Pak. J. Bot., 53(6): 2135-2142, 2021. DOI: http://dx.doi.org/10.30848/PJB2021-6(37)

IN SITU GERMPLASM CONSERVATION OF *GOSSYPIUM RAIMONDII* ULBRICHT ("ALGODONCILLO") IN THE LAMBAYEQUE REGION (PERU)

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Abstract

Cotton belonging to the genus *Gossypium*, Malvaceae family, is a natural source for the textile industry. It is an economically important crop that is grown worldwide but can also be used as an ideal model system for several physiological studies in cell elongation and differentiation. *Gossypium raimondii* is a diploid species from the Peruvian flora and is a progenitor of allotetraploid cottons. However, this species is considered to be critically endangered and therefore, this study aimed to evaluate the population of "algodoncillo" (*G. raimondii*) across an area of 2.3 ha in La Colmena (Chongoyape-Lambayeque). One hundred and 53 adult plants were registered and a population of 342.28 plants was estimated by natural regeneration. A large number of plants were recorded in El Potrero with around 85 individual shrubs; otherwise, in El Alto plants were recorded with the highest relative height and largest diameter with around 5.06 and 8.73 cm, respectively. In addition, plants with the highest branching rate (3.65 branches) and consequently the largest number of green and ripe fruits in dehiscence (621.38 / plant) were recorded in La Quebrada which has also been the only place where an estimated 348.20 naturally regenerated plants were found. La Colmena is the only place in the Lambayeque region where *G. raimondii* can be found, making it the only natural place of *in situ* conservation. Based on what is indicated in "El Libro Rojo de las Plantas Endémicas del Perú/Red Book of Endemic Plants of Peru" and the criteria of the International Union for Conservation of Nature (IUCN), *G. raimondii* would fall under the category of Endangered species (EN), hence the need for its *in situ* conservation and to test other methods for germplasm conservation is high.

Key words: Algodoncillo, Cotton, Green and mature fruits, Nutural regeneration, Plants evaluation.

Introduction

The genus *Gossypium* belongs to the order Malvales, Malvaceae family (APG IV, 2016; Christenhusz and Byng, 2016), which includes polyploidy species, presenting five tetraploid (2n = 4x = 52) and 45 diploid species (2n = 2x = 26) (Zhu and Li, 2013) distributed across all arid and semi-arid regions of America, Africa and Oceania. *Gossypium* diploid species comprise 8 sub-genomes A-G and K, which distribute in Oceania subgenome C and G, Africa subgenomes A, B, E and F, and in America subgenome D (Brubaker *et al*., 1999).

Gossypium raimondii Ulbricht, also called algodoncillo, algodón silvestre, milkweed, wild cotton, and the vernacular Amazonian names, haupge (piro), huasmuén (pano), huasmué (cunibo) or urúch (jíbaro) (Soukup, 1970), is a shrub of arid to semi-arid environments which has been used since prehispanic times as a source of fiber. It was recognized by INRENA (currently SERFOR/Servicio Forestal y de Fauna Silvestre) as a species in a "Critical" state (Chanco *et al*., 2006). *G. raimondii* is a diploid species, which from the evolutionary point of view is parental (DD, 2n = 2x = 26), but was also considered as a contributor to subgenome D and putative progenitor for tetraploid cotton (Zhu and Li, 2013). This is an ideal genus to investigate evolutionary stages of genomes in polyploidy individuals (Brubaker *et al*., 1999).

G. raimondii is a possible contributor to subgenome D for fiber-producing cotton species, including *G. hirsutum* and *G. barbadense* (Zhu & Li, 2013), those two, in particular, presenting two optimal characteristics for the textile industry. Due to industrial interest, the sequencing and assembly to the genome of *G. raimondii* were performed using a new generation of sequencing methods, the products of which showed 40,976 protein-coding genes. It is known that transposable elements have an impact on genome size, thus, the genome of *G. raimondii* has approximately 57% (778-Mb of total length) of transposable elements (Wang *et al*., 2012; Paterson *et al*., 2012).

