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COLLECTION, FIBER COLOR CHARACTERIZATION AND GERMPLASM CONSERVATION OF NATIVE COTTON *Gossypium barbadense* L. IN PERU

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ABSTRACT

Cotton is an important fiber crop, with special significance in the historical, cultural, and socio-economic development of the planet. In Peru, the great color variability of cotton fibers has allowed the commercialization of numerous textile products throughout the centuries. At present, cotton species native to South America, such as *Gossypium barbadense*, are constantly threatened by anthropogenic factors, and thus there is a clear need for species conservation. Therefore, this study aimed to collect *G. barbadense* germplasm in Peru, characterize the germplasm by fiber color, and evaluate the application of different methods of germplasm conservation. The evaluated methods were seed bank, field techniques, and *in vitro* culture. Two hundred seventy-six samples of *G. barbadense* were collected between 2017 and 2021 and classified by fiber color into monochrome and polychrome of white (114 samples), orange (107) and brown (41). The conservation methods showed

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that the seed bank samples ranged from 50 g to 1 kg, while 95% of the field accessions survived. In addition, seedlings presented positive developmental responses when they were grown *in vitro* under modified MS culture, sucrose 2.0%, vitamins, glycine 2.0 $mgL^{-1,}$ and $AgNO_3$ 2.0 mg L^{-1}. This study provides evidence of the color variability of *G. barbadense* distributed throughout Peru, and confirms that this species can be managed and conserved using germplasm conservation methods.

Keywords: accessions, colored fiber, micropropagation, native cotton, seed bank.

INTRODUCTION

Cotton (family Malvaceae) has been one of the most valuable resources since the origin of civilizations and even today remains as one of the most important crops in the biosphere (Wendel et al., 2010). Its textile fibers are of considerable economic benefit, which is why numerous biotechnology programs around the world have been working on the genetic improvement of cotton species (Wegier et al., 2016). However, anthropogenic factors such as deforestation or land-use change limit the conservation of its species, mainly wild or domesticated species of significant historical-social interest, such as *Gossypium barbadense* L. (Wendel and Grover, 2015).

G. barbadense, known as 'native cotton' in Peru, is a cotton allotetraploid species (4n = 52, $[AD]_2$ genomes) native to South America and widely distributed in Mesoamerica (Wendel et al., 2010). Its global production yield is relatively low (accounting for ~8%) compared to 'Upland cotton' [*Gossypium hirsutum* L.] (accounting for ~90%) (Liu et al., 2015). However, in recent years, appreciation for its superior quality fiber has attracted the attention of researchers seeking to unravel its phylogenetic relationship with other kinds of cotton, as well as implement breeding technologies and species conservation (e.g., Almeida et al., 2009; Hoffmann et al., 2018; Fan et al., 2020).

Historically, *G. barbadense* was extensively used by the pre-Inca cultures of the north coast of Peru (e.g., Mochica indigenous culture), who explored the variety of colors of its raw material for the manufacture of textile products (Dillehay et al., 2007). Such diversity of colors has already been portrayed two centuries ago by naturalists such as Charles Darwin, Joseph Dombey, Antonio Raimondi, among others, who collected and described the colors white, beige, brown, lilac, blue, green, and some exotic tones (CONAM, 2005). Currently, new botanical collections allow characterizing small populations of native cotton (e.g., López et al., 2018). However, they are insufficient to characterize a national pattern. A similar event occurred in Mexico, considered the center of origin and genetic diversity of upland cotton (*G. hirsutum*), the most important source of natural fiber in the world (Alavez et al., 2021). This domestication process possibly began in the northwest of the Yucatán Peninsula, and then spread to other regions inside and outside of Mexico (Vega et al., 2023).

Characterizing the color of the fibers of *G. barbadense* is an important strategy to preserve the genetic inheritance of its populations (Carvalho et al., 2014). Likewise, working on the conservation of their germplasm, evaluating different methods, is a way to maximize the success of their yield (Wegier et al., 2016). In this sense, the use of biotechnological techniques such as micropropagation and conservation of germplasm has proven to be an important ally in the cultivation of *G. barbadense* (Triplett et al., 2008; Rojas-Idrogo et al., 2013; Pierce et al., 2019). However, in Peru, there are still few research groups dedicated to working under this approach with native cotton species (Delgado-Paredes et al., 2021). Indeed, although the sexual seed of native cotton, due to its recalcitrant nature, cannot be conserved *ex situ* in germplasm banks, *in situ* conservation by native communities is still a common practice both in the Amazon regions and on the northern coast of Peru. Even though this activity is poorly documented, *in situ* conservation is conducted by rural communities in localities such as Monsefú, Mórrope, Mochumí, Túcume (Lambayeque), Catacaos (Piura) and Moche (La Libertad). The inhabitants of all these localities have a very strong ancestral link with the ancient pre-Columbian cultures that developed in the place. Therefore, this study aimed to collect germplasm of *G. barbadense* in Peru, characterize the germplasm by fiber color, and evaluate the application of different methods of germplasm conservation.

