Pak. J. Bot., 53(4): 1259-1270, 2021. DOI: http://dx.doi.org/10.30848/PJB2021-4(43)

GERMPLASM COLLECTION, *IN VITRO* CLONAL PROPAGATION, SEED VIABILITY AND VULNERABILITY OF ANCIENT PERUVIAN COTTON (*GOSSYPIUM BARBADENSE* L.)

GUILLERMO E. DELGADO-PAREDES[1,2*], CECILIA VÁSQUEZ-DÍAZ[2], BORIS ESQUERRE-IBÁÑEZ[2], ALEXANDER HUAMÁN-MERA[2,3] AND CONSUELO ROJAS-IDROGO[1,2]

[1]Facultad de Ciencias Biológicas, Universidad Nacional Pedro Ruiz Gallo, Ciudad Universitaria Nº 391, Lambayeque, Perú
[2]Laboratorio General de Biotecnología, Universidad Nacional Pedro Ruiz Gallo, Ciudad Universitaria Nº 391, Lambayeque, Perú, and
[3]Facultad de Ingeniería Forestal y Ambiental, Universidad Nacional de Jaén, Jr. Cuzco Nº 259, Jaén, Perú. (Present address).
**Corresponding author's email: guidelg2015@yahoo.es; cvd_28@hotmail.com; kamijoo002@gmail.com; alexander.h.mera@gmail.com; crojasi2002@yahoo.es*

Abstract

Gossypium barbadense, known as 'native cotton', 'brown cotton', 'algodón del país', 'Sea Island', 'Egyptian o extra-long staple cotton' is probably originated from west Peruvian Andes. *G. barbadense* has been used since ancient times by the ancient habitants of pre-Columbian civilizations. The objective of the work was the collection of germplasm, study the *In vitro* clonal propagation and the viability of seeds, as well as the vulnerability of the species. The germplasm collection was carried out in several locations in the Lambayeque region and around the Piura, Cajamarca, and La Libertad regions. The fieldwork approximately added 160 accessions and various fiber colors. Cotyledonary nodes were isolated from seven days old *In vitro* germinated seeds and grown in MS culture medium supplemented with 2.0 mg/L $AgNO_3$, formulation where the seedlings reached 12 months of conservation with minimal vitrification and browning of the culture medium. Tests were carried out on the viability of the seed, reaching average germination rates between 50 and 70% in seeds collected no greater than one year, so it is possible to consider them as recalcitrant seeds. Surveys conducted in Morrope, a locality with very ancestral customs, determined a dramatic decrease in the percentage of women weavers with a waist loom of 63% (great-grandmothers and grandmothers, over 40 years of age) to 33% (mothers and aunts, among 20 to 40 years of age) and 8% (sisters, under 20 years of age). The vulnerability of the species would be related to the loss of colors and fiber tones and in the ethnobotanical aspect with the loss of the ancestral tradition in the use of the waist loom.

Key words: Germination and recalcitrant seeds; Germplasm conservation; *In vitro* morphogenic process; Native cotton; Silver nitrate.

Introduction

The genus *Gossypium* was named by Linnaeus in the middle of the 18th century and belongs to the Tribe Gossypieae, Family Malvaceae, and Order Malvales (Smith, 1995; APG IV, 2016). The genus *Gossypium* comprises more than 50 recognized species, distributed in arid and semiarid areas of the tropics and subtropics. Four of these 50 species of *Gossypium* were independently domesticated to take advantage of their fibres. Two of these four are diploids, *Gossypium arboreum* and *G. herbaceum*, with 2n = 2x = 26 and are distributed in the old world, Africa - Asia. The last two are allopolyploids, *G. hirsutum* (upland cotton) and *G. bar*badense (pima cotton), with (2n = 4x = 52) and with a restricted range to the new world (The Americas). The *Gossypium* allopolyploids have AD genome and belongs to the primary gene pool (Wendel *et al*., 2010; Wang *et al*., 2012; Wendel & Grover, 2015). According to Wendel & Grover (2015), probably the allopolyploid condition appeared during the beginning of the early Pleistocene (the last 1 to 2 millon years), as a consequence of biographic past events such as transoceanic dispersion of an A genome species of *Gossypium* from Old to the New World and following hybridization with a native D genome diploid. Evlutionary events that conducted the diversification of these *de novo* allopolyploid originated the emerges of seven new species of cotton within three modern lineages, these evolutionary events gave rise agronomically important cotton species suchs as *G. hirsutum* L. and *G. barbadense* L.