Analysis of the genes involved in the initiation of the fiber tissue was investigated. Genes sucrose synthase (*SusB*, *Sus1* and *SusD*) and genes 3-ketoacyl-CoA synthase (*KCS2*, *KCS13* and *KCS6*) showed a higher expression in *G. hirsutum* than in *G. raimondii*, suggesting that the *Sus* and *KCS* gene families may be necessary for the initiation and elongation of the fiber (Wang *et al*., 2012). Likewise, the *CDN1* gene family was detected, which participates in the biosynthesis of gossypol, a metabolite that, when accumulated in pigmented glands can act against pathogens and herbivores (Wang *et al*., 2012). Another study in molecular biology was carried out, for example, 78 putative *MAPKKK* (Mitogen-activated protein kinase kinases) genes were identified in *G. raimondii* and their phylogenetic relationship, genomic distribution and motif of conserved proteins were characterized (Yin *et al*., 2013). In the same study, *MAPKKK* expression patterns in the state of fiber initiation and leaf maturation were also analyzed, which might be related to the elongation of the fiber tissue (Yin *et al*., 2013).

Genetic sources are used in the research on biodiversity and conservation as well as the adaptability of species, taxonomy, and systematics. A source of information on all the genes present in an individual is referred to as the germplasm, which can be maintained for a long period and regenerated when necessary (Bhatia, 2015). Likewise, since there is some variation in the genetic structure of individual specimens, this variation can result in the loss of important information from previous generations. Therefore, the conservation of this successful germplasm is an important aspect to preserve the genetic pool of primitive species, endangered trade or any existing species (Bhatia, 2015).

One way to preserve germplasm is through in situ conservation, which refers to preserving genetic resources of crop species, especially wild relatives in the form of natural populations by establishing biosphere reserve sites with restricted access as sanctuaries or natural parks. This method of conservation can be complemented with floriculture, horticulture and farm conservation, conserve biodiversity in farmer´s fields as an integral part of agricultural activities (Svalbard Global Seed Vault, 2018; Bhatia, 2015).

In the present decade, *G. raimondii* has been recognized as an invaluable source of genes that can potentially be useful in the genetic improvement of cultured cotton, whether by fusion of protoplasts or genetic engineering. Observations made in their natural habitat have shown great tolerance to stressful conditions of salinity and drought, as well as the infection of various pests and diseases. This species also shows great compatibility with the other Peruvian native cotton species, *Gossypium barbadense* (Delgado-Paredes *et al*., 2021). Additionally, *G. raimondii*, although it has a very short fiber, the olive-green color gives it a very special characteristic.

The present study aims to evaluate the current state of conservation *in situ* of what would be the only, and perhaps the last, refuge of the "algodoncillo" (*Gossypium raimondii* Ulbrich) in La Colmena (Chongoyape-Lambayeque, Peru). Determining the number of adult plants, the number of adult plants per re-growth, natural regeneration, ramifications per plant, and the number of fruits per plant can help to evaluate or determine the most appropriate strategy to conserve the germplasm of this important native cotton species, endemic to northern Peru.

Materials and Methods

Plant material: Plant material was obtained from specimens of *Gossypium raimondii* including material from adult plants, regrowths of adult plants and young plants of natural rejuvenation. All the plants were evaluated between June 01 and August 02, 2019 and around 95% of the adult plants were in a fruiting stage with both green fruits and mature fruits in dehiscence. The plants have been previously identified by Dr. Guillermo E. Delgado-Paredes, botanist at Universidad Nacional Pedro Ruiz Gallo (UNPRG), Lambayeque-Peru, and a voucher specimen (No 18736) is preserved at the Herbarium Pedro Ruiz Gallo (HPRG).

Study area: The study area is located in La Colmena (Chongoyape-Lambayeque, Peru) (Fig. 1), along the road that leads from Chongoyape (Chiclayo, Lambayeque) to the Tocmoche district (Chota, Cajamarca), Peru, covered an extension total area of about 2.3 ha (23,000 m^2), between the coordinates displayed in (Table 1). In this area, four minor study areas were identified called: 1. El Potrero (575.0 m^2), 2. El Alto (165.0 m^2), 3. La Quebrada (3,795 m^2) and 4. El Camino (233.4 m^2) (Fig. 2). In El Potrero, El Alto and La Quebrada only adult plants were found and in El Camino only adult plants from regrowth were found. Only in La Quebrada were specimens from natural regeneration plants been sampled measuring around 0.30 to 1.00 m in height.

