PAB Pesquisa Agropecuária Brasileira 60 years

Genetics/ Original Article

ISSN 1678-3921

Journal homepage: www.embrapa.br/pab

For manuscript submission and journal contents, access: www.scielo.br/pab

Gossypium barbadense maintenance and coexistence *in situ* with genetically modified cotton in the state of Mato Grosso, Brazil

Abstract – The objective of this work was to determine which cotton genetic resources are present in the state of Mato Grosso, Brazil, to assess the risks associated with the *in situ* conservation of these materials, including gene flow from genetically modified (GM) crops, and to determine the effectiveness of the exclusion zones for genetically modified cotton. Four expeditions were performed inside and outside the exclusion zones, to collect geographical and morphological data, as well as the use of these species by the population, and data on the *in situ* maintenance practices at each site. The presence of GM proteins was determined in some plants within the exclusion zones, and in all plants outside the exclusion zones. *Gossypium barbadense* was the most frequent species, primarily cultivated in urban and rural backyards for medicinal purposes. No evidence of hybridization with *G. hirsutum* was observed, based on morphology or on GM protein expression, even in regions with long-term coexistence. The species *G. barbadense* is the most important cotton genetic resource in Mato Grosso state. The exclusion zones are not necessary to protect the genetic diversity of the species in the state. The decline of medicinal use of the species is the primary threat to its conservation, and preservation efforts should focus on maintaining its traditional uses.

Index terms: biosafety, gene flow, genetic resources.

Conservação e coexistência *in situ* de *Gossypium barbadense* com algodoeiros geneticamente modificados, no estado do Mato Grosso, Brasil

Resumo – O objetivo deste trabalho foi determinar quais recursos genéticos de algodoeiro estão presentes no estado de Mato Grosso, Brasil, para avaliar os riscos associados à conservação *in situ* desses materiais, inclusive o fluxo gênico de culturas geneticamente modificadas (GM), para determinar a eficácia das zonas de exclusão para algodão geneticamente modificado no estado. Quatro expedições foram realizadas dentro e fora da zona de exclusão, para coletar dados geográficos e morfológicos, dados sobre uso pela população, além de práticas de manutenção *in situ* em cada local. Determinou-se a presença de proteínas GM em parte das plantas, dentro das zonas de exclusão, e em todas as plantas fora das zonas de exclusão. *Gossypium barbadense* foi a espécie mais frequente, cultivada principalmente em quintais urbanos e rurais, para fins medicinais. Não houve evidência de hibridização com *G. hirsutum*, com base na morfologia ou expressão de proteínas GM, mesmo em regiões com longa coexistência. A espécie *G. barbadense* é o recurso genético mais importante de algodoeiro em Mato Grosso. As zonas de exclusão são desnecessárias para proteger sua diversidade genética no estado. O declínio

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Received
February 17, 2025

Accepted
July 23, 2025

How to cite
BARROSO, P.A.V.; HOFFMANN, L.V.; LIMA, M.M. de A.; CAVALCANTI, J.J.V.; CAVALIERI, S.D.; FILIPIAKE, A.L.; CARDOSO, K.C.M.; LIMA, L.M. de. *Gossypium barbadense* maintenance and coexistence *in situ* with genetically modified cotton in the state of Mato Grosso, Brazil. **Pesquisa Agropecuária Brasileira**, v.61, e04155, 2026. DOI: https://doi.org/10.1590/S1678-3921.pab2026.v61.04155.

do uso medicinal é a principal ameaça à conservação, e os esforços de preservação devem priorizar a manutenção de seus usos tradicionais.

Termos para indexação: biossegurança, fluxo gênico, recursos genéticos.

