



TRANSFERABILITY OF MICROSATELLITE MARKERS FROM GENUS *CERCIS* TO *BAUHINIA FORFICATA* SUBSP. *PRUINOSA* (FABACEAE): A TOOL FOR GENETIC CHARACTERIZATION

TRANSFERIBILIDAD DE MARCADORES MICROSATÉLITES DEL GÉNERO *CERCIS* A *BAUHINIA FORFICATA* SUBSP. *PRUINOSA* (FABACEAE): UNA HERRAMIENTA PARA LA CARACTERIZACIÓN GENÉTICA

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SUMMARY

Background and aims: *Bauhinia forficata* subsp. *pruinosa* is a native South American tree widely used in traditional medicine due to its hypoglycaemic properties, attributed to glycosylated flavonoids. Little is known about the genetic diversity of its populations, therefore, the aim was to evaluate the transferability of microsatellite (SSR) markers developed for the genus *Cercis* to *B. forficata* subsp. *pruinosa* and to characterize them.

M&M: Fifteen SSR markers developed for *Cercis canadensis* and *Cercis chinensis* were tested in 52 individuals from its distribution range of Argentina. Total genomic DNA was extracted from leaf tissue using the CTAB method. PCR amplification was performed under standardized conditions, and fragments were analyzed to estimate the number of alleles and genetic diversity parameters.

Results: Six SSR markers successfully amplified and generated 34 alleles, with 3–7 alleles per locus. Genetic diversity parameters showed moderate levels of genetic variability (mean $H_e = 0.481$; $H_o = 0.502$), and the average inbreeding coefficient ($F_{is} = -0.138$) indicated a slight excess of heterozygotes.

Conclusions: Some microsatellites developed for *Cercis* can be transferred to *B. forficata* subsp. *pruinosa*, providing a low-cost tool for preliminary population genetic studies, contributing to future conservation strategies and the sustainable use of native plant resources with phytopharmaceutical potential.

KEY WORDS

Conservation, genetic diversity, microsatellites, phytopharmaceutical potential, transferability.

RESUMEN

Introducción y objetivos: *Bauhinia forficata* subsp. *pruinosa* es un árbol nativo de Sudamérica utilizado en la medicina tradicional por sus propiedades hipoglucemiantes, atribuidas a flavonoides glicosilados. Se sabe poco sobre la diversidad genética de sus poblaciones, por ello, el objetivo fue evaluar la transferibilidad de marcadores microsatélite (SSR) desarrollados para el género *Cercis* a *B. forficata* subsp. *pruinosa* y caracterizarlos.

M&M: Quince marcadores SSR desarrollados para *Cercis canadensis* y *Cercis chinensis* fueron probados en 52 individuos de su área de distribución en Argentina. El ADN genómico total fue extraído del tejido foliar utilizando el método CTAB. La amplificación por PCR se realizó bajo condiciones estandarizadas, y los fragmentos se analizaron para estimar el número de alelos y parámetros de diversidad genética.

Resultados: Seis marcadores SSR amplificaron exitosamente y generaron 34 alelos, con 3 a 7 alelos por locus. Los parámetros de diversidad genética mostraron niveles moderados de variabilidad genética ($H_e = 0,481$; $H_o = 0,502$), y el coeficiente de endogamia promedio ($F_{is} = -0,138$) indicó un ligero exceso de heterocigotos.

Conclusiones: Algunos microsatélites desarrollados para *Cercis* pueden transferirse a *B. forficata* subsp. *pruinosa*, proporcionando una herramienta de bajo costo para estudios preliminares de genética de poblaciones contribuyendo a futuras estrategias de conservación y uso sostenible de recursos vegetales nativos con potencial fitofarmacéutico.

PALABRAS CLAVE

Conservación, diversidad genética, microsatélites, potencial fitofarmacéutico, transferibilidad.