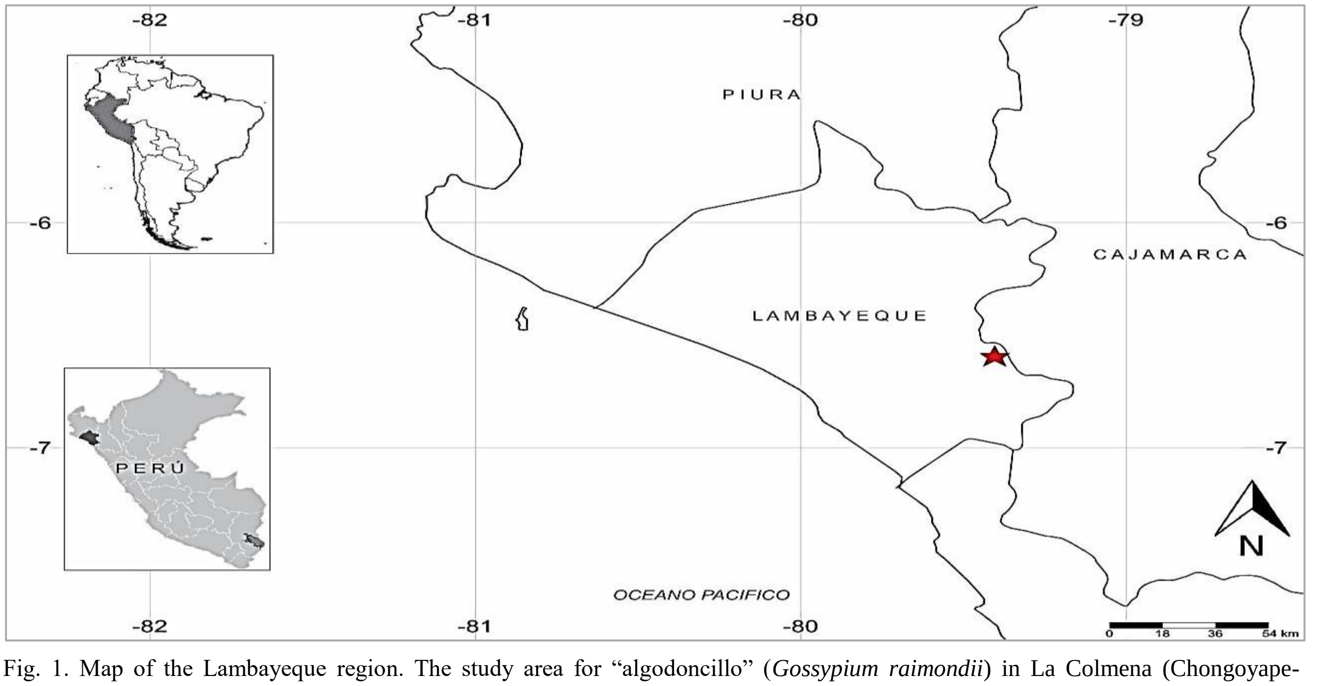

Fig. 1. Map of the Lambayeque region. The study area for "algodoncillo" (*Gossypium raimondii*) in La Colmena (Chongoyape-Lambayeque, Peru) is indicated.

Table 1. Coordinates in UTM of the study area in the evaluation of "algodoncillo" plants (*G. raimondii*) in La Colmena (Chongoyape-Lambayeque, Peru).

No	Study area	Universal transverse mercator (UTM) coordinates	Extension (m^2)
	Places		
1.	El Potrero	17M 676450 9270772	575.00
		17M 676448 9270795	
		17M 676474 9270782	
		17M 676471 9270804	
2.	El Alto	17M 676404 9270773	165.00
		17M 676401 9270781	
		17M 676386 9270773	
		17M 676388 9270764	
3.	La Quebrada	17M 676528 9270886	3,795.00
		17M 676493 9270826	
		17M 676539 9270793	
		17M 676573 9270854	
4.	El Camino	17M 676438 9270933	233.43
		17M 676424 9270976	
		17M 676445 9270938	

El Potrero, is a small agricultural area used for grazing cattle, goats and sheep. The Alto is an even smaller area containing clay soil of a slight salinity. This area is often used for agriculture providing shade and shelter for poultry. El Camino, is an area of a triangular shape on the edge of the Chongoyape - Tocmoche road, numerous adult plants of regrowth that serve as a live fence of an agricultural plot. La Quebrada is the most extensive sampling site formed by an unconsolidated bed of stones of different sizes, where between the clearings of vegetation, the silty land provides excellent growing conditions for plants that thrive by natural regeneration.