Introduction

Upland cotton [*Gossypium hirsutum* (L.) r. *latifolium* Hutch.] is a cultivated plant native to Mexico and Honduras. In Brazil, it is commercially grown in farms with different technological profiles, in regions with diverse edaphoclimatic conditions. Since the mid-1990s, the Cerrado biome (a Brazilian savanna) has been the primary location for cotton cultivation. The planted area in 2023/2024 season was estimated at 1709.6 thousand hectares planted in 339 municipalities, across 16 Brazilian states (IBGE, 2024). Brazil is also an important center of diversity for the genus *Gossypium*. Three cotton species exist in the country, all of them allotetraploid and sexually compatible with cultivated cotton. In addition to upland cotton, *Gossypium hirsutum* r. *marie-galante* (*mocó* cotton) is also present, primarily found in the Northeast Brazil of and cultivated on a large scale, until the end of the 1980s. The second species is *Gossypium mustelinum*, the only cotton native species occurring exclusively in the wild form and found in the Northeast Region of the country (Barroso et al., 2021). The third species − *Gossypium barbadense* − is found almost throughout the entire national territory (Barroso et al., 2005b). The species *G. barbadense* was cultivated by Indigenous Peoples, before Europeans arrived in Brazil, and it was quickly adopted as a cultivated species by the colonizers. Although this species is native from Southern Ecuador and Northern Peru (Viot & Wendel, 2023), Brazil is considered a center of its diversity. Its importance as a cultivated species gradually declined from the second half of the 19th century (Giband et al., 2010) and, currently, *G. barbadense* is not commercially cultivated for fiber production in the country.

Cotton was the second crop in Brazil to be approved for the commercial cultivation of genetically modified (GM) cotton varieties. In 2005, the National Technical Commission on Biosafety (CTNBio), Brazil's GM biosafety authority, approved the commercial cultivation of GM cotton at a time when the country had a limited experience with GM crops. Aware of the great variability present in Brazilian cotton, CTNBio was very cautious about the conservation of *Gossypium* diversity, and determined the creation of an exclusion zone, where GM cotton could not be cultivated. Similar approaches were previously implemented by GM authorities from the USA, with the prohibition of commercial culture of *Bt* cotton in Hawaii, Puerto Rico, US Virgin Islands, and some part of Florida (US-EPA, 2001, Pierce et al., 2023). The intention was to preserve the Brazilian variability of both native and naturalized Brazilian *Gossypium* species from possible adverse effects resulting from gene flow with GM cotton. The original exclusion zone encompassed the entire Amazon and Pantanal biomes, for the conservation of *G. barbadense*, and a small portion of the Northeast Region for the conservation of *G. mustelinum* and the *mocó* cotton. The exclusion zone was established as a dynamic biosafety measure, designed to be adaptable to new information and advancements in knowledge (Barroso et al., 2005b). Following this principle, changes were made. The entire area of the states Tocantins, Roraima, and Rondônia − which were contained originally within the exclusion zone − obtained authorization from the CTNBio for the cultivation of GM cotton in 2013, 2015, and 2018, respectively. Subsequently, in 2023, the CTNBio also authorized the removal of exclusion zone areas in the Santana do Araguaia municipality, in the state of Pará. In early 2023, CTNBio initiated a discussion to assess the relevance and effectiveness of the zones in protecting *Gossypium* biodiversity. To substantiate its decision, CTNBio requested that Embrapa (Brazilian Agricultural Research Corporation) could conduct studies, to assess the importance of exclusion zones in the *in situ* conservation of *Gossypium* biodiversity in the country, with particular reference to gene flow.

Mato Grosso state is the largest cotton producer in Brazil, and about 33% of its territory was within the exclusion zone, covering 31 municipalities in the northern part of the state and the Pantanal biome. In GM cotton permitted area, the state cultivated 1.23 million hectares in 2022, representing almost 72% of Brazilian acreage. The extensive cotton cultivation in Mato Grosso state, with its designated exclusion zones, offers a valuable model for evaluating the risk of gene flow from GM cotton. This can help assess the effectiveness of exclusion zones as a biosafety strategy

to preserve the genetic integrity of *in situ Gossypium* germplasm in Brazil.