Gossypium barbadense, know as 'algodón nativo', 'algodón pardo', 'algodón del país' (INIPA, 1985), 'Sea Island', 'Egyptian o extra-long staple cotton' (Hutchinson & Manning, 1945), and *G. hirsutum*, known as 'Upland cotton', both *Gossypium* are the two widespread crop species. *G. barbadense*, natural from South America and spread out into Mesoamerica and the Caribbean. The modern lineage of *G. barbadense* cultivated in the United States, probably was originated from west Peruvian Andes (Hutchinson & Manning, 1945; Percy & Wendel, 1990). Although *G. barbadense* is important because of its high-quality fiber (lenght, fineness and strenght), only the worlwide production of this species is around 4.5% nowdays (Abdullaev *et al*., 2017).

Cotton as all plant developed a metabolic system that avoid the attack of predators, between chemical compounds produced. Cotton contains the gossypol, a polyphenolic compound that is produced in the pigment glands of adult plants, and it is possible found inside roots, leaves, and seeds. Gossypol could cause adverse repercussions on human health if the concentration in plasma is high, but investigations in human medicine

have had significant outcomes (Coutinho, 2002). Cyclopropenoids fatty acids (malvalic, sterculic and dihydrosterculic acids) in the seed and tannins in the leaves were also detected (Mansour *et al*., 1997). On the other hand, the extracts of *G. barbadense* are used in alternative medicine as treatment of hypertension, fungal infections, and as an abortificiant or emmenagogue (menstruation stimulant) (Tropilab Inc, 2007). Also, gossypol exhibits toxicity to human melanoma cells (Blackstaffe *et al*., 1997), and *In vitro* tests showed that gossypol decreased the increase of populations of both *Trypanosma cruzi*, which causes Chagas disease (Montamat *et al*., 1982) and *Entamoeba histolytica*, the causal agent of amoebiosis (Gonzalez-Garza *et al*., 1989). Australian Government have published a comprehensive information on beneficial phytochemicals properties of gossypol from both, *G. hirsutum* and *G. barbadense* (Australian Government, Version 2.1 April 2013).

The traditional medicine of Peruvian Northern coast uses *G. barbadense* as treatment of some human diseases such as the "evil eye" of children, manifested with permanent crying as a sign of pain or discomfort and for the case is rubbed several times the body of the infant with some native cotton fuzz and then throws himself into a street in the form of a cross to ward off evil. In the protection of the "mollera" or fontanelle, placing a few fuzz of native cotton of the newborn baby under a hat in order to prevent it from colds. In the treatment of eczema caused by fungi (dermatomycosis) and in this case the green boll, previously scratched with a knife, is rubbed for several days on the eczema. In the healing of small wounds, the ashes of the burned fuzz are applied, as many times as necessary, until the total healing of the wounds, and in the treatment of "spider licks", which are small blisters that form on the edge of the lips and for that matter the blisters are washed, burst gently and the ashes of the brown cotton burned fuzz are applied until healing (Rodríguez, 1985).

In the field of molecular genetic studies of cotton, amplified fragment length polymorphism fingerprinting (AFLP) was applied to survey the genetic diversity and geographic pattern of primitive South American *G. barbadense*. The objective was establishing a posible link to its pre-Columbian expansion, and in this effort gene bank material of three diploid (*G. raimondii*, *G. arboreum*, and *G. herbaceum*) and four allotetraploid cotton species (*G. hirsutum*, *G. mustelinum*, *G. tomentosum* and additional *G. barbadense*) was added for inter- and intra-specific comparison (Westengen *et al*., 2005). Likewise, genetic diversity in the 29 accesions (15 from Peru, 13 from Brazil, and the cultivar Pima S7) were analyzed using 29 microsatellite markers, and based on these analyses, it is verified that there is similar variability level between the Brazilian and the Peruvian accesions (Rodrigues *et al*., 2016). Studies using genetic diversity approaches of *G. barbadense* from several germplasm accesions (up to 200) and phenotypic relationship revelead high level of intra-variability in *G. barbadense* populations and strong association between genetic structure and major fiber quality traits (Abdullaev *et al*., 2017).