Sampling method: Sampling in the four study areas was carried out by direct counting of all the individuals. Additionally, in the La Quebrada area, three samples were counted using the square method (1 m^2), to determine the natural regeneration. Thus, in the first area of 4 m^2, two evaluations were carried out; in the second area of 25 m^2, three evaluations were carried out and in the third area of 24 m^2, two evaluations were carried out.

The plant height was determined by a rigid measuring tape, with the plant diameter being determined 10 cm above the ground. The number of branches was also counted 10 cm above the ground. The number of fruit per branch was determined with the average of 15 branches on 15 different plants, chosen randomly in all sampling areas. Likewise, the plant coverage was determined by the average of 25 plants, at a random selection.

Results and Discussion

Plants evaluation: Table 2 shows that the largest number of samples/shrubs of *G. raimondii* were recorded in El Potrero (85) and the lowest number in El Alto (7). The highest relative mean of plant height and diameter were recorded in El Alto with a mean of 5.06 m and 8.73 cm, respectively. The lowest plant height and diameter were recorded in El Camino with 2.60 m and 1.59 cm, respectively. Likewise, the highest branching rate per adult plant was recorded in La Quebrada (3.65 branches), which made it possible to register the largest number of green and ripe fruits in dehiscence per plant (621.88). The coverage of the plants was between 3.0 to 9.0 m (data not shown in table). The main morphological characteristics of *G. raimondii* are shown in Figs. 3 and 4.

These results would be related to the fertility of the soils, the greater competition between plants, the number of branches produced and the greater availability of light. In El Potrero, the high number of counted plants is due to the high organic fertility of the soils. A possible explanation for this could be the excrement of cattle, goats and sheep. However, although the plants reach an average height of about 3 m, the number of branches produced was relatively low (1.93), producing a smaller number of fruits per plant (328.42). In El Alto, several plants were registered with a height between 3.40 to 7 m and a diameter between 4.14 to 18.78 cm, despite the soil nutrition composition not being very rich. However, poultry droppings and the fact that a water irrigation canal runs near the site could have influenced the formation of very tall and vigorous plants. The light may have negatively influenced the El Camino ~~the~~ plants resulting in lower heights and smaller stem diameters. The latter could be explained by the plants being of regrowths that grew in the shade of several tree species such as "algarrobo" (*Prosopis limensis*, Fabaceae), "faique" (*Vachellia macracantha*, Fabaceae), and "sauce" (*Salix humboldtiana*, Salicaceae). In La Quebrada, because it was an open space, the light would have positively influenced the pollination, fertilization and formation of fruits and seeds, registering the greatest number of fruits relative to the number of counted individuals.

The progressive destruction of natural ecosystems in South America and especially the Seasonally Dry Tropical Forest (SDTF) ecosystem of Northern Peru, has led to the identification of priority conservation areas. These areas are based on floristic inventories containing high numbers of endangered and endemic species (Banda *et al*., 2016; Marcelo-Peña *et al*., 2016). The SDTF is the natural habitat of *G. raimondii*. At present several of the ancient natural places of germplasm dispersion and conservation of *G. raimondii* no longer exist. Germplasm conservation alternatives are *In vitro* conservation and *in situ* conservation since *ex situ* conservation in the form of a sexual seed, is still uncertain given the ambiguity of the seed being recalcitrant or orthodox.

A document about the status and contents of the global cotton germplasm resources, made in eight major cotton germplasm collections in the world, reported that the number of classified *Gossypium* species (around 50 species included in the primary, secondary and tertiary gene pool) are not maintained in all these collections, and several are underrespresented and vulnerable to extinction (Campbell *et al*., 2010). This report indicated that *G. raimondii* germplasm was found in small number in Australia, Brazil, China, France, India, Russia, Uzbekistan and United States collections. However, in the United States there is the largest collection of *Gossypium*, but no mention of the state and the conservation strategy.