The objective of this work was to determine which cotton genetic resources are present in Mato Grosso state, Brazil, to assess the risks associated with the *in situ* conservation of these materials, including gene flow from genetically modified crops, and thus determine the effectiveness of the exclusion zones for genetically modified cotton in the northern and in the Pantanal regions of the state.

Materials and Methods

To identify online information on *Gossypium*, including occurrence locations other than upland cotton in Mato Grosso state, Brazil, searches were conducted in databases. For information on herbaria, the following databases were consulted: SpeciesLink (Canhos et al., 2022) and Reflora (Pinheiro et al., 2024). *Gossypium* data from germplasm repositories were retrieved from Albrana (https://www.cnpa.embra.br/albrana/) and Alelo (Alves & Azevedo, 2018). Google Scholar was used to search for information available in scientific publications.

Three criteria were used to define the expedition routes, as follows: to include both exclusion zones in Mato Grosso state (northern region and Pantanal); to include regions with significant cotton production (all outside the exclusion zone); and to include the municipalities in Mato Grosso with the highest cotton production (IBGE, 2024).

The information from each criterion was cross-referenced using the geographic information system QGIS 3.36, and four expeditions were determined to be conducted, as follows: in the northern region of Mato Grosso, in October 2023, within the designated exclusion zone; in the Pantanal region, in October 2023, also within the exclusion zone; in the northern and western portions of the Cerrado biome, outside the exclusion zone, in December 2023; and in the central-southern portion of the Cerrado biome, outside the exclusion zone in January of 2024.

Plant locations were determined through direct observation, reports from local residents, and searches in rural properties and urban households. At each site where cotton plants were identified, a questionnaire was applied containing the following information on: geographic location (municipality, coordinates, and address or property name); population type (dooryard, wild, roadside, feral, spontaneous, landrace); plant uses (medicinal, lamp wick, asepsis/cleaning, artisanal spinning and/or weaving, ornamental, agricultural, and unknown); adult and juvenile plants per site; declared original seed provenance; possible risks (animal grazing, habitat degradation, gene flow, expansion of crops, change in cultural habits, and civil works); agricultural practices (planting, control of diseases/pest, fertilization, harvest, cotton ginning, commercialization); presence of diseases and pest insects (caterpillars, boll weevil, mites, viruses, ramularia leaf spot, ramulosis, target spot, etc.); predominant vegetation (urbanized vegetation, rural anthropogenic environment, riparian forest, and other natural environments); presence of reproductive structures (flowers, closed bolls, and open bolls); average height and age.

Additionally, cotton plants were evaluated in the field for 27 descriptors, a subset of descriptors of Consultative Group on International Agricultural Research - CGIAR (IBPGR, 1985). The descriptors encompassed leaf, stem, flower, fruit, and seed traits. Seasonal limitations prevented assessment of all characteristics in every plant.

The identified plants were evaluated *in situ* for the presence of transgenic proteins using commercial kits (Homer Labs) for the detection of Cry1Ac, Pat/Bar, and Cp4Epsps. These proteins were chosen because they are present, either individually or in combination, in all GM cultivars in Brazil. Analyses for the presence of transgenes were conducted on all plants (adult and young) identified outside the exclusion zone. Within the exclusion zone, assessments were conducted by sampling about 50% of the locations.

