTATATTGCC	TTTCCATAGAGCTTTCTTGC	507	$(TTA)_4$
Msc080[a]	CTATGAAAGCTTGGGTATGG	TATCTACAACCACCACCTCC	136	$(GGT)_6$
Msc098[b]	CGGGAATAGAAGAGACTTCA	CTTTGTTCAATCTTTCACCC	117	$(GTTG)_3$
Msc099[a]	ATGAAGTGTTGATTGCTGGT	CGAATCGCAACCTAATTAAC	504	$(GAA)_4$
Msc100	CCATCTTGGAAAGTAGGTTG	TCAACCTTAGGAGTGGAAGA	133	$(TCT)_4$
Msc102	CTACCTGATTGTGTGTGACG	GTAGCTCAATCGCCATAATC	302	$(TA)_9$
Msc105	AGTATATGCGACGTATGGCT	CCTCAATGTTGGAGGAGTAA	476	$(TA)_7$
Msc106	ACAACCAGAGGTTCACAGAC	TTGAGAAAGAAATCCTGGAC	397	$(TA)_6$
Msc108	CACATGTTGCTCACGTTTAC	TCCCATGTGTTCACACTAAA	520	$(CT)_8$
Msc120[a]	TGATGAGTAAGTATGCGTCG	GAAATTCTTCTCCCAGGACT	395	$(CCT)_5$
Msc121[a]	TGGTAAAGAAGGGAGAATGA	AAATTCTGTCCCTGTTGTTG	426	$(TAAA)_3$
Msc124[b]	TCTTGCTCAGACCGATAAAT	AGGATGGGTAGATGTCACAG	292	$(ATCA)_3$
Msc127[a]	GTCCGAGTTTCACCAATTTA	TGCACAAACTTAATGGAGAC	235	$(TTTA)_3$
Msc138	CAACGGATCTATTCTAACGG	GAGCATTGAGAGTGGAAGAG	174	$(GAT)_4$
Msc151	AATATGGGCACATCAAAGTC	TCTCCTCCTTGTATGCCTAA	131	$(CAT)_4$
Msc158	ATTGTAGACGACGGTGAATC	CATCCCTAATTCCTCAATCA	496	$(AT)_9$
Msc159[a]	TCCGCTGTTTACTCTGAGAT	GTGTTCCTATCTTGCTTTCG	200	$(TGCTT)_3$
Msc160[b]	ATCGTATGAGCTCGCATC	TCTATACAGATTGCCATCCC	101	$(TCT)_5$
Msc161[b]	AGCTCTCTTCTTCGTCCTTT	GCCATATTCAAACGGATCTA	112	$(TCT)_5$
Msc165	CCTCCAACCCTCTTCTAACT	CACAATACGAGATGGTTCAA	247	$(TG)_7$
Msc173	ACGACATTTCATTTCCTGAG	CTCTCCTCAACCTCACTCTG	171	$(TGA)_4$
Msc174[a]	CAGAGTGAGGTTGAGGAGAG	CCCAATTAAGCTGCAATATC	467	$(ATTT)_3$
Msc175	TCATATGTCTTGAGCATCCA	ACGAGACAAGAGACCAAGAA	256	$(TTG)_4$
Msc179	TCATCTCATCTCACCACTGA	TTCAACTCAAGTGCATCAAG	194	$(TGA)_4$
Msc184	CCAAGATAATTCCCTCAGACT	CATCCTCCTTATGATTCGAG	146	$(AAG)_4$