Furthermore, it was indicated that without global, collaborative efforts, the rarest and most unique cotton germplasm resources, as is the case with *G. raimondii*, are vulnerable to extincion (Campbell *et al*., 2010).

One of the best *ex situ* conservation strategies for wild and domesticated germplasm is *In vitro* conservation preserved in genetic banks, as demonstrated by Hernández-Terán *et al*., (2019) in their study on *In vitro* performance of *Gossypium hirsutum* in Mexico using different genetic backgrounds demonstrating its implications for germplasm conservation. Based on the research shown in this paper, the authors believe that La Colmena (Lambayeque) is a priority area for the *in situ* conservation of *G. raimondii*. Likewise, we believe that *ex situ* conservation carried out by reliable organizations such as the Svalbard Global Seed Vault is a priority recognized in international law and policy (Westengen *et al*., 2013).

Is *Gossypium raimondii* an endangered species?: In a preliminary report about the cotton~~s~~ of Peru (Ferreyra (1985), it was indicated that *G. raimondii* was discovered by the famous Italian naturalist Antonio Raimondi near the Nanchoc Mountains (Contumazá, Cajamarca). This botanical material was described by the German botanist E. Ulbrich in 1932. In an expedition led by R. Ferreyra, between 1983 and 1984, various specimens were found on the north bank of the Chicama river (La Libertad). Possible remnants of the floods that occurred during the 1983 El Niño Phenomenon. However, a large population of *G. raimondii* was found in the Santa Ana Valley (Cascas-Contumazá, Cajamarca), where it was known from an important population of *G. raimondii* (Ferreyra, 1986). El Niño is a climatological pattern that describes the unusual warming of surface waters in the eastern tropical Pacific Ocean; the "warm phase" of which ~~posing~~ pose a greater influence on meteorological cycles and is called the El Niño-Southern Oscillation (ENSO).

In the course of the same expedition, some specimens were found near the Huertas river (Chilete-Contumazá, Cajamarca). However, in Nanchoc, the place of the first Raimondi collection, no additional specimen was found (Ferreyra, 1986). In a recent collection made along the north coast of Peru, between December 2012 and February 2013, individuals specimens of *G. raimondii* were collected in the districts of Chongoyape (Lambayeque), San Benito and Chilete (Cajamarca) and Cascas (La Libertad). It was reported that the populations of *G. raimondii* in San Benito (Ravines of San Benito and Santa Ana) were very dense in volume area and made up of thousands of plants. However, here too agricultural activity was progressively deforesting these areas that are replacing them with the cultivation of corn. In the case of Chilete and Chongoyape, the populations of *G. raimondii* were very small (MINAM, 2012).