MATERIALS AND METHODS

Germplasm collection

Between 2017 and 2021, the research group of the Institute of Biotechnology-Universidad Nacional Pedro Ruiz Gallo (IB/UNPRG), has carried out several collections of *G. barbadense* germplasm in different locations in Peru, belonging to the regions of Piura, Lambayeque,

Cajamarca, La Libertad, Amazonas and Ucayali; some of the most representative localities being Sechura (5°34′ S; 80°49′ O), Mórrope (6°33′ S; 80°10′ O), Potrerillo (6°33′ S; 79°12′ O), San Pedro de Lloc (7°26′ S; 79°30′ O), Trujillo (8°60′ S; 79°20′ O), Bagua (5°38′ S; 78°32′ O), Chachapoyas (6°14′ S; 77°52′ O) and Pucallpa (8°24′ S; 74°36′ O) (Fig. 1). In most cases, individuals were located on highways, rural fields, farms, gardens, cemeteries, and parks. In some regions (e.g., Lambayeque), individuals were in small private plots of crops, in the Túcume Site Museum (6°31′ S; 79°51′ O) and UNPRG campus (6°43′ S; 79°54′ O). Seeds with fibers were collected, assuming they were a single accession when the individuals were very close and the fiber was the same color.

Germplasm color characterization

The color of cotton fiber accessions was compared with the UltracolorPlus+® color chart with RGB – HEX codes, and their respective values were also estimated on the Pantone® scale. Through this comparison, samples with a uniform and mixed tonality (two or more combined tones) were classified. A table of color comparison was used (Fig. 2H); the groups of shades of cotton fibers are shown, including the corresponding color codes.

Germplasm conservation

To conserve *G. barbadense* germplasm, three methods were used: seed bank, field bank, and *in vitro* germplasm bank. For seed bank, the seeds were preserved with their fibers after a rigorous cleaning process, removing plant debris, seeds damaged by insects, fungal specks, and other impurities. It was not possible to determine humidity, although the seeds were dried in a natural aerated environment and under shady conditions for one month. Samples were stored in transparent polyethylene containers, hermetically closed, in an area with 580 lx (lumen/m^2) of illumination at 12 h and room temperature of 18-22 °C in autumn and winter and 24-28 °C in spring and summer.

For field bank, fifty samples of the most representative cotton fiber colors were established at a rate of three plants per accession and with a spacing of 2.5 m, in a particular field in Pomalca District (Lambayeque region). On the other hand, 25 accessions were established at a rate of five plants per accession and 3 m, in the greenhouse of the IB/UNPRG. The plants received periodic irrigations, fertilization, pruning, and phytosanitary controls.

For *in vitro* germplasm bank, five seeds per accession were used. Fibers were previously