Results and Discussion

Secondary data provide enough information on *Gossypium* species in Mato Grosso. Herbarium databases recorded 58 specimens of *Gossypium* collected in Mato Grosso. Among these, 31 are identified as *G. barbadense*, 11 as *G. hirsutum*, 1 as *G. arboreum*, 4 as *G. herbaceum*, and 11 remain unidentified at the species level (*Gossypium* spp.). *G. arboreum* or *G. herbaceum* are diploid species that were never cultivated or found in natural habitats in Brazil. To verify the accuracy of the classification,

photos of the exsiccatae were requested from herbarium curators. The image analysis suggests that the original classification may have been incorrect, and the specimens are likely *G. barbadense*. Thirty articles on the scientific literature mention cotton outside crop fields in Mato Grosso state and describe it as a medicinal plant. The species *G. hirsutum*, *G. barbadense*, *G. herbaceum*, and *Gossypium* spp. were the most commonly referenced species, listed in descending order of frequency. Once again, a misclassification must have occurred, with *G. herbaceum* and *G. arboreum* likely being plants of *G. hirsutum* or *G. barbadense*. Germplasm bank databases contain information on 180 *Gossypium* accessions collected in Mato Grosso state. Out of these, 177 are *G. barbadense*, two are *G. hirsutum* r. *marie-galante* (referred to as *mocó* cotton in Brazil and widely cultivated in the Northeastern Semiarid Region until the late 1980s), and *latifolium* (the upland cotton grown on Brazilian farms). Data from herbarium, literature, and germplasm databases were congruent: outside the cultivated fields in Mato Grosso, *G. barbadense* predominates. Almost all these plants, found mainly in backyards and exclusively in human-modified environments, are used for medicinal purposes, never occurring in wild habitats.

The four expeditions covered 10,747 kilometers and visited 47 out of the 141 municipalities in the state of Mato Grosso, out of which 22 municipalities are located within the exclusion zone of genetically modified cotton (Figure 1). The surveyed municipalities are distributed across Pantanal, Cerrado, and Amazon biomes, four of the state's five mesoregions, and 14 of its 22 microregions. A total of 465 locations with non-commercially cultivated cotton were identified: 103 in the northern exclusion zone, 92 in the Pantanal exclusion zone, and 271 outside exclusion zones. Cotton crops in the sampled municipalities outside the exclusion zone totaled 910,000 ha, in the 2022/2023 season, representing 55.2% and 76.4% of all cotton cultivated in Brazil and Mato Grosso state, respectively. In urban areas, they were predominantly found in districts and older or lower-income neighborhoods. In rural areas, they were almost exclusively located on smallholding properties and rural residences. All plants were found exclusively in highly human-modified areas, and none was observed in natural environments. The absence of cotton plants in undisturbed areas corroborates the statements of technicians and residents consulted in the state. All unanimously affirmed having never seen or heard of wild or native cotton plants in Mato Grosso state.

The most frequently identified cotton species was *G. barbadense*. It was present at 453 of the 466 sites, representing 97.2% of the plants. In 448 locations, the plants were found in backyards, in both rural and urban areas. In five locations, the plants were classified as spontaneous, occurring on roadsides or vacant lots. No cultivated fields of this species were found. Few plants were maintained at each location, with the presence of one or two individuals being most common. In all municipalities, both in urban and rural sites, *G. barbadense* is maintained by owners primarily for its medicinal use. The frequency of medicinal use was similar in the three regions of the state, with values equal to or close to 100% (Figure 2). In some locations, the produced cotton was also used for asepsis and cleaning (similarly to the use of pharmacy cotton) and, less frequently, for making lamp wicks. In three locations, the plants were not maintained for medicinal uses only. The observed morphological variations were present across the entire state, both in