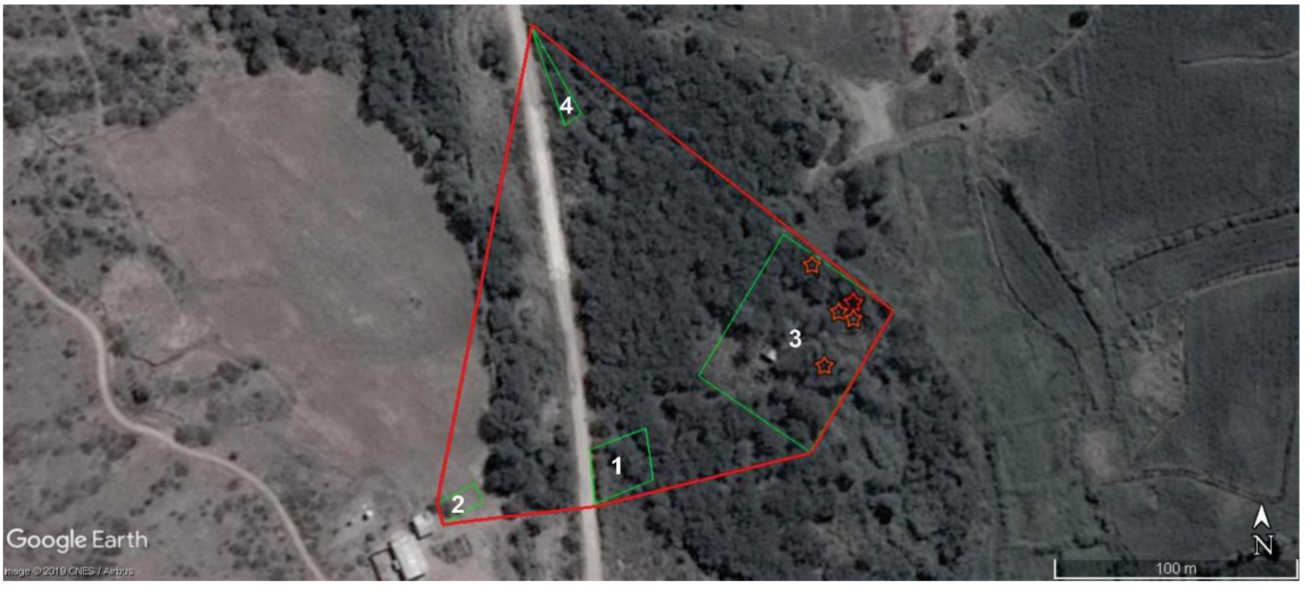

Fig. 2. Study area of "algodoncillo" (*G. raimondii*) in La Colmena (Chongoyape-Lambayeque, Peru), showing the following places: 1. El Potrero, 2. El Alto, 3. La Quebrada and 4. El Camino. The figure (red pentagon) indicates the place for natural regeneration.

Table 2. Evaluation of "algodoncillo" plants (*G. raimondii*) in La Colmena (Chongoyape-Lambayeque, Peru).

No.	Study place/extensión (m^2)	Plants evaluated (No)	Plant height (m)	Plant diameter (cm)	Branches/plant (No)	Fruits/plant (No)
1.	El Potrero (575.00)	85	3.01	3.02	1.93	328.42
2.	El Alto (165.00)	7	5.06	8.73	1.86	316.10
3.	La Quebrada (3,795.00)					
	- Adult plants	23	2.98	3.17	3.65	621.88
	- Natural regenera-tion (53 m^2)	348.28 (6.57 plants/m^2)	< 1.0	< 1.0	0.0	0.0
4.	El Camino (233.43)	38	2.60	1.59	2.71	461.25
	Total	153 (adult plant) and 342.28 (natural regeneration				

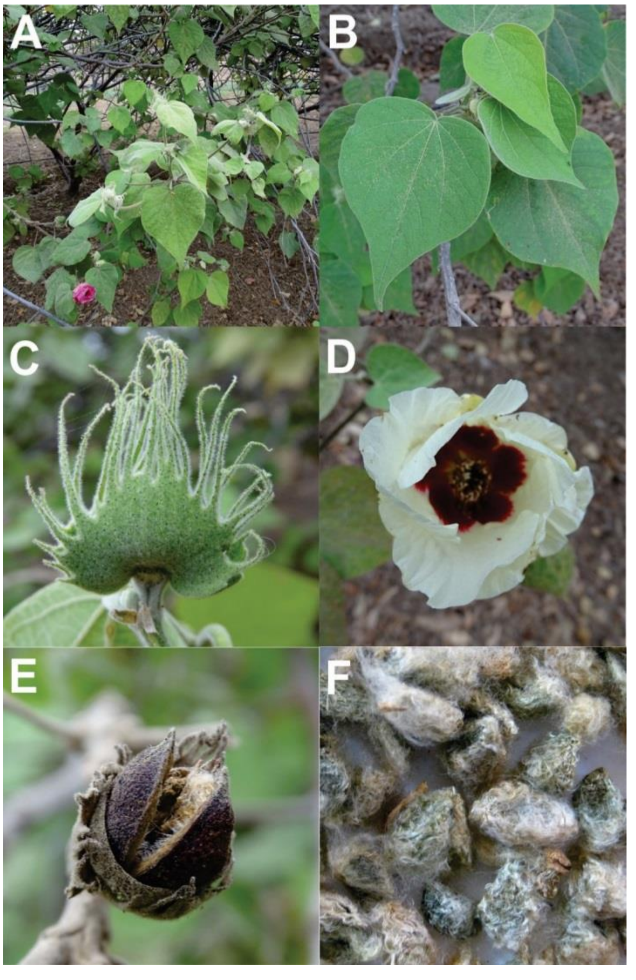

Fig. 3. Morphological characteristics of “algodoncillo” (*G. raimondii*). A. Plant in its natural environment, B. Leaf characteristic, C. Green fruit, D. Flower after anthesis, E. Fruit in dehiscence, and F. Mature seeds.