Biotechnology techniques such as plant tissue culture is an outstanding tool that permit culturing several explants from a plant, this including cells and organs, or protoplasts in controlled basic medium with the aim of originating a new plant. Within the several methods of plant tissue culture exist culture of somatic cell, shoot tip, protoplast, anther or pollen, likewise somatic hybridization (Qin & Liu, 2006). Plant tissue culture as an auxiliary technique was suggested for studies in genetic transformation of plants as a strong system of regeneration (Firoozabady & DeBoer, 1993), though most studies in *In vitro* cotton tissue culture were performed in upland cotton (*G. hirsutum*). In the clonal propagation, cotyledonary nodes obtained from aseptically raised seedling of cotton cultivar NIAB-99 were cultured on modified MS media supplemented with 0.25 mg/L KIN and produced maximum number of shoots (3.43 shoots/explants) (Rauf *et al*., 2005). Shoot tip of approximately 2.0, 4.0 and 6.0 mm, from *In vitro* germinated seedlings of 22 cotton (*G. hirsutum*) varieties were cultured on basal MS salts, vitamins and without plant growth regulators, and shoot and root formation was observed in all varieties (Rashid *et al*., 2004). In another similar study, multiple shoots from shoot tip explants excised from 5-7 day-old seedlings cultured *In vitro* of two cotton cultivars of *G. arboreum* and *G. hirsutum*, were obtained in MS medium supplemented with 2.0 mg/L BA, in *G. arboreum* and MS medium supplemented with 1.0 mg/L BA and KIN 1.0 mg/L, in *G. hirsutum* (Sanghera *et al*., 2012). From embryo apex explants of *G. hirsutum* cv. Narashima was obtained high-frequency of regeneration using the method of multiple shoot induction, additionaly for better results the MS medium was supplemented with 2.0 mg/L BAP and 2.0 mg/L KIN (Pathi & Tuteja, 2013). Likewise, multiple shoot induction was achieved on MSB_5 medium supplemented with N^6-benzyl adenine (BA), kinetin (KN), thidiazuron (TDZ), Pluronic F-68 or silver nitrate; however, $AgNO_3$ at 2 mg/L produced the greatest number of shoots (22.2 shoots per cotyledonary node explant), with no phenolic secretion (Kumar *et al*., 2016).

The studies, regardless the kind of regeneration of plants, via organogenesis and/or somatic embryogenesis, are numerous although the majority carried out in *G. hirsutum.* Callus formation in ovules from *G. hirsutum* and *G. barbadense* it was observed in several combinations of KIN and IAA, but only rootlike structures were observed in some cultivars whereas shoot formation was not observed (Efe, 2005). Regeneration, via somatic embryogenesis and organogenesis from cotyledonary leaves and hypocotyls explants, in different cultivars of *G. hirsutum* and *G. arboreum*, in culture medium supplemented with 2iP, was observed (Khan *et al*., 2006). *In vitro* studies on organogenic potential from several explants of some important cotton species incluiding *G. arboreum* and *G. hirsutum* in MS culture medium supplemented with 1.0 mg/L 2,4-D for callus induction and 1.0 mg/L BAP for shoots regeneration were performed (Yasmin *et al*., 2016). In another study a suitable callus induction and plant regeneration protocol of cotton cultivar in comparison to non cultivar Coker (*G. hirsutum*) was development (Bandyopadhyayi & Sen, 2016).

In others cotton species such as *Gossypium bickii*, multiple shoots induction through organogenesis (cotyledonary nodes) was made using MSB_5 medium supplemented with the combination of 4.0 mg/L BAP and 0.1 mg/L TDZ, additionally analysis of RAPD's confirmed the genetic homogeneity of the diploid mother plants with the regenerated plants and offsprings (Yang, 2010). In geral, in a recent literature review, conducted in *G. hirsutum*, Ashan & Majidano (2014) concluded that the factors affecting the tissue culture response in *Gossypium* were genotype, donor plant, growth regulators, type of sugar, culture medium, temperature and subculture timing.

The main objectives of the present work were: (1) the germplas collected (seeds) and evaluation of the distribution of ancient Peruvian cotton specimens (*G. barbadense*) in the Lambayeque region and surrounding regions of Piura, Cajamarca and La Libertad; (2) *In vitro* clonal propagation by cotyledonary nodes; (3) the viability of seeds, and (4) and the vulnerability of the crop through to various factors of human influence as as the destruction of its natural environment and the decreasing number of rural women weaving with waist loom.