Msc187	GAAAGGTACCCAGAGGCTAT	ACCTGATTGTTTGTGTGGTT	130	$(AT)_9$
Msc193	GACCGGAGGGAGTATTATTT	GGAATTGCGTTACATGAGAT	137	$(AT)_8$
Msc196	TAGTTCTGGAAGCTATGGGA	TGCCCTTGAATAGAAACTGT	447	$(GCA)_4$
Msc198	AGATGCAATATTAGGGCTCA	GATCTCCTTCTACACCCACA	190	$(CCA)_4$
Msc199[a]	CACAATTCCACTCAATGTCA	ATTGTGAAATGGTTAGGTGC	208	$(CAAT)_3$
Msc200	CACATCGGTTCCTAAGACAT	AGGGAGAGTGAGGATAAAGG	315	$(ATT)_4$
Msc204	ATCAGCTCGAATTTAGGGTT	TCAAAGGGATGATGATTAGG	121	$(TTC)_4$
Msc206	ATGAGTGGGTTAGCAGAGTG	TGAATGCAAGTCTGTGTGAT	189	$(AT)_8$
Msc217[b]	TGAGTCGGAGCTATCATCTT	AGAGGTCCAGAGATAAAGGG	161	$(CTT)_5$
Msc218[a]	AAAGAAACTCAAGGTCCCTC	TCCTCATCATCCTCAGACTC	179	$(AGA)_5$
Msc219[a]	GATTATGGCTTGCTTCTGTC	CCAATGTTACTCAAGCCAAT	175	$(GCTT)_3$
Msc220	TCTAACTCTTCTGCCTCAGC	AAGAAGATTGTGCTGTCGAT	427	$(TTC)_4$
Msc225	ACAAACCAAGAAACACCAAC	GATGACTTAGAACGGGATCA	408	$(AT)_6$
Msc229[b]	GAGAAAGGATATGATGCAGG	GTTGCTCGGAAATTATAAGG	140	$(AATA)_4$
Msc230	CATATGTCAAATGCCTTTCC	ATAAATTGACTGATGGGTGC	151	$(TA)_7$
Msc231[a]	GTGGATTGTTAGGCATGAAT	AATGCAAGAGTGATATGGGT	504	$(CAAAA)_3$
Msc240	GAAATGTAGTTGGTTGACCG	ATTCTTCTTCTCCTTCGTCC	239	$(CCT)_4$
Msc248[a]	GTGATTGGGATATTGAGGAA	GGTCCCTGTCTACAATCAGA	166	$(CGGC)_3$
Msc253[a]	TGTTGAGAGTGTGCATTCAT	GTTGTATTGATGGAAAGGCT	321	$(CATTC)_3$
Msc255	CAAAGCAAATCAAGTGGC	CCAGCAGTGACTACAACAGA	241	$(TGT)_4$
Msc256[a]	GATTGATCTCATTGGACTGG	TCATTTCTTCATCCTTCACC	493	$(AAGA)_3$
Msc259	TCCCTGATTGAAATGAAGAC	AGTTGAGAGTTGAGAAGGAAGA	219	$(TG)_6$
Msc266[a]	GCTGCTTATCTTTCTCTTGG	GTGCCTGACACATACCTCTT	219	$(GAT)_5$
Msc267	AGAAGATCAAGGAAAGGAGC	ATAAGTTCAATGGGAGCAGA	256	$(AAG)_4$