In “El libro rojo de las plantas endémicas del Perú”, *G. raimondii* was determined as EN (Endangered). This was based on a survey from plants that were collected in the 19th century, in the Chicama river basin (La Libertad) (Chanco *et al*., 2006). However, there are currently no individuals found either in the Chicama river or in Guadalupe (La Libertad), a district close to Chicama. It was initially reported that the populations of *G. raimondii* would not be threatened with a higher risk of extinction because they were found in uninhabitable terrain that was unfit for agriculture (Ferreyra, 1986; MINAM, 2012), which would correspond to the localized populations in Chilete and Chongoyape. This statement would not be so true since the El Niño Phenomenon of recent years (1982-1983, 1997-1998 and 2016-2017), where devastating and serious damage was caused to the flora and fauna of the country, especially in the biodiverse of riparian habitats or in ravines, both reported habitats for of *G. raimondii*. To this, we must add the effects of Climate Change and pollution. Furthermore, observations have confirmed that in San Benito and Santa Ana, agriculture would be displacing the wild populations of *G. raimondii* (MINAM, 2012) (Fig. 5).

A taxon is classified as “Endangered” when the most accurate evidence indicates that it meets any of the following criteria (A to E), and it is therefore considered to be facing a very high risk of extinction in the wild. A. Reduction in population size based on several criteria relating to habitat fragility, B. Geographic range in the form of either B1 (extent of occurrence) or B2 (area of occupancy) or both, C. Population size estimated to number fewer than 2,500 mature individuals and F. Quantitative analysis showing the probability of extinction in the wild is a least 20% within 20 years or five generations, whichever is the longer (up to a maximum of 100 years) (Anon., 2012). Based on these criteria, the "algodoncillo" (*G. raimondii*) would fall under the category of endangered species (EN), so the necessary measures should be taken for its conservation in its natural environment (*in situ*). Likewise, other methods of germplasm conservation should be tested, such as sexual seed conservation and *In vitro* conservation.

A similar situation to that occurring in Peru with *G. raimondii* would be occurring in Mexico where the increased human population, modernization and urbanization have drastically reduced the habitats and survival potential of *G. hirsutum*. Even *in situ* conservation of some of the populations of *G. aridum*, *G. barbadense*, *G. gossypioides*, *G. hirsutum*, *G. laxum*, *G. lobatum*, and *G. schwendimani*, and one undescribed wild diploid *Gossypium* taxon is threatened (Ulloa *et al*., 2006). In recent years, important studies have been published on world cotton germplasm resources (Abdurakhmonov, 2014), carried out in various countries like Australia (Stiller and Wilson, 2014), China (Jia *et al*., 2014), India (Narayanan *et al*., 2014), Pakistan (Rahman *et al*., 2014), United States (Percy *et al*., 2014) and Uzbekistan (Abdurakhmonov *et al*., 2014), which highlights the importance of the introduced collections of *G. raimondii* and the need to conserve this invaluable genetic resource.

Conclusions

In this study, the unique “algodoncillo” population (*Gossypium raimondii*), occupying an area of 2.3 ha, was evaluated in the La Colmena region (Chongoyape-Lambayeque, Peru). In this place 153 adults, over a meter high, with green fruit and fruit in dehiscence were registered (June 01 and August 02, 2019). A total of and 348 young individuals were estimated by natural regeneration, which was usually less than one meter high. The population has all the characteristics of a species to be classified under the IUCN category EN (Endangered). At the study site, it is possible to develop or establish a germplasm conservation model *In situ* while considering other conservation options, in the form seeds or *In vitro* cultures.

Acknowledgement

The authors are grateful to Prof. Jorge V.W. Cachay-Wester and Dr. Yero R. Kuethe, University of Bristol, Department of Earth Sciences, the United Kingdom for English improvements. This research was funded by the General Biotechnology Laboratory-VRINV (UNPRG).

Fig. 4. A. Fruits and seeds of *G. raimondii* and B. Comparison between seeds of *G. barbadense* (native brown cotton) and *G. raimondii* ("algodoncillo"). Seed length-width of *G. barbadense* = 9.4-5.0 mm and *G. raimondii* = 5.2-3.6 mm.