Materials and Methods

Germplasm collection: The collection of native cotton germplasm (*G. barbadense*) was carried out in numerous localities of the Lambayeque region and in localities of the surrounding places of regions of Piura, Cajamarca and La Libertad. The collected samples or accessions consisted of seeds included the fuzz and lint fibers, from 50 g to more than 1.0 kg, depending on the availability of the sample. Occasionally, seed donations were received, with or without fibers, which generally corresponded to plants eradicated or that were in a vegetative or flowering state. In most cases, germplasm from a single plant was collected from those that were generally very isolated from each other. When there was more than one plant, very close and of the same speck color, the number was counted and taken as a single sample collected. All the samples collected had their corresponding Accesion Data that included georeference positions.

In vitro* clonal propagation*: Samples used in present work were from eight accesions of seeds belong to the Ancient Peruvian Cotton Germplasm Bank of the Universidad Nacional Pedro Ruiz Gallo, Lambayeque (Peru). The seeds were linteless, mature, and healthy. They were collected from the mother plant with one to two months old. The surface of seeds was sterilized with 70% ethanol for one min and thoroughly washed with sterile distilled water. Later the seeds were treated with sodium hypochlorite (NaOCl) (5.25% of Clorox®, commercial bleach solution) for 10 min. Then the seeds were washed thoroughly with sterile water from 3 times with the objective of removing the NaOCl, and cultured on MS mineral salts (Murashige and Skoog, 1962) supplemented with 2% sucrose and 0.7% agar-agar. After 2-4 weeks, the isolated cotyledonary nodes with 1-2 cm hypocotyl with partial cotyledons were cultured in a vertical upright position in MS mineral salts supplemented with the vitamins 1.0 mg/L Thiamine. HCl and 100 mg/L m-inositol, 3% sucrose and N-6-benzylaminopurine (BAP) (2.5 and 0.5 mg/L) and silver nitrate ($AgNO_3$) (2.0 mg/L). The pH of medium was adjusted to 5.8 before autoclaving. The culture was maintained in light conditions (75 μmol m^{-2} s^{-1}) of 16/8 h and temperature of 25ºC ± 2ºC. All the steps above were performed aseptically into a laminar flow machine.

Seed viability: To determine the viability of the seeds, samples were taken from several years of collection, seeds with and without fibers were considered. In the case of seeds with fibers, the total of lint, this was removed before cultivation. The selected seeds presented the best morphological and phytosanitary characteristics. Seed culture was performed in three systems: (a) Petri dishes with filter paper moistened with distilled water sterilized and permanently maintained moisture. In this system seeds were disinfested with benzomil® 500 0.2% (Benomyl), and performed at a rate of 10 seeds per three Petri dishes, (b) Test tubes (150 x 25 mm) supplemented with MS culture medium plus vitamins, 2.0% sucrose and 0.7% agar-agar. Seeds were disinfested with 70% ethanol and 0.25% NaOCl active chlorine, and c) Pots in greenhouse supplemented with soil and fine sterilized sand (1:1). The incubation was carried out at 26°C ± 2°C of temperature in dark conditions. The evaluation was made 7 and 15 days after sowing. It was important to considering the seeds germination with the appearance of the radicle and a root length greater than 2.0 cm. The experiments were performed three times.

Survey to women weavers with waist loom system: This survey was conducted to 125 students of the second (13 years old) and fourth (15 years old) year of secondary from Educational Institution Inca Garcilazo de la Vega of Morrope (Lambayeque). The survey recorded the age and skill and wisdom in weaving with waist loom, great grandmothers, grandmothers, mothers, aunts and sisters, both of the maternal line and the paternal line.

Results and Discussion

Germplasm collection: 157 accessions of germoplasm of native Cotton (*G. barbadense*) from the Lambayeque region and surrounding regions in northern Peru (Piura, Cajamarca and La Libertad) were collected to January 12th 2019 (Table 1; Fig. 1). The largest number of accessions (21) was collected at the Botanical Garden of UNPRG, the provenance and origin of these genetic materials is unknown although it was known that were collected and cultivated at the Botanical Garden of UNPRG between 2012-2014. Among the collections made in the field, the ones carried out on the Chiclayo – Morrope (and surrounding) and Lambayeque – Chiclayo – Cumbil – Catache – Munana (Cajamarca) routes with 16 accessions, respectively, and Lambayeque – Carhuaquero – Llama (Cajamarca) with 14 accessions stand out. Most of the samples were collected in 1 to 3 plants, usually found on the edges of paved roads, rural roads, uncultivated land and even in areas with a strong presence of salts and permanent drought soils. Only in one locality of Huaca de Banderas (On the route Lambayeque – Túcume – Pacora – Jayanca) was found on the edge of a rural road around 35 cultivated plants, in good phytosanitary status and with high production of