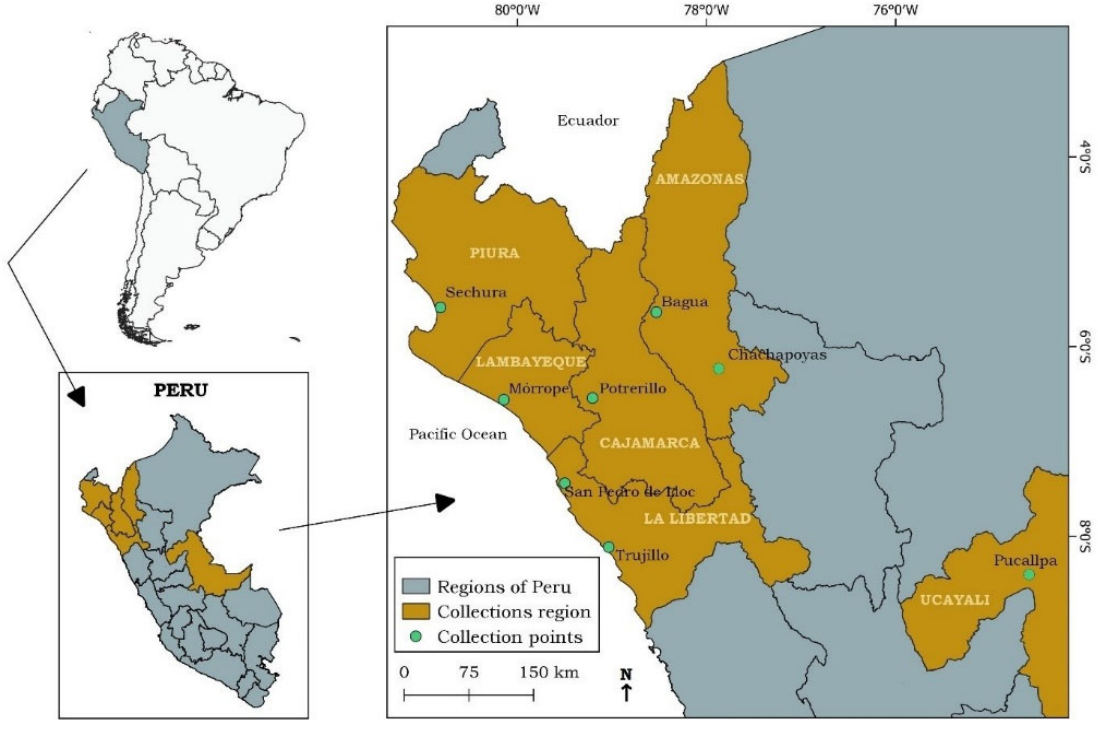

Fig. 1. Location of collection areas of *G. barbadense* sampled in Peru in the period 2017-2021.

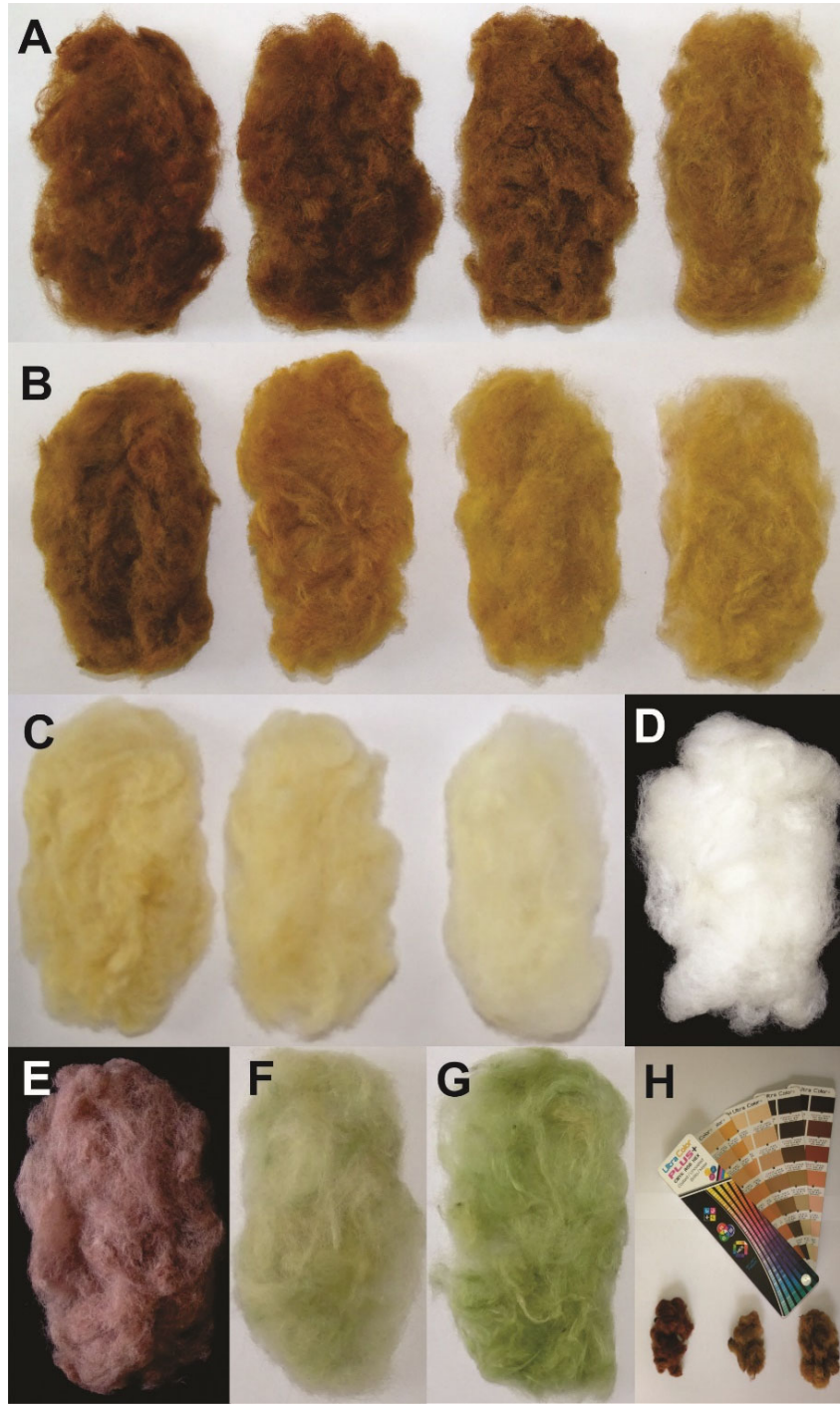

Fig. 2. Frequent shade colors of *G. barbadense* cotton fibers: A. Brown shades, B. Orange shades, C. Cream shades, D. White, E. Lilac, F-G. Greenish (possibly hybrids with *G. hirsutum*), H. Color comparison. All fiber samples were collected by the authors in Peru.