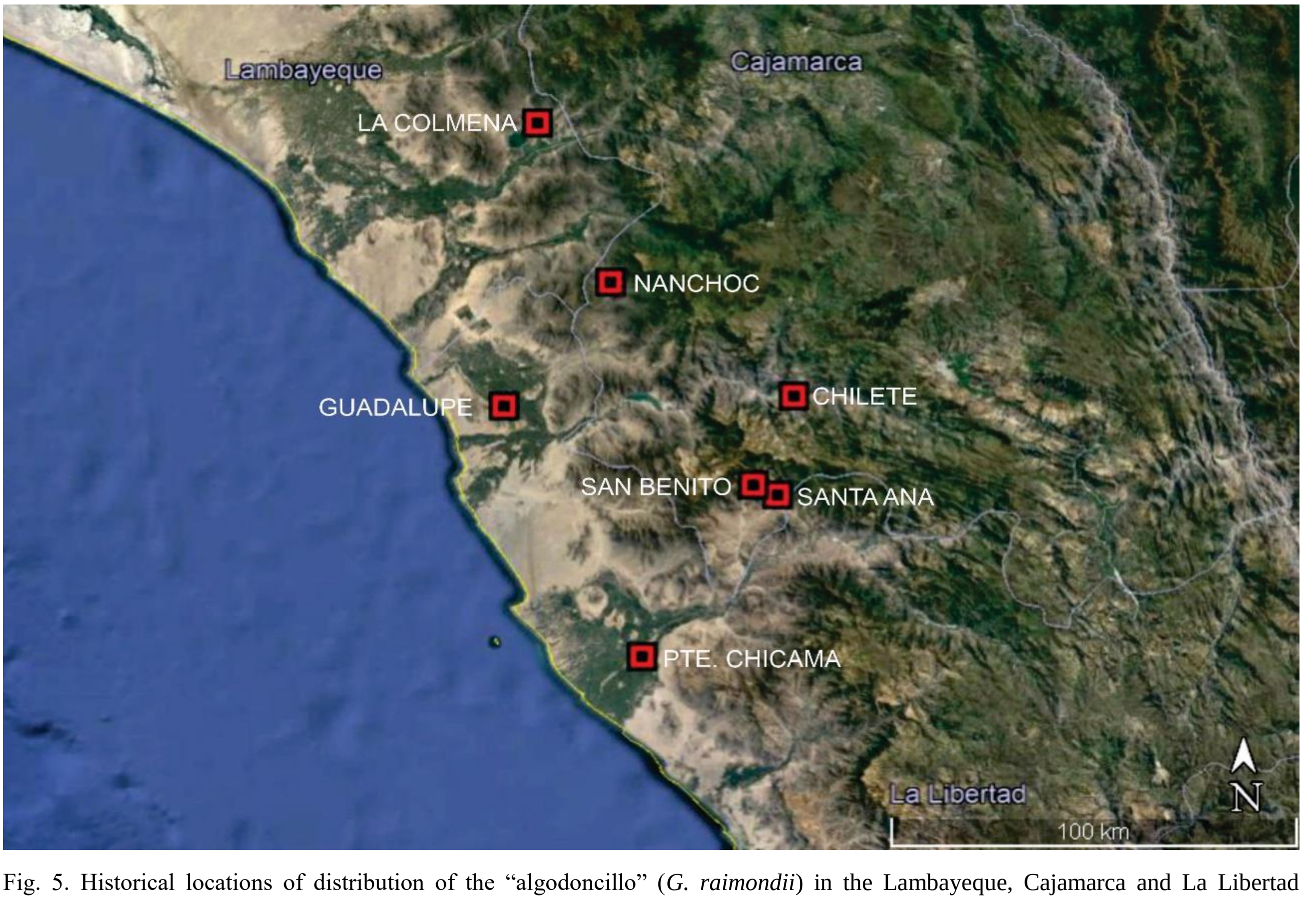

Fig. 5. Historical locations of distribution of the "algodoncillo" (*G. raimondii*) in the Lambayeque, Cajamarca and La Libertad departments. In Nanchoc, Guadalupe and Pte. Chicama this is not found; in Chilete, some individuals may exist but is yet to be confirmed, and in La Colmena, San Benito y Santa Ana, several hundreds of individuals have registered.

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(Received for publication 2 March 2020)