dark Brown specks in color. Out of the sampling area, only seven accessions were incorporated, four from the locality of Bagua (Amazonas region) and three from the locality of Pucallpa (Ucayali region), both regions from of the Peruvian Amazon. A relevant fact was that in most cases the plants were strongly attacked by fungi and cotton stainer bug (*Dysdercus peruvianus*), in other cases the plants had been cut from the root and in other cases totally burned, as observed in the samples collected on the Lambayeque – Carhuaquero – Llama (Cajamarca) route (Fig. 2a), where by coincidence a large number of samples were collected, both in number and variety of fiber colors. Isolated plants were also found in lands with serious salinity problems (Fig. 2b).

In a recent study on collection and conservation of native cotton (*G. barbadense*) carried out between december 2012 to february 2013 on the north coast of Peru (regions of Tumbes, Piura, Lambayeque, La Libertad, Cajamarca, Ancash and Lima) 106 collected samples (accessions) were reported, and only 38 accesions corresponded to the Lambayeque region. They were collected in four routes: Chiclayo - Monsefú - Zaña - Nanchoc (Cajamarca), Chiclayo - Tumán - Chongoyape, Chiclayo - Mórrope - Jayanca - Olmos and Chiclayo - Ferreñafe - Pítipo, without indicating the number of accessions collected in each of these four routes (MINAM, 2013). In this study the anonymous authors determined that the geographical unit of sampling was the district and comparing its results with another study conducted in all Peru (Westengen, 2004). They concluded that at least on the north coast of Peru the results of the collections are similar. In relation to the study presented, where up to January 12, 2019 about 75% of the territory of the Lambayeque region has been explored, the number of accessions collected (157) and with a wide variety of fiber colors, can be considered very satisfactory although insufficient.

***In vitro* clonal propagation:** In order to establish an efficient *In vitro* clonal propagation protocol for eight seeds accesiona of *G. barbadense* the seeds germination was observed after 5-7 days incubation under dark conditions. The maximum germination frequency was 90-100%.

The frequency of shoot length and shoots and nodes formation was influenced by the BAP concentration and the $AgNO_3$. The results are shown in Tables 2, 3, 4 and 5. The highest proportion of explants forming adventitious shoots (5.6 per explant) was obtained with media containing 2.5 mg/L BAP (Table 2). Lower concentration of BAP (0.5 mg/L) yielded in reduced number of multiple shoots (3.3 per explant) (Table 4); however, the highest proportion of explants forming nodes (12.8 per explant) was obtained with media containing 2.0 mg/L $AgNO_3$; likewise, in this same culture medium, the greatest elongation of the shoot (10.9 cm) was observed and root formation was 100% (Table 3), while in treatments with 2.5 mg/L BAP and 2.0 mg/L $AgNO_3$ + 0.5 mg/L BAP no root formation was observed (Tables 2 and 4). On the other hand, the survival rate of the cultures was 61.9 and 64.4%, in the treatments with 2.5 mg/L BAP (Table 2) and 2.0 mg/L $AgNO_3$ and 0.5 mg/L BAP (Table 4), respectively, but only in 6 months of culture, while in the treatment with 2.0 mg/L $AgNO_3$, up to 90 and 58.8% of crop survival was achieved in 9 and 12 months of culture (Table 3). So, higher concentrations of BAP (2.5 mg/L) or lower concentrations of BAP (0.5 mg/L) supplemented with $AgNO_3$ (2.0 mg/L) negatively affected the shoot elongation, roots formation and and the survival rate of *In vitro* cultures. Among the most relevant morphological and physiological characteristics observed in *In vitro* plants that did not exceed the survival rate for more than 6 months were the following: Dead plants, deep cracks at the base of the stem, apical necrosis and slightly vitrified, and scarce green plants (Table 5).