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INTRODUCTION

Bauhinia forficata subsp. *pruinosa* (Voguel) Fortunato & Wunderlin, commonly known as “*pezuña/pata de vaca*”, is a Fabaceae tree native to southern South America. This species has a wide distribution in central and northern Argentina; and it can also be found in Bolivia, SE and S of Brazil, Paraguay and Uruguay. It is characterized by its large white flowers and well-defined bilobed leaves, which gives rise to its common name “*pata de vaca*” (cow’s foot). It is a spiny tree with arching branches that can reach 8-10 m in height (Fortunato, 1986).

The genus *Bauhinia* L. has several representatives that are used in South American traditional medicine, particularly for analgesic and hypoglycaemic purposes. Glycosylated flavonoid compounds, derived from quercetin and kaempferol, are responsible for these therapeutic activities (Santos *et al.*, 2019; Fortunato & Nores, 2022). There are three native species in Argentina that have records of these medicinal properties, such as *B. forficata* subsp. *pruinosa*, *B. uruguayensis* Benth. and *B. bauhinioides* (Mart.) J. F. Macbr. This study focuses on *B. forficata* subsp. *pruinosa* due to its well-documented hypoglycaemic activity, which is popularly used in patients with type II diabetes without reported toxicity (Pepato *et al.*, 2004).

In general, raw materials for medicinal preparations are obtained from extractive activities in wild populations. This practice leads to the loss of genetic resources, ecosystem disruption and lacks guarantees as to the quality of commercialized products. Additionally, plant biodiversity is being increasingly threatened by climate change, which causes habitat loss for native and endemic species (Bellard *et al.*, 2012). Therefore, the development of tools to describe the diversity and structure of plant populations is essential for establishing conservation plans.

Microsatellites, also known as SSRs (simple sequence repeats), are widely used in plant genetic variability studies. They are selected for their broad distribution across the genome, codominant nature, ability to detect multiple alleles, high degree of polymorphism, reproducibility, ease of transferability between species and also for being a more cost-effective option (Morgante &

Olivieri, 1993; Powell *et al.*, 1996; Varshney *et al.*, 2005). When it comes to SSR transferability, it is important to consider the phylogenetic distance between species. The closer the families, genera, and species are, the more effective the transferability will be (Montúfar-Galárraga & Barreiro, 2010).

It is important to note that SSRs have not been developed in *B. forficata* subsp. *pruinosa*. However, there are SSRs developed in related taxa: *Cercis canadensis* L. (Wadl *et al.*, 2012) and *C. chinensis* Bunge (Gong *et al.*, 2012), for which there is a history of transferability to *C. chuniana* F. P. Metcalf, *C. chingii* Chun, *C. glabra* Pamp., *C. griffithii* Boiss., *C. occidentalis* Torr. ex A. Gray, *C. racemosa* Oliv., *C. siquastrum* L. and *Bauhinia brachycarpa* Wall. ex Benth. sub nom: *B. faberi* Oliv. (Wadl *et al.*, 2012). The genus *Cercis* L., includes species widely recognized. *Cercis canadensis* is native to North America and is cultivated globally as an ornamental tree. Another species, *Cercis chinensis*, a garden plant, is native to China (Gong *et al.*, 2012; Wadl *et al.*, 2012). According to Poggio *et al.* (2008), Caesalpinioideae species show a large variation in somatic number, with $2n = 14, 16, 20, 22, 24, 26$, and 28 . Genera with $2n = 14$ ($x = 7$), such as *Cassia*, *Senna* and *Cercis*, would indicate that polyploidy occurred early in the evolutionary process. It should be noted that *Cercis* would be the genus with the most basal origin in Fabaceae (Goldblatt, 1981; Lewis *et al.*, 2005). In this context, Poggio *et al.* (2008) propose that in the subfamily Cercidoideae, *Bauhinia* ($2n = 28$) was generated by hybridization and polyploidy from the ancestor *Cercis* ($2n = 14$). This hypothesis is further supported by phylogenetic analyses of *Bauhinia variegata* L., which reinforce its proposed allopolyploid origin (Zhong *et al.*, 2022).

Based on the close phylogenetic relationship between *Cercis* and *Bauhinia*, we hypothesize that SSR markers already developed for some species of *Cercis* could be partially transferred to *B. forficata* subsp. *pruinosa*, and that these markers will be informative for characterizing the genetic diversity of its populations. Therefore, the aim of this study is to evaluate the transferability of SSRs from *Cercis* species to *Bauhinia forficata* subsp. *pruinosa*, and to characterize them using individuals from its natural distribution range in Argentina.