removed manually under the best physiological and phytosanitary conditions. In a laminar flow chamber with sterilized air, the seeds were disinfected with 70% ethyl alcohol for one minute and sodium hypochlorite (Clorox® commercial bleach with 5.0% active chlorine) in a 1:1 ratio for ten minutes and then rinsed for three times with sterile distilled water. The seeds were grown in MS culture medium (Murashige and Skoog, 1962) supplemented with the vitamins 1.0 mg/L thiamine HCl and 100 mg/L m-inositol, 2.0% sucrose and gelled with 0.7% agar-agar, contained in test tubes of 150x25 mm with 25 mL of culture medium. The pH of the culture medium was adjusted to 5.8±0.1 with HCl and KOH, before the incorporation of the gelling agent and sterilized in an autoclave. The culture was carried out at the rate of one seed per glass container. Evaluations were made after 15 to 30 days of culture, determining the number of germinated and contaminated seeds. After 1-2 months of seeds germination, the seedlings (seed germination plantlets) were used as explants, specifically, the shoot tips with the cotyledonary node. Previously, the cotyledons and the better constituted nodal segments, were removed at the rate of one explant per tube (150 x 25 mm test tube containing 25 mL of culture medium). These explants were used in *in vitro* germplasm conservation in modified MS culture medium (1/2 concentration of the macronutrients: ammonium nitrate, potassium nitrate, magnesium sulfate, potassium acid phosphate, and calcium chloride). In addition to the components of the seed germination culture medium, volumes of 0.5 mg/L nicotinic acid, 0.5 mg/L pyridoxine, 2.0 mg/L glycine and 2.0 mg/L $AgNO_3$ were added. It is important to highlight that the seeds were carefully cleaned, eliminating any remains of fiber in order to minimize microbial contamination.

In seed germination, the incubation environmental conditions were adjusted to a temperature of 26 °C±2 °C, relative humidity 85%, photoperiod 16/h, and irradiance with cold white fluorescent lamps of 10 μmol $m^{-2}s^{-1}$. Volumes of 70 μmol $m^{-2}s^{-1}$ and 35 μmol $m^{-2}s^{-1}$were used in the micropropagation and germplasm conservation, respectively; the explants were transferred after the remaining three months in micropropagation conditions. *In vitro* germplasm conservation evaluations were carried out 4 months after the culture was established. For this method, the evaluations corresponded to the development of functional attributes of the seedling, such as height, nodes, leaves, root, and vegetative state. To verify significant differences between the accessions, all attributes were subjected to analysis of variance (ANOVA) with Tukey's test (Haynes, 2013), in R 4.0.5 software (R Core Team, 2022).

RESULTS

Germplasm collection and color characterization

A total of 276 samples of native cotton were collected in the period 2017-2021. The highest number of collection records (107) was obtained in 2018, while the lowest number of records was obtained in the years 2020 and 2021 (24 and 31, respectively), of which 2 samples (greenish fiber) corresponded to possible hybrids with *G. hirsutum* and the rest to *G. barbadense*. From the collections, the largest number of the collected samples corresponded to white color (114), and eight frequent shades also were found in all the fiber samples evaluated: dark orange (36), orange (44), light orange (27), dark brown (10), brown (8), reddish brown (23), cream (6) and lilac (6) (Fig. 2). These shades are present in the cotton fiber samples in a monochrome form or combined, giving the samples a polychromatic appearance where white is generally the dominant color. Subsequently, the same samples were evaluated in other colorimetric scales and their approximate equivalencies were determined (Table 1).

The regions with the highest number of collected samples were Lambayeque (156), Cajamarca (44), and Piura (30) (Table 2).

Germplasm conservation

The 274 collected samples of *G. barbadense* make up the current IB/UNPRG germplasm bank. Accessions ranged from 50 g to 1.0 kg (Fig. 3A). To date, the day samples have not presented problems with pests and the containers were only opened to carry out propagation treatments in the field and *in vitro* (germination tests).

In the field, despite soil salinity problems, 95% of the cultivated samples survived, reaching up to 2 m in height, flowering, and bearing fruit (Fig. 3B). The seeds were used both for the refreshment of the seed germplasm bank and for donations among farmers in the region as an activity of social responsibility of the university.