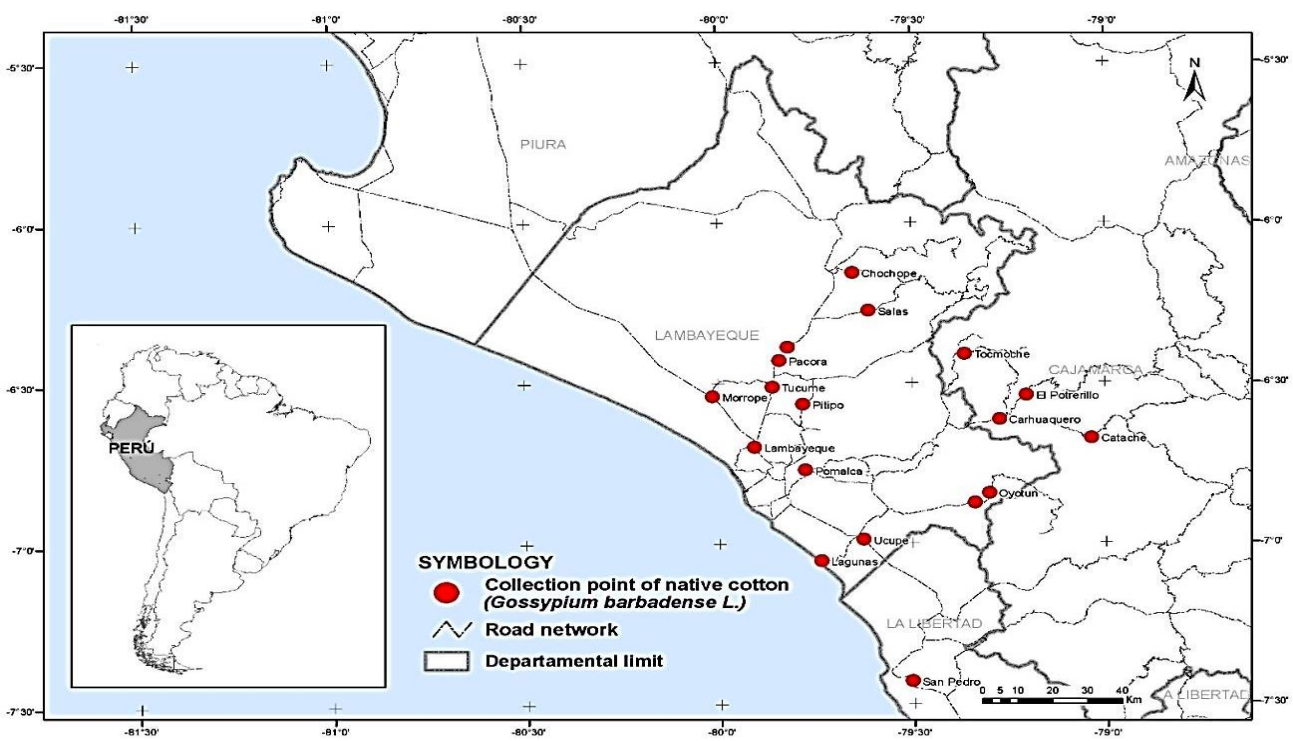

Fig. 1. Collection map of germplasm of ancient Peruvian cotton (*G. barbadense*) in the Lambayeque and surrounding regions.

Table 1. Collection of germplasm of ancient Peruvian cotton (*G. barbadense*) in the Lambayeque region and surrounding regions.

No.	Collection route	Samples collected (No.)	Collection code	Collection date
1.	Lambayeque – Mórrope (Fanupe)	04	001-004	17/07/2017
2.	Lambayeque – Túcume (Museo de Sitio)	06	005-010	15/08/2017
3.	Lambayeque	01	011	25/08/2017
4.	Lambayeque – Chiclayo – San Carlos – Carhuaquero – Potrerillo (Cajamarca)	14	012-025	25/11/2017
5.	Lambayeque – Mórrope (caseríos)	16	026-041	05/01/2018
6.	Ciudad Universitaria (UNPRG–Lambayeque)	02	042-043	11/01/18
7.	Lambayeque – Chiclayo – Motupe – Chóchope	05	044-048	12/01/2018
8.	Lambayeque – Túcume – Pacora – Jayanca	08	049-056	13/01/2018
9.	Lambayeque – Chiclayo – Lagunas – Úcupe	04	057-060	06/02/2018
10.	Botanical Garden (UNPRG – Lambayeque)	21	061-081	16/02/2018
11.	Lambayeque – Chiclayo – Oyotón – Nueva Arica – La Florida (Cajamarca)	09	082-090	25/02/2018
12.	Lambayeque – Jayanca	03	091-093	01/03/2018
13.	Lambayeque – Salas – Pilasca	03	094-096	12/05/2018
14.	Lambayeque – Chiclayo – Mocupe – Pacasmayo – San Pedro (La Libertad)	08	097-104	02/08/2018
15.	Pucallpa (Carretera Federico Basadre, Ucayali)	03	105-107	09/08/2018
16.	Lambayeque – Pilasca – Salas	01	108	27/08/2018
17.	Lambayeque – Mórrope – Playa San Pedro	01	109	29/08/2018
18.	Lambayeque – Chiclayo – Cumbil – Catache – Munana	16	110-125	15/09/18
19.	Lambayeque – Motupe – Chóchope – Chiñama	01	126	13/10/18
20.	Lambayeque – Chiclayo– Pósope –Tinajones – Tocmoche – Miracosta – Pampas (Cajamarca)	03	127-129	16/1018
21.	San Juan (Bagua – Amazonas)	02	130-131	15/11/18
22.	Lambayeque – Chiclayo – Boró – Pomalca	01	132	27/11/18
23.	Ciudad Universitaria (UNPRG-VRINV, Lambayeque)	01	133	28/11/18
24.	San Juan (Bagua – Amazonas)	02	134-135	05/12/18
25.	Lambayeque – Chiclayo – Ferreñafe – La Calzada –Mochumí Viejo – Pítipo – Laquipampa	09	136-144	08/12/18
26.	San Nicolás (Carretera a San José – Fundo Santa Rosa)	01	145	18/12/18
27.	Lambayeque – Chiclayo – Boró – Pomalca	02	146-147	27/12/18
28.	Lambayeque – Chiclayo – San Carlos Alto – Sexi (Cajamarca)	01	148	28/12/18
29.	Lambayeque – Chiclayo — Carhuaquero – San Carlos – El Potrerillo – Sexi (Cajamarca)	09	149-157	12/01/19