MATERIAL AND METHODS

Plant Material and DNA isolation

A total of 52 samples of *B. forficata* subsp. *pruinosa* were collected from the species distribution range. Voucher specimens from these collection sites were deposited into the SI and BAB herbaria (Thiers, 2016) (Table 1, Fig. 1).

The selected individuals in each collection site are spaced 10 m apart to avoid the potential presence of clones, given their habit of asexual reproduction through gemmiferous roots. For each individual, 5-10 young leaves were collected and stored in plastic bags with silica gel to dry and preserve the DNA. The samples were then stored at -20 °C until further processing.

Total genomic DNA was extracted from leaf tissue by grinding 20 - 40 mg of each sample into a fine powder using a TissueLyser (Qiagen). The extraction was performed using the CTAB method (Doyle & Doyle, 1987), with modifications. DNA quantification was performed by spectrophotometry (NanoDrop 2000 Spectrophotometer, Thermo). This was based on measuring absorbance at 260 nm, 1 µL of each sample was loaded for measurement.

The purity index was determined as the ratio of absorbance at 260 nm to that at 280 nm. Values between 1.80 and 2.00 indicate high DNA purity. DNA samples were diluted with ultrapure water to achieve the required concentrations (10 ng/µl).

Table 1. Geographical location and sampling details of collection sites of *Bauhinia forficata* subsp. *pruinosa*.

Population	N	Province	Dpto.	Date	Collector	Latitude coordinates	Longitude coordinates
BAI	5	Buenos Aires	Hurlingham	21-III-2025	Elisei, A. 189 (SI)	34° 36' 65" S	58° 40' 59" O
ERC1	8	Entre Ríos	Concordia, Concordia	08-XI-2022	Elisei, A. 100 (SI)	31° 22' 33.78" S	57° 59' 47.58" W
ERC2	15	Entre Ríos	Colón, Parque Nacional El Palmar	08-XI-2022	Elisei, A. 108 (SI)	31° 52' 10.56" S	58° 12' 28.8" W
C1	2	Córdoba	Capital	29-I-19	Fortunato, R.H. 13614 (BAB)	31° 43' 27.3" S	64° 24' 18.9" W
CH	3	Chaco	San Fernando, Barranqueras	13-IX-2022	Fortunato, R.H. 14842 (SI)	27° 29' 39" S	58° 55' 79" W
MR	19	Misiones	San Pedro	3-II-2026	Helou s/n (SI-241045)	26° 43' 52.31" S	53° 53' 09.87" W

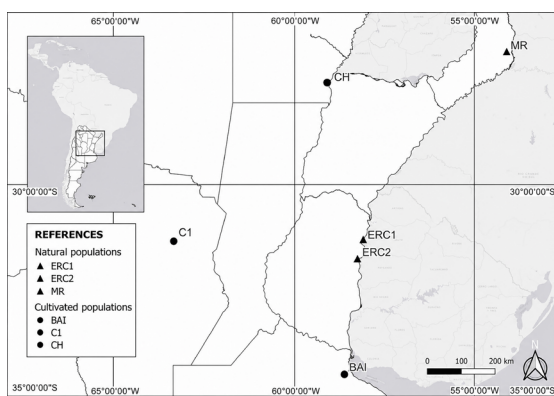


Fig. 1. Geographic coordinates and sampling information of collection sites of *Bauhinia forficata* subsp. *pruinosa* across Argentina. **BAI** (INTA Hurlingham, Buenos Aires), **ERC1** (Concordia, Entre Ríos), **ERC2** (El Palmar, Colón. Entre Ríos), **C1** (Córdoba), **CH** (Chaco), **MR** (San Pedro, Misiones).