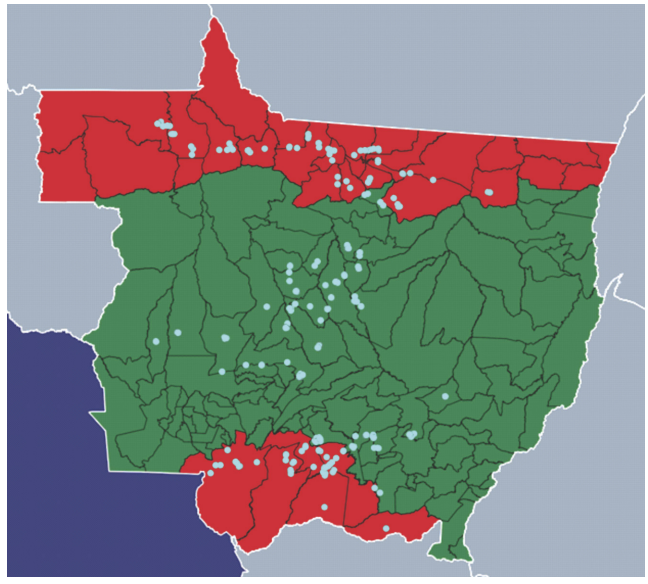

Figure 1. Spatial distribution of *Gossypium* spp. identified during the expeditions, in the state of Mato Grosso, Brazil, inside and outside the exclusion zone of genetically modified cotton. Red: inside the exclusion zone. Green: outside the exclusion zone.

areas within and outside the exclusion zone. The plants exhibited morphological characteristics typical of *G. barbadense*, and all tests carried out to check for the presence of genetically modified proteins in the species yielded negative results (Table 1). Thus, no detectable gene flow from upland cotton into *G. barbadense* was observed.

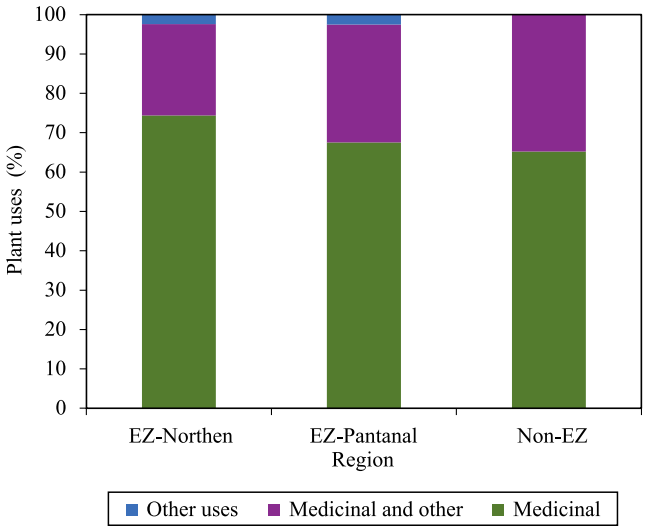

Figure 2. Percentage of reported uses for *Gossypium barbadense* categorized by collection region in the state of Mato Grosso, Brazil. EZ-Northen: northern exclusion zone of genetically modified cotton; EZ-Pantanal region, exclusion zone of genetically modified cotton in municipalities with areas in the region of Pantanal biome; Non-EZ, locations outside the exclusion zone of genetically modified cotton.

Besides *G. barbadense*, *G. hirsutum* was found at 12 sites (2.6%), mainly in backyards and vacant lots. Two races, both derived from improved genotypes, were identified: *marie-galante* (*mocó*) and *latifolium* (upland). A *marie-galante* plant was found in a backyard, in the municipality of Diamantino, used as a medicinal plant. Two possibly mocó plants were discovered in a backyard, in the municipality of Poconé, originating from a seed exchange fair held by a family farming organization. Outside cultivated fields, upland cotton plants were observed in eight locations: two vacant lots and six backyards. Five upland cotton plants maintained in backyards were also used as medicinal plants, and one was kept as an ornamental. The individuals who maintained these upland cotton plants had different profiles: they were aware of the existence of more than one type of cotton and planted upland cotton due to lack of access to *G. barbadense* seed; they were unaware of the existence of more than one cotton species. Upland cotton was also observed on roadsides, primarily outside the exclusion zones, but also, on a much smaller scale, within the exclusion zone. Outside the exclusion zones, seed scattering occurs through two main mechanisms: the transportation of raw seed cotton (pre-ginning) from crop fields to cotton ginning mills, and the transportation of cottonseed (post-ginning) from cotton mills to livestock feed locations. Inside the exclusion zones, roadside plants originated from cottonseed spills during transport to cattle farms. All upland cotton plants tested positive for one or more transgenic proteins, while one mocó and two probable mocó plants tested negative.