The *in vitro* germplasm bank was preserved (until November/2021), with 98 samples of *G. barbadense* (Fig. 3C). Thirteen randomly selected accessions grew and developed after four months of cultivation (Fig. 3D). Plant height ranged from 5.6 cm (Gb-212) to 18.4 cm (Gb-158), showing significant differences. A similar response was observed in the number of nodes and leaves formed as well as in the development of the root systems, where no root formation was observed in only one accession (+) (Gb-238), while in most of the accessions were formed from 2 to 5 roots (++ and +++) (Table 3).

Table 1. Frequent color tones in the cotton fiber samples collected in the study and their approximate equivalencies in other colorimetric scales.

	Color Codes			
Color group	**Ultracolor**	**PMS**	**HEX**	**RGB**
Reddish brown	056F	P35-8C	BE4D00	171,95,50
Dark Brown	054K	P29-14C	925A2E	146,90,46
	054LL	725U	7A4B2D	122,75,45
Brown	054J	P26-12C	A77D55	167,125,85
	054G	P25-11C	CD9765	205,151,101
Dark orange	006K	7412C	D47D2D	212,125,45
Orange	006D	7411C	E0A262	224,162,98
Light orange	006B	P20-10C	E7B982	231,185,130
	006E	P14-13C	E7B565	231,181,101
Cream	054A	7506U	F5E2C8	245,226,200
	054C	726 U	EED3B2	238,211,178
	007A	7401 U	F7E4BB	247, 228, 187
White	-	P179-1C	FFFFFF	255, 255, 255
Lilac	045H	479 U	AA8672	170, 134, 114
	045I	4745 U	C7AFA4	199, 175, 164
	045K	P 23-2C	D6C3B5	214, 195, 181
	056I	4675 U	D4B8A0	212, 184, 160
	045G	4725 U	B09387	176, 147, 135

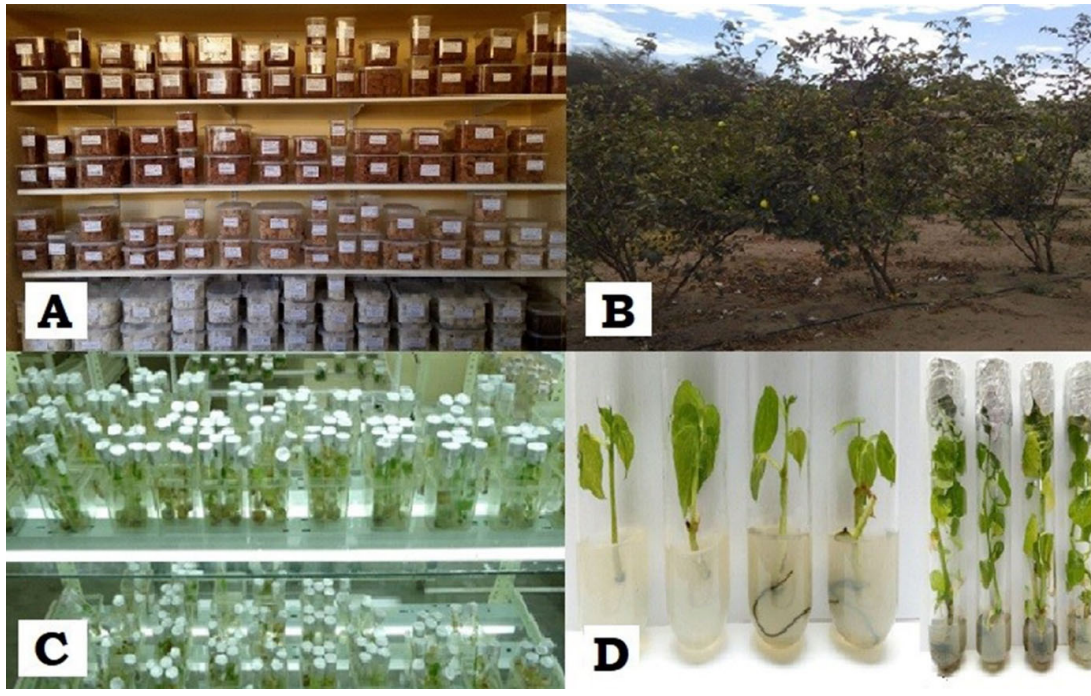

Fig. 3. Observation of the different methods of germplasm conservation of *G. barbadense* samples collected in Peru in the period 2017-2021. A) Germplasm bank of *G. barbadense* seeds. B) Germplasm bank of the core collection in the greenhouse of the UNPRG. C) Initial growth *in vitro*. D) Accessions after 4 months of *in vitro* culture.

Table 2. Color shades of *G. barbadense* collected in Peru in the period 2017-2021.