SSRs transferability

In order to test the transferability, we used six SSR markers of *Cercis chinensis* (Gong *et al.*, 2012) and nine SSRs of *Cercis canadensis* (Wadl *et al.*, 2012) (Table 2). They were selected based on their close phylogenetic relationship between the genera *Cercis* and *Bauhinia* (Zhong *et al.*, 2022). The evaluation included assessing amplification success, resolution clarity, and polymorphism levels of the SSR markers. Then, the genetic variation of the identified polymorphic loci was characterized and evaluated.

Different polymerase chain reaction (PCR) conditions were tested on each primer to optimize the transferability. The PCR reaction was performed in a 12 µl reaction volume consisting of 20-40-60 ng of DNA, 1.5 mM - 2.5mM - 3mM - 3.125mM of MgCl₂, 0.25mM of dNTPs, 0.5 mM of each primer, 0.5 U of Taq DNA polymerase (INBIO HIGHWAY) and 1X of Taq reaction buffer. The PCR thermal

Table 2. Characteristics of 15 SSR markers originally developed for *Cercis* species and tested for transferability to *Bauhinia forficata* subsp. *pruinosa* (Gong *et al.*, 2012; Wadl *et al.*, 2012).

<i>Cercis chinensis</i>			
Name	GenBank	Repeat	Primer
CC09	JQ624656	(TG)8	F:*ATGGACAGTCCAAAAAGGG R:ACATGCACAAAGGAACACC
CC332	JQ624657	(AC)8(AC)5	F:*TTTGAAGAAACACTGTATGC R:AATGGAGATTTATTGAGAAG
CC585	JQ624659	(GT)7	F:*ATGACTCCCAAAGTATGCT R:TTCAGAAATATTACCGTGC
CC689	JQ624660	(GT)7	F:*AGATGGGAAAGGAAACAA R:ATGAACGAAGCCAATGAT
CC716	JQ624662	(AC)7	F:**CAGGATCAAATCCACAAG R:AAAGAAATCATTCAAAGCA
CC735	JQ624663	(TG)6(GA)7	F:**AAAGAAAAAGCAAACAAGC R:TCACAGGGAAACAGAATCA
<i>Cercis canadensis</i>			
Name	GenBank	Repeat	Primer
CCAN164a	GU252911	(AAAT)6	F:CCTCTAAAACTCAGGGTGCACTTTAC R:CAATATGAATACCATCGAAGCCTG
CCAN176a	GU252918	(AG)6	F:GACTGCAAATCGGAAAGAAAGAAG R:TCCTTCAGGGATGCATTTAAGAAC
CCAN180a	GU252921	(CT)5	F:GATTTCAAAGCCCTGTTAGTGCC R:TTATTGTGTTTGTAAGTAGCGCC
CCAN461a	GU253032	(AG)12	F:TCACAAACAGGAAAAGGGAGATG R:CATTTCAGAATTCAGAGCAATCCC
CCAN921a	GU171392	(CT)20	F:ATTCGTCACCTTCCATTAAACCC R:CTTTGTTTGCAGTTGCAGAGGTG
CCAN941spa	GU253197	(AG)8	F:TTTTGGTGGGATTGTATATGTGGG R:CTTCCACCCACTCACTTCTTGAAC
CCAN942a	GU253198	(CT)10	F:CAGTACTCAAACACCAACCTCGTG R:CCACCACGTGGAAAGCATATAAAG
CCAN1006a	GU253212	(ACT)6	F:GAGTCCACTGATTGCGGCAC R:TTTGACAACATAGGAACAGAGAGTTGG
CCAN1240b	GU253251	(CT)4	F:TCTACGCCATTCTCTTCATCTTC R:GTGCTTTCAGTCATGGTGATTTTG

profiles for the SSR markers were adjusted from the original protocols (Sraphet *et al.*, 2011). The PCR program applied consisted in: 94 °C for 4 minutes, followed by 35 cycles at 94 °C for 40 seconds, 45-50-55 °C for 60 seconds, 72 °C for 60 seconds, and a final extension at 72 °C for 7-10 minutes. PCR products were separated by electrophoresis on 6 % denaturing polyacrylamide gels and visualized by silver staining as described by Benbouza *et al.* (2006). A 25 bp molecular marker (Inbio Highway) was used to estimate the molecular size of the amplified fragments.