[a]Synthetized unlabeled primers for PCR selection; [b]synthetized unlabeled primers for PCR selection and synthetized primers for polymorphism analysis.

DNA Polymerase (Invitrogen). Amplification program was 95°C for 2 min; 35 cycles of 45 s at 95°C, 1 min at 55°C, and 2 min at 72°C; and a final extension of 5 min at 72°C on Veriti thermocyclers (Applied Biosystems). The PCR products were analyzed on 2% agarose gels. From 31 SSR locus PCR selections, 11 loci were then assessed for polymorphism in 444 samples of *Mimosa scabrella.*

Genotyping using Multiplex Panels and polymorphism analysis validation

The Multiplex Panels were developed with Autodimer (Vallone and Butler, 2004) and Multiplex Manager (Holleley and Geerts, 2009) with up to four loci per panel (Table 2). PCRs were performed in a 12.5- to 25-μL volume reaction with 1 ng template DNA, 1X PCR buffer Tris-HCl, 2.5 mM $MgCl_2$, 0.2 mM dNTP mix, 0.05 to 0.12 μM of each primer (Table 2) and 1 U Platinum Taq DNA Polymerase (Invitrogen). DMSO (10%) was added for Msc003 and Msc161. PCR amplification was also performed on Veriti thermocyclers (Applied Biosystems) under the following conditions: 95°C for 10 min; 25 cycles of 45 s at 95°C, 1 min at 55°-56°C, and 2 min at 72°C; and a final extension of 30 min at 72°C. Fluorescent dyes of each forward primer, primer concentration, and annealing temperature are described in Table 2. Fluorescently labeled PCR products were separated by capillary electrophoresis using the ABI 3130 Genetic Analyzer (Applied Biosystems) with internal size standard GeneScan600 LIZ (Applied Biosystems). Peak interpretation, allele size calling and genotyping were performed with the Gene Mapper® software ID-X v. 1.2 (Applied Biosystems). Descriptive statistics, for the nuclear SSR marker, like allelic richness, the number of alleles at a locus (N_A); genotypic richness, the number of genotypes with four alleles per locus (G); the observed and expected heterozygosities (H_O and H_E) and fixation index (F) were all calculated using Autotet (Thrall and Young, 2000) assuming autopolyploidy. H_E and F were computed under random mating and chromosome segregation [H_E(Ce)] and [F(Ce)], and under random mating and some level of chromatid segregation [H_E(Cd)] and [F(Cd)], considering maximum double reduction with $\alpha = 1/7$. For cpSSR, haplotype frequencies were calculated using GenAlEx 6.5 (Peakall and Smouse, 2012).

Table 2. Characteristics of 11 SSR labeled primers and panels developed for *Mimosa scabrella*.

Locus	Multiplex panel	Forward primer dye	Allele size (bp)	Primer concentration (μM)	Ta (°C)	BLASTn top hit	E value	GenBank accession No.
Msc098*	1	VIC	113	0.05	56	No hit	-	KY082898
Msc045	1	VIC	267-283	0.05	56	Chloroplast genome (*Acacia exocarpoides*)	3E-114	KY310532
Msc124	1	FAM	286-300	0.05	56	Chloroplast genome (*Pararchidendron pruinosum*)	2E-136	KY310534
Msc217*	1	FAM	156	0.05	56	No hit	-	KY310536
Msc229*	2	PET	138	0.11	56	No hit	-	KY034416
Msc041*	2	NED	422	0.07	56	Chloroplast genome (*Prosopis glandulosa*)	3E-100	KY310533
Msc009	2	FAM	499-514	0.12	56	Chloroplast genome (*Acacia formidabilis*)	1E-40	KY271088
Msc072*	2	VIC	430	0.07	56	No hit	-	KY273304
Msc161*	3	NED	108	0.2	56	No hit	-	KY310537
Msc003	3	NED	195-224	0.1	56	No hit	-	KY310535
Msc160*	Single	FAM	95	0.05	55	No hit	-	KY310538

*Monomorphic loci; Ta = annealing temperature.

RESULTS AND DISCUSSION

Characterization of microsatellites and validation for *Mimosa scabrella*

Illumina paired-end sequencing generated a total of 1,431,034 reads with 237,704,384 bases. After trimming and quality control read checks, 42,546 contigs were assembled.

Contigs screening generated 290 SSR loci, trinucleotide repeats were the most common (32.4%), followed by tetranucleotide (23.15%), dinucleotide (19.0%), mononucleotide (17.6%), pentanucleotide (6.25%), and hexanucleotide (1.7%) repeats. The number of perfect, imperfect, and compound motif repeats corresponds to 252, 2, and 16 loci. From all SSR loci identified, we designed 211 primer pairs, 65 primer pairs were approved after *in silico* primer quality assessment, and a subset of 31 were synthesized. All 31 primer pairs were tested on agarose, 19 generated amplicons in the expected size, and from these, 11 primer pairs were synthetized with fluorescent dyes in forward primer. All samples successfully amplified and showed at least one allele in common between mother trees and progeny samples in all 11 markers. Based on capillary electrophoresis data for the 444 *M. scabrella* samples, 4 loci were polymorphic (P = 36%) in adult and progeny individuals: Msc009, Msc045, Msc124, and Msc003, with allele richness (A) of 4, 3, 2, and 11, respectively, considering all sampling. These polymorphic markers are hexa-, penta-, tetra-, and trinucleotide motifs. A similarity search of amplicon contigs was conducted using BLASTn with E value cutoff of 1 x 10^{-5} (Table 2) Of the 11 markers, Msc009, Msc041, Msc045, and Msc124 sequences showed significant similarity with chloroplast genomes of some Mimosoideae species and all samples of these markers displayed only one allele per sample and the same allele between mother trees and progenies, revealing maternal inheritance of chloroplast genome for *M. scabrella*. Msc003 displayed from one to four alleles per sample, showing its tetraploid nature, as described by Dahmer et al. (2011). Only identified allele peaks were considered for heterozygote genotype, without considering the allelic dosage for incomplete heterozygotes (Narayan et al., 2015).