Collection regions	Color/shades	Collected samples (No)/Year					
		2017	2018	2019	2020	2021	Total
Amazonas	Orange	0	2	0	0	0	2
	Light orange	0	2	5	0	0	7
	Dark orange	0	0	0	0	0	0
	Cream	0	0	1	0	0	1
	White	0	0	8	0	0	8
		0	**4**	**14**	**0**	**0**	**18**
Cajamarca	Orange	6	0	8	0	1	15
	Light orange	1	0	1	0	0	2
	Dark orange	0	3	4	0	1	8
	Cream	0	0	0	0	0	0
	White	7	6	5	0	1	19
		14	**9**	**18**	**0**	**3**	**44**
Lambayeque	Brown	3	0	0	0	0	3
	Dark brown	0	1	0	0	0	1
	Reddish brown	4	8	3	1	0	16
	Lilac	2	3	1	0	0	6
	Orange	4	13	1	4	0	22
	Light orange	2	7	0	2	0	11
	Dark orange	4	10	1	1	0	16
	Cream	1	3	0	1	0	5
	White	5	43	4	14	10	76
		25	**88**	**10**	**23**	**10**	**156**
La Libertad	Brown	0	0	0	0	5	5
	Dark brown	0	0	0	0	9	9
	Reddish brown	0	0	0	0	0	0
	Lilac	0	0	0	0	0	0
	Orange	0	0	0	0	0	0
	Light orange	0	0	0	0	4	4
	Dark orange	0	0	2	0	0	2
	Cream	0	0	0	0	0	3
	White	0	3	0	0	0	3
		0	**3**	**2**	**0**	**18**	**23**
Piura	Brown	0	0	0	0	0	0
	Dark brown	0	0	0	0	0	0
	Reddish brown	0	0	7	0	0	7
	Lilac	0	0	0	0	0	0
	Orange	0	0	5	0	0	5
	Light orange	0	0	0	0	0	0
	Dark orange	0	0	10	0	0	10
	Cream	0	0	0	0	0	0
	White	0	0	7	1	0	8
		0	**0**	**29**	**1**	**0**	**30**
Ucayali	Orange	0	0	0	0	0	0
	Light orange	0	3	0	0	0	3
	Dark orange	0	0	0	0	0	0
	Cream	0	0	0	0	0	0
		0	3	0	0	0	3
Total samples/year		**39**	**107**	**73**	**24**	**31**	**274**

Table 3. Accessions of *G. barbadense* conserved *in vitro* conditions after four months of culture, using cotyledonary nodes as explants.

Accessions	Height (cm)	Nodes (N°)	Leaves (N°)	Roots	Physiological state
Gb-037	7 ± 0ab	5.5 ± 0.4a	6 ± 0a	+	VG
Gb-054	16 ± 0.9def	10 ± 0bcd	11 ± 0.9bc	++	VG
Gb-057	12.6 ± 1.2cd	10 ± 1.7bcd	9.8 ± 1.3bc	++	VG
Gb-092	14.4 ± 2.1cdef	11 ± 1.3cd	12.2 ± 1.6c	+++	VG
Gb-126	9.8 ± 3.6abc	10 ± 2.2bcd	10 ± 3bc	++	G
Gb-133	13.6 ± 1.0cde	10.8 ± 1cd	11.2 ± 1.6bc	+++	VG
Gb-158	18.4 ± 1.9f	13 ± 0.6d	12.4 ± 0.8c	+	VG
Gb-189	18 ± 1.3ef	11 ± 1.3cd	11.8 ± 1bc	+++	VG
Gb-193	10.2 ± 2.9bc	8.2 ± 2.2abc	9.6 ± 1.9bc	++	VG
Gb-200	14 ± 1.8cdef	9.2 ± 3.2abcd	9.6 ± 1.9bc	+++	VG
Gb-207	16.5 ± 1.3def	11 ± 0.9cd	10 ± 0bc	+++	VG
Gb-212	5.6 ± 1.3a	6.2 ± 0.8ab	8.4 ± 0.9ab	+	G
Gb-238	10 ± 2.9abc	9.6 ± 2.2bcd	5.4 ± 1.4ab	-	G
Average	**12.8 ± 3.9**	**9.7 ± 1.9**	**9.8 ± 2.1**	**++**	**VG**

Root development: -, without root formation; +, 1 root formed; ++, 2-3 roots formed; +++, 4-5 roots formed. Root length: 1-5 cm. Physiological condition: VG: Very good (non-vitrified plantlets and little or no defoliation) and G: Good (non-vitrified plantlets and moderate defoliation). In the columns, means followed by different letters indicate a significant difference according to the Tukey's test ($P<0.05$).

DISCUSSION

Scientific explorations carried out in the archaeological strata of the northern coast of Peru determined that Peruvian cotton has been cultivated in this region for 4,500 years, constituting the oldest industrial crop in the Andean area (Bird, 1948; Lostaunau, 1985). Although the domestication process has not been fully explained, it has probably occurred in the north and northwest of Peru and southern Ecuador (Stephens, 1975; Fryxell, 1980).