To analyse genetic variability of SSRs, descriptive parameters of genetic variability were estimated, including number of alleles per locus (N_a), number of effective alleles per locus (N_e), expected heterozygosity (H_e), observed heterozygosity (H_o) and inbreeding coefficient (F_{is}) using GenAlex 6.5 (Peakall & Smouse, 2006). The

polymorphic information content (PIC) of the SSRs markers was estimated using Cervus 3.0.3 software (Kalinowski *et al.*, 2007).

RESULTS

A total of 15 SSR markers were analysed, of which seven successfully amplified. Among these, six exhibited polymorphic patterns (CC735, CCAN176a, CCAN180a, CCAN461a, CCAN921a and CCAN1006a) and only one was monomorphic (CC09). The transferred polymorphic SSRs produced a total of 34 alleles, ranging from 3-7 alleles per marker (Table 3). The markers CCAN180a, CCAN176a, and CCAN921a exhibited the highest number of alleles (7), making them the most informative, while CCAN1006a had the lowest number of alleles (3).

To optimize the amplification of transferred SSRs, PCR conditions were adjusted. The DNA concentration, MgCl_2 concentration, annealing temperature and final program length were modified to enhance transferability. Finally, a concentration of 3mM MgCl_2 was used for markers CCAN180a, CCAN176a and CCAN461a, while a concentration of 3.125 mM MgCl_2 was used for markers CCAN1006a, CCAN 921a and CC735. A single PCR program was used for all markers, with an annealing temperature of 50 °C. The lengthening of the final extension step to over 10 minutes enhanced amplification consistency, likely due to improved temperature-sensitive nature of primer-template hybridization.

The observed and expected allele size ranges varied across loci (Table 4). For instance, CCAN180a was expected to range from 83-104 bp, but observed fragments ranged from 105 to 154 bp. This discrepancy may reflect species-specific mutations or nonspecific primer binding due to partial homology between template and primer sequences.

The analysis of genetic variability across all polymorphic loci (Table 3) revealed moderate to low levels of diversity, with an average H_e of 0.481. The highest H_e was observed at locus CCAN461a (0.605), while the lowest was recorded at CCAN176a (0.378). Observed heterozygosity (H_o) ranged from 0.060 (CCAN176a) to 0.916 (CCAN461a), with an average of 0.502. Intermediate H_o values were found at the remaining loci. Particularly, loci CCAN461a and CC735 exhibited high H_o values,

suggesting a high degree of polymorphism.

The average F_{is} , which ranges from -1 to 1, was low and negative (-0.138), indicating a low excess of heterozygotes. The loci exhibiting a positive F_{is} value were CCAN176a (0.819) and CCAN921a (0.566). In contrast, loci such as CCAN180a showed F_{is} values near zero, consistent with equilibrium expectations.

The average PIC was 0.576, classifying this marker set as highly informative. The most informative loci were CCAN180a, CCAN176a, and CCAN921a ($\text{PIC} > 0.5$), while CCAN1006a, CCAN461a, and CC735 were considered moderately informative ($\text{PIC} < 0.5$).

DISCUSSION

Given the absence of previous population-level genetic studies on *B. forficata* subsp. *pruinosa*, this study introduces a set of transferred SSR markers that could serve as a fundamental tool for genetic analyses (Barbará *et al.*, 2007). These low-cost markers will allow the assessment of genetic variability in further population genetic studies.

The 40 % success rate of SSR transfer from *Cercis chinensis* and *Cercis canadensis* to *B. forficata* subsp. *pruinosa* reflects moderate cross-species utility. Despite the close phylogenetic relationship between the genera *Cercis* and *Bauhinia*, the transferability rate remains relatively low, consistent with patterns seen in other genera. For example, in *Arachis* L. (Fabaceae), SSRs developed for

Table 3. Genetic variation at six polymorphic SSRs markers in 52 individuals of *Bauhinia forficata* subsp. *pruinosa*. Abbreviations= Na: Number of alleles per locus, Ne: Number of effective alleles per locus, He: expected heterozygosity, Ho: observed heterozygosity, Fis: Inbreeding coefficient, PIC: Polymorphic information content.