Table 1. Results of tests conducted to detect the presence of genetic modified proteins (Pat/Bar, Cry1Ac, and Cp4Epsps), in municipalities located within the exclusion zone of genetically modified cotton (northern Mato Grosso state and the Pantanal region), and in municipalities situated outside that zone.

Species	Result	Exclusion zone in the northern Mato Grosso state	Exclusion zone in the Pantanal region	Nonexclusion zone	Total
Gossypium barbadense	Positive	0	0	0	0
	Negative	49[(1)] (125)[(2)]	49 (65)	258 (762)	356 (952)
Gossypium hirsutum (upland)	Positive	2 (3)	2 (2)	6 (9)	10 (14)
	Negative	0	0	0	0
Gossypium hirsutum (*mocó*)	Positive	0	0	0	0
	Negative	0	0	1 (1)	1 (1)
Gossypium spp.	Positive	0	0	0	0
	Negative	0	1 (2)	0	1 (2)

[(1)]Number of sites where the test was performed. [(2)]Number of plants evaluated.

Outside the cultivated fields, *G. barbadense* is the predominant cotton species in Mato Grosso state; other cotton types are rarely found in the same habitats. All plants of *G. barbadense* were found in highly human-altered environments, and there was no evidence of wild or feral *Gossypium* in the state. The morphological traits, the way the plants are maintained, and their uses are similar to those found in other Brazilian states (Almeida et al., 2009; Castro et al., 2016; Hoffmann et al., 2018; Barroso et al., 2023), as well as in other countries (Arriel et al., 2023; Morales-Aranibar et al., 2023). Thus, *G. barbadense* is the most valuable cotton genetic resource in Mato Grosso state.

The species *G. barbadense* and upland cotton are sexually compatible, allowing of the transfer and expression of transgenes in interspecific crosses. This transferability has been exploited in breeding programs in the USA, to develop genetically modified extra-long staple cultivars of *G. barbadense* in that country (McCarty et al., 2021). The potential for transgene transfer to Brazilian *G. barbadense* was investigated by Hoffmann et al. (2013), who showed that the transgene can be transferred through interspecific crosses, and that it is expressed and segregates according to Mendelian patterns. Therefore, the presence of GM proteins in *G. barbadense* is a reliable indicator of gene flow from GM upland cotton. Considering that almost all cotton crops in Mato Grosso state have been cultivated with genetically modified genotypes for over a decade, interspecific hybridizations between upland cotton and *G. barbadense* would result in morphological variations and positive tests for GM proteins. Despite the theoretical possibility of crossing mainly outside the exclusion zone, the results showed that all plants of *G. barbadense* remain genetically isolated from GM upland cotton, indicating that if any gene flow occurs, its frequency is extremely low.

The species *G. barbadense* is considered an autogamous species, with high frequency of selfing. Cross-pollination under natural conditions is mediated by insect pollinators. Although it is sexually compatible with *G. hirsutum* and they share pollinators, the two species maintain their integrity, even when occurring sympatrically. This can be partially explained by the combined effects of partial pre- and post-zygotic barriers (Johnston et al., 2005; Pereira et al., 2012; Viot & Wendel, 2023). The rate of gene flow is also a function of bees' ability to perform pollination, which decreases, as the distance between cotton plants increases (Loureiro et al., 2016; Yann et al., 2018). Typically in Mato Grosso state, *G. barbadense* is cultivated within urban areas and on rural smallholdings, often far from large farms where GM cotton is grown. This distance generally exceeds the foraging range of pollinating bees, thereby creating geographic isolation, hindering cross-pollination with upland cotton. Furthermore, obstacles like trees, walls, and houses, at *G. barbadense* occurrence sites, further limit pollinator foraging range. Finally, *G. barbadense* and upland cotton are phenotypically different. The hybrids are morphologically intermediate and can be recognized by some plant owners, who retain only those that have the characteristics of *G. barbadense*. The combined factors outlined likely explain why gene flow between GM cotton and *G. barbadense* was not detected. Based on the results obtained, gene flow apparently does not represent a real risk for the *in situ* maintenance of *G. barbadense* diversity in any of the Mato Grosso regions.