Significant departure from Hardy-Weinberg equilibrium was detected at Msc003 locus (P < 0.001) under chromosome and chromatid segregation for adults and progenies. Allele richness, genotypic richness, and H_O were 5, 4, and 0.394 for adults and for progenies they were 11, 16, and 0.309. For adults, under random mating and chromosome segregation, F(Ce) and H_E(Ce) were 0.478 and 0.175. Considering random mating and some level of chromatid segregation, F(Cd) and H_E(Cd) were 0.446 and 0.116. For progenies, F(Ce) was 0.296, H_E(Ce) 0.438, F(Cd) 0.245, and H_E(Cd) 0.409.

Four different haplotypes were found for Msc009, Msc045, and Msc124 cpSSRs, two in common between adult and progeny groups, and the other two were private to the adult group. The haplotype 1, formed by the alleles 514 bp (Msc009), 283 bp (Msc045), and 286 bp (Msc124), was the most common both for adult and progeny groups with frequencies of 0.788 and 0.762. The haplotype 2, formed by 499, 274, and 300 bp alleles, was the second most common, with frequencies of 0.152 and 0.238 for adult and progeny groups, respectively. Haplotype 3, formed by 507, 267, and 286 bp alleles, and haplotype 4 formed by 502, 267, and 286 bp alleles had only one occurrence in adults, both with frequency of 0.030. Polymorphism detection in chloroplast genome is limited due to low mutation rate and does not recombine (Provan et al., 2001). Informative chloroplast microsatellite of non-model species can be time intensive (McPherson et al., 2013) and first microsatellites developed for *M. scabrella* included three polymorphic cpSSRs.

The 11 SSR markers were 100% cross-amplified in five Mimosoideae species, ranging from two loci in *Calliandra tweedii* Benth. to all 11 loci in *M. taimbensis* Burkart (Table 3). Msc003 marker displayed polymorphism for *M. taimbensis*.

Next-generation sequencing has enabled detection of many SSR loci and marker development for nuclear and chloroplast sequences. The validated SSR set developed in this study has the potential to be applied for population genetic studies, will enhance molecular

research on the species for marker-assisted selection and develop conservation strategies for this highly human managed species. The validated SSR set can also be used in other Fabaceae-Mimosoideae species because of its high cross-amplification and as SSR reference for other polyploidy species. However, most of the microsatellite markers obtained in the present study still need to be further validated.

Table 3. Cross-species transferability test of *Mimosa scabrella* microsatellites for five Fabaceae-Mimosoideae family species.

Loci	CTW (N = 5)	MBM (N = 5)	MRM (N = 2)	MTB (N = 5)	PTR (N = 5)
Msc229	-	-	+	+	-
Msc041	-	-	+	+	-
Msc009	-	+	+	+	+
Msc072	-	-	+	+	-
Msc160	-	-	-	+	-
Msc045	+	+	+	+	+
Msc098	-	-	+	+	+
Msc217	-	-	-	+	+
Msc124	+	+	+	+	+
Msc161	-	-	-	+	-
Msc003	-	-	-	+	-

CTW = *Calliandra tweedii* Benth.; MBM = *Mimosa bimucronata* (DC.) Kuntz; MRM = *Mimosa ramosissima* Benth.; MTB = *Mimosa taimbensis* Burkart; PTR = *Parapiptadenia rigida* (Benth.) Brenan; N = number of individuals.

Conflicts of interest

The authors declare no conflict of interest.

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