Locus	Na	Ne	Ho	He	Fis	PIC
CCAN180a	7	2.016	0.467	0.477	-0.086	0.721
CCAN176a	7	1.932	0.060	0.378	0.819	0.705
CCAN1006a	3	1.868	0.766	0.473	-0.806	0.404
CCAN921a	7	2.123	0.168	0.442	0.566	0.714
CCAN461a	5	2.183	0.916	0.605	-0.710	0.458
CC735	5	1.597	0.634	0.512	-0.611	0.451
	34	1.953	0.502	0.481	-0.138	0.576

Table 4. Comparison of expected and observed allele size ranges for SSRs transferred from *Cercis* to *Bauhinia forficata* subsp. *pruinosa*. Abbreviations= bp: base pairs.

Locus	Expected allele size range (bp*)	Observed allele size range (bp*)
CCAN180a	83-104	105-154
CCAN176a	137-146	108-142
CCAN1006a	107-123	99-104
CCAN921a	69-119	108-117
CCAN461a	130-179	117-129
CC735	213	117-126

Glycine Willd. showed only a 28 % success rate (He *et al.*, 2006). Amplification failure of certain loci may be attributed to nonspecific primer binding or insufficient sequence homology between the template and the target regions, despite high DNA quality. This limitation has also been observed in other studies on intergeneric SSR transferability studies (Chirinos-Arias & Jiménez, 2015; Zhong *et al.*, 2022). These findings underscore the importance of developing genus- or species-specific markers to increase resolution and reproducibility.

Genetic diversity analyses showed lower observed heterozygosity (H_o) than expected (H_e) at loci CCAN180a, CCAN176a, and CCAN921a, suggesting potential inbreeding. This pattern was particularly evident at loci 176 and 921, where these results were consistent with high and positive F_{is} values, as observed in similar SSR transferability studies in *Elsholtzia stauntonii* Benth. (Zhang *et al.*, 2022). Conversely, CCAN1006a, CCAN461a, and CC735 showed H_o values higher than H_e , indicating a greater frequency of heterozygotes. These results differ from intergeneric microsatellite transfer studies in *Foeniculum vulgare* Mill., which reported low variability was observed in the amplified loci (Aiello *et al.*, 2020).

Regarding the F_{is} , most loci showed deviations from Hardy-Weinberg equilibrium, which could be attributed to the characteristics of the analysed material in this study. These results could improve by including additional populations in further studies using the same SSR markers.

The relatively high mean PIC values indicate that the amplified loci are highly informative for analysing genetic variability in *B. forficata*

subsp. *pruinosa*. According to Botstein *et al.* (1980), markers with PIC values greater than 0.5 are considered highly informative, as seen for CCAN176a, CCAN921a and CCAN180a in this study (PIC= 0.705, 0.714, 0.721, respectively).

Finally, it is important to note that, although sequencing of the amplicons was not performed, the reproducibility, polymorphism, and amplification profiles observed are consistent with typical SSR behaviour. However, the possibility of non-specific amplification cannot be completely ruled out, particularly in loci showing size deviations, and should be considered when interpreting the results.

CONCLUSION

The transferability of SSRs between related species is a valuable strategy for advancing the genetic characterization of *B. forficata* subsp. *pruinosa*. These markers can support future analyses of population structure and may help identify loci linked to production of flavonoids with pharmacological interest.

The characterization and conservation of native germplasm are essential for the potential domestication and agricultural introduction of this species. If appropriate technological processes and development strategies are implemented, *B. forficata* subsp. *pruinosa* could become the basis for a marketable phytopharmaceutical product. This would not only promote sustainable use of biodiversity but also contribute to improving the quality of life in rural communities through the rational and integrated exploitation of native resources.

AUTHOR CONTRIBUTIONS

AME: Analysis, collection of plant material, collection and interpretation of data, preparation of figures, writing of the manuscript. FM: Writing of the manuscript. MVI: Analysis and interpretation of data. RHF: Design of the research, collection of plant material. MVL: Design and execution of the research; analysis, interpretation of data, writing of the manuscript.

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