Table 2. Influence of 2.5 mg/L BAP on *In vitro* multiplication from cotyledonary nodes of seedlings of ancient Peruvian cotton (*G. barbadense*).

Accesion/ (Fiber color)	Shoot length (cm)	Number of shoots (Nº)	Number of nodes (Nº)	Roots (+/-)	Survival (06 m) (%)	Survival (09 m) (%)
Gb-001/Light brown	5.5	5.0	10	-	100.0	0.0
Gb-004/Light brown	5.7	4.3	9.0	-	100.0	0.0
Gb-005/ Cream	6.2	4.5	11	-	100.0	0.0
Gb-006/Fino colorado	5.8	6.0	12	-	50.0	0.0
Gb-007/Fifo (Light purple)	5.8	6.5	11.7	-	70.0	0.0
Gb-008/Brown	6.2	7.0	14	-	0.0	0.0
Gb-009/Dark brown	5.3	7.0	12	-	50.0	0.0
Gb-010/ Bombasi (Brown)	6.6	4.8	11.5	-	25.0	0.0
Total	**5.9**	**5.6**	**11.4**	**-**	**61.9**	**0.0**

(+/-), +, with roots; -, without roots

Table 3. Influence of 2.0 mg/L $AgNO_3$ on In vitro multiplication from cotyledonary nodes of seedlings of ancient Peruvian cotton (G. barbadense).

Accesion/(Fiber color)	Shoot length (cm)	Number of shoots (Nº)	Number of nodes (Nº)	Roots (+/-)	Survival (09 m) (%)	Survival (12 m) (%)
Gb-001/Light brown	9.5	2.5	7.0	+	100	50
Gb-004/Light brown	9.5	3.7	10.7	+	80	30
Gb-005/Cream	11.5	4.0	15.7	+	80	40
Gb-006/Fine colorado	12.3	4.0	14.0	+	100	100
Gb-007/Fifo (Light purple)	9.6	2.8	14.5	+	90	60
Gb-008/Brown	10.8	3.0	10.0	+	90	50
Gb-009/Dark brown	13.4	3.0	15.0	+	80	40
Gb-010/Bombasi (Brown)	10.8	4.0	15.3	+	100	100
Total	**10.9**	**3.4**	**12.8**	**+**	**90**	**58.8**

(+/-), +, with roots; -, without roots

Table 4. Influence of 2.0 mg/L $AgNO_3$ and 0.5 mg/L BAP on *In vitro* multiplication from cotyledonary nodes of seedlings of ancient Peruvian cotton (*G. barbadense*).