Characterizing genetic variability is an essential step for species conservation (Wendel et al., 2010). In this context, this study proposed to collect, characterize the color of the fibers and conserve one of the most historically and socio-economically explored cottons of the north coast of Peru. Here, different accessions of *G. barbadense* presenting a high color variability are currently monitored and conserved under different germplasm conservation techniques.

Seed collections of *G. barbadense* began in 2017 and continue to date (2022). The lowest collection numbers were recorded in 2020 and 2021 due to restrictions on collection excursions imposed by the State of Health Emergency because of the COVID-19 pandemic. In some cases, more than one route has been made on the same collection route, and in most cases, it was not possible to find the plants that were initially collected.

In Peru, the most recent collections of *G. barbadense* germplasm were carried out in past decades, with 88 accessions reported by the Universidad Nacional de Piura (UNP) (MINAM, 2012); 106 accessions were collected, of which 95 corresponded to *G. barbadense* (89.6%), with the highest number of accessions in Lambayeque (45 accessions) (MINAM, 2013). In that work, it is also mentioned that when reviewing the information from the herbaria of the UNP, UNPRG and Universidad Nacional Mayor de San Marcos and the collections made by O.T. Westengen, this researcher collected 390 accessions of the genus *Gossypium*, of which 239 were collected in the seven regions of the north coast of Peru (MINAM, 2013).

One of the major challenges in the study of *G. barbadense* is the definition of the colors of the fiber. Locally, the shades of native cotton fiber have received different vernacular names, for example: cream, brown, white, 'fifo' (lilac), 'fino colorado' (fine red), green, 'bayo' (a shade of cream), 'bombacín' (a shade of orange), and 'colombino' (light orange shades) (CITE-SIPAN, 2008). In the Cotton Descriptors (IBPGR, 1985), only white, green, gray, and brown (tan) are mentioned as a fuzz color, while the fiber color is only white, cream, bright brown, and brown (IBPGR, 1985). Gil and López (2015) indicated that only seeds with green fiber presented

hairiness (fluff) on the seeds. Accordingly, these seeds are likely to belong to hybrid specimens of *G. hirsutum* cultivar Del Cerro with *G. barbadense*, while the seeds with lilac and brown fiber, which lacked this hairiness, belong to *G. barbadense*. Only molecular biology studies can fill this gap in knowledge. *G. hirsutum* cultivar Del Cerro was introduced in Lambayeque probably at the beginning of the 20th century (Vreeland, 1985). In a study on crosses made between *G. barbadense* and *G. hirsutum*, genotypes with fibers of various colors were obtained and called chocolate brown, orangish, yellowish, grayish, and dark brown (Carvalho et al., 2014), which can be compared with the colors reddish-brown, orange, cream, 'fifo' and dark brown, respectively, reported in the present study. In other studies, the fiber colors of the collected native cotton accessions were not defined (MINAM, 2013). Only in the study by CONAM (2005), a list of colors was included but without indicating that it was drawn up based on established standards or scales. Recently in Peru, the frequent colors of native cotton have been cataloged through descriptors, and eight categories for fiber color have been established. These are the same categories suggested to be purchased with colorimetric charts such as the RHS (Royal Horticultural Society) to get the approximate color (Manco et al., 2022). In the present study, the characterization was made by estimating the color in four colorimetric scales (Ultracolor, Pantone, RGB, HEX). However, it is recommended to prepare flat textile samples of each fiber color tonality in good condition (discarding fibers discolored by solar overexposure) to use colorimeters for a more accurate measurement of color (Kasajima, 2019). This is a more technical estimate, compared to direct observation.

It has been indicated that *G. barbadense* seeds cannot be preserved for long periods since they quickly lose viability. This occurs when they are preserved in natural environmental conditions (seeds with fiber), without refrigeration, or subjected to desiccation (Delgado-Paredes et al., 2021). In this regard, tests on the prediction of longevity in seeds have been carried out in *G. hirsutum* (Usberti et al., 2006) but not in *G. barbadense*. Under natural environmental conditions of conservation, seeds can preserved up to two years, provided that the seeds are with fiber 'kapok' (Delgado-Paredes et al., 2021). Several authors have regarded the kapok as an important accessory that protects the seed, increasing its chances of survival and germination, especially in species of the genera *Eriotheca*, *Cochlospermum*, *Ochroma*, and others (Linares-Palomino and Ponce-Álvarez, 2005) from the seasonally dry tropical forest of Peru and Ecuador.