The limited experience with GMOs in Brazil and the existing knowledge gaps in 2005 led CTNBio to establish a GM cotton exclusion zone. At that time, it was not possible to accurately measure the risk of biodiversity loss of native or naturalized *Gossypium* species in Brazil, due to gene flow with GM cotton. CTNBio acted correctly, observing the precautionary principle as mandated by Brazilian law and ratified international treaties. However, the assessments conducted in the present study have shown that the exclusion zones represent an excessive biosafety measure in Mato Grosso state, imposing unnecessary restrictions on the state's farmers. Therefore, they can be removed without significant impacts on the *in situ* conservation of *Gossypium* diversity in Mato Grosso.

The real risk to *in situ* conservation is the change of cultural habits of the population segment that uses *G. barbadense*. During the expeditions, there were no reported cases of textile use for *G. barbadense* fibers. The culture associated with this use has been described as lost, with numerous accounts from mothers, grandmothers, and great-grandmothers who spun and wove *G. barbadense* fibers in the past. The small fields with thirty or more plants of *G. barbadense*, found in the past in Mato Grosso state, (Barroso et al., 2005a) disappeared together with artisanal spinning and weaving. Since the Sistema Único de Saúde (SUS) −

the Brazilian Unified Health System − was established in 1990, the proportion of Brazil's population with access to public healthcare has significantly risen (Ortega & Pele, 2023). Not only the SUS provides medical and hospital care, but it also supplies medication for treatments. As a consequence, the access to chemical drugs, can be considered the factor causing the weakening of medicinal plant use (Leite et al., 2021), including cotton. When asked, individuals who maintained cotton plants in their homes reported a decreasing trend in the use of medicinal plants across generations: they use fewer than their parents, and more than their adult children. The decline in the use of *G. barbadense* for medicinal purposes may negatively impact on its *in situ* conservation, similarly to way the availability of inexpensive industrialized textiles led to the disappearance of small fields of the species. If *G. barbadense* is no longer used as a medicinal plant, its status may change from a useful plant to a 'weed' in people's yards. To effectively conserve the diversity of *G. barbadense in situ* in Mato Grosso state, efforts should prioritize the maintenance of the cultural connections between the state's population and the species.

Conclusions

1. *Gossypium barbadense* is the main genetic resource of cotton in the state of Mato Grosso, Brazil, maintained by the local population as a medicinal plant.

2. The genetically modified exclusion zone is not a necessary biosafety measure to protect *Gossypium* diversity from gene flow from genetically modified (GM) cotton in the state of Mato Grosso.

3. The adequate *in situ* conservation of *G. barbadense* in the state of Mato Grosso depends on the maintenance of the uses and traditions associated with the species by the state's population.

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Chief editor: Edemar Corazza
Edited by: Célia Tremacoldi

Data availability statement

Data available upon request: research data are only available upon reasonable request to the corresponding author.

Declaration of use of AI technologies

During the preparation of this work, the author(s) used Gemini in order to review the English version of the text. After this use, the author(s) reviewed and edited the content as needed and take(s) full responsibility for it.

Conflict of interest statement

The authors declare no conflicts of interest.

Acknowledgments

To André Luis Filipiake, for his contributions during the field data collection; and to Instituto Matogrossense do Algodão, for financing, in part this study (SAIC Nº 20100.24/0015-7).

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