Conservation in the field is another alternative for the preservation of an agronomic crop, as is done in potatoes with the name of *ex situ* conservation (MINAM, 2019). However, very few of these collection sites meet the minimum requirements to be considered germplasm banks (MINAM, 2017), as is the case of community banks, since it requires adaptation to international standards, articulation of activities, access to information, among others, that guarantee efficient conservation, research, and use. Although a germplasm bank in the field has some advantages such as being established in its natural environment and thus being subject to the pressure of evolutionary factors inherent to its natural environment, it also has important disadvantages, such as having an area with good farmland, land rotation, permanent availability of water, fertilizers, and pesticides, as well as dealing with natural external factors such as frost and eventual threats such as fire and looting. In Peru, other initiatives to conserve native cotton germplasm through field cultivation include the collections of INIA (Instituto Nacional de Innovación Agraria) in its experimental stations, highlighting the cotton collection in the Lambayeque region (Vista Florida Station) and San Martín region (El Porvenir Station), where genetic improvement and the promotion of new varieties for their use within known cultivars have even been experimented with the experimental stations of UNP (Universidad Nacional de Piura) in Piura region; and the collection of native cotton and 'algodoncillo' (*G. raimondii*) from UNPRG (Universidad Nacional Pedro Ruiz Gallo). Furthermore some local farmers have made efforts to conserve native cotton by growing this crop on their farms and borders (MINAM, 2020).

In a study published in 2010, the description of the cultivated tetraploid species, *G. barbadense* and *G. hirsutum*, as well as wild primary, secondary, and tertiary cotton *Gossypium* species in eight major cotton germplasm collections, was reported, developing and establishing several management strategies of conservation (Campbell et., 2010). A specific case is the *in situ* conservation of the genetic resources of *G. barbadense*, which is considered essential and complements *ex situ* conservation, as occurs in three different municipalities situated in Brazilian Cerrado, Goias state, including the quilombo Kalunga community (Cardoso et al., 2023). Certainly, orthodox seed gene banks should have equipment for seed dehydration and cold chambers that reach negative temperatures such as -10 ^{o}C and -20 ^{o}C; such facilities are not always

found in institutions where the conservation of traditional plant genetic resources, such as *G. barbadense*, is neglected.

Establishing a formulation of a micropropagation culture medium and germplasm conservation for numerous accessions of an *in vitro* germplasm bank is a permanent activity of adjustment and improvement. In the case of *G. barbadense*, the formulation of the culture medium, for micropropagation and conservation, is subject to permanent adjustments to increase the conservation months from 9 to 12 at least, as well as to tend to the sub-cultures increase from 3 to 5, without loss in their ability to elongation of the shoot and root. It is also important to minimize the rate of vitrification or hyperhydricity, a complex physiological phenomenon that occurs in certain species during long-term *in vitro* conservation (Sreedhar et al., 2009). In this study, not only the effect of $AgNO_3$ is being tested, but also the supplement of new vitamins (pyridoxine and nicotinic acid) and amino acids (glycine) as well as a drastic modification of the macronutrients of MS mineral salts. The use of various biotechnological techniques developed by tissue culture would be important in solving several problems that could not be solved, even with the application of conventional biotechnological techniques. These techniques would be the culture of zygotic embryos, cryopreservation using zygotic embryos, somatic embryos and stem tips as explants, callus induction, indirect organogenesis and somaclonal variation, indirect somatic embryogenesis, microspores, and anthers culture, which would allow producing haploid plants, protoplasts fusion, to obtain somatic hybrids, both of intraspecific (*G. barbadense* x *G. barbadense* and *G. raimondii* x *G. raimondii*) and interspecific (*G. barbadense* x *G. raimondii*) crosses.

CONCLUSIONS

Two hundred and seventy-four samples of *Gossypium barbadense* were collected in Peru in the period 2017-2021. The samples included the three basic color tones brown, white, and mixed. The methods of germplasm conservation showed positive responses of development *in vitro* and in the field. This study will serve as a basis for state organizations and private entities to improve management and conservation strategies for *G. barbadense*.

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Authors' contribution

The authors declare active participation in the bibliographic review by Guillermo Delgado-Paredes and Consuelo Rojas-Idrogo; in the development of the methodology: Guillermo Delgado-Paredes, Consuelo Rojas-Idrogo, Cecilia Vásquez-Díaz, Pilar Bazán-Sernaqué and Felipe Zuñe-Da Silva; in the discussion of the results: Guillermo Delgado-Paredes, Consuelo Rojas-Idrogo, Boris Esquerre-Ibañez and Felipe Zuñe-Da Silva; in review and approval of the final version of the article: Guillermo Delgado-Paredes, Consuelo Rojas-Idrogo, Cecilia Vásquez-Díaz, Boris Esquerre-Ibañez, Pilar Bazán-Sernaqué, Pedro Custodio-Ayasta and Felipe Zuñe-Da Silva.

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