Accesion/(Fiber color)	Shoot length (cm)	Number of shoots (N°)	Number of nodes (N°)	Roots (+/-)	Survival (06 m) (%)	Survival (09 m) (%)
Gb-001/ Light brown	9.1	2.5	10.5	-	50.0	0.0
Gb-004/ Light brown	5.7	3.5	9.3	-	70.0	0.0
Gb-005/ Cream	6.1	5.0	11.3	-	0.0	0.0
Gb-006/ Fine colorado	6.1	3.7	11.3	-	100.0	0.0
Gb- 007/ Fifo (Light purple)	8.3	3.7	10	-	70.0	0.0
Gb-008/ Brown	6.5	2.5	6.5	-	100.0	0.0
Gb-009/ Dark brown	8.9	2.0	7.0	-	50.0	0.0
Gb-010/ Bombasi (Brown)	8.0	3.3	8.5	-	75.0	0.0
Total	**7.3**	**3.3**	**9.3**	**-**	**64.4**	**0.0**

(+/-), +, with roots; -, without roots

Table 5. Influence of 2.5 mg/L BAP (T1), 0.5 mg/L BAP - 2.0 mg/L $AgNO_3$ (T2) and 2.0 mg/L $AgNO_3$ (T3) on *In vitro* multiplication from cotyledonary nodes of seedlings of ancient Peruvian cotton (*G. barbadense*).

Trat.	Shoot length (cm)	Number of shoots (N°)	Number of nodes (N°)	Roots (+/-)	Survival (%) (months)	Survival (%) (months)	Morphologics and physiologic characteristics
T1	5.9	5.6	11.4	-	61.9 (06 m)	0.0 (09 m)	**Plants 6-month-old** Dead plants; apical necrosis, browning and cracking at the base of the stem
T2	10.9	3.4	12.8	+	90 (09 m)	58.8 (12 m)	**Plants 12-month-old** Green plants; slight cracks at the base of the stem; slight apical necrosis; some plants with slight vitrification
T3	7.3	3.3	9.3	-	64.4 (06 m)	0.0 (09 m)	**Plants 6-month-old** Dead plants; deep cracks at the base of the stem; apical necrosis and slightly vitrified; scarce green plants

Table 6. Seed germination and viability of several years of collection of ancient Peruvian cotton (*G. barbadense*)[a].

No.	Characteristics of the seeds/ Culture conditions	Year of collection	Response (%)	
			Germination	Viability
1.	Ten accesions of seeds preserved with fuzz in tightly closed plastic bags of various colour of lint. Seeds with mechanic escarification and whitout mechanic escarification. Culture conditions: Petri dishes	2018	60.0	40.0
2.	Eight accesions of seeds preserved with fuzz in tightly closed plastic bags of various colour of lint. Seeds with mechanic escarification. Culture conditions: Pots in the greenhouse	2018	50.0	45.0
3.	Ten accesions of seeds preserved with fuzz in tightly closed plastic bags of various colour of lint. Seeds with mechanic escarification. Culture conditions: *In vitro* cultures in test tubes	2018	70.0	70.0
4.	Six accesions of seeds preserved with fuzz in tightly closed plastic bags of various colour of lint. Seeds with mechanic escarification and whitout mechanic escarification. Culture conditions: Petri dishes	2008-2009	0.0	0.0
5.	Five accesions of seeds preserved with fuzz in tightly closed plastic containers of various colour of lint. Seeds with mechanic escarification and whitout mechanic escarification. Culture conditions: Petri dishes	2012-2014	0.0	0.0

[a]Treatments with 10-15 seeds evaluated/accession

In several studies cytokinins (BAP, KIN, 2iP and TDZ) has been reported to propagated and regenerated cotton plants, specially in *G. hirsutum*. In early studies on *In vitro* clonal propagation of cotton, specifically on multiple shoot induction and plant regeneration from embryonic axes of cotton, was established that higher concentration of growth hormone yields fewer shoots (Morre *et al*., 1998). Additionally, was established that BAP or KIN cytokinins were responsible for the induction of the higher number of shoots (Agrawal *et al*., 1997; Hemphil *et al*., 1998). Age and size of explants is the most important factor in cotton stem-tip culture. Shoot tip of seedlings with more than 6.0 mm in size and 10 days old showed best response for shoot and root formation on MS basal media, vitamins, 3.0% sucrose and without phytohormones (Rashid *et al*., 2004). Induction and regeneration of multiple shoots from shoot tip explants excised from 5-7 day-old seedlings cultured *In vitro* was observed in MS medium supplemente with 1.0 to 2.0 mg/L BA and a maximum number of shoots (3.9 shoots/explant) (Sanghera *et al*., 2012). Cotyledonary nodes obtained from aseptically raised seedling were

cultured on modified MS media supplemented with different concentrations of KIN, and produced maximum number of shoots (3.43 shoots/explant) when cultured with 0.25 mg/L KIN (Rauf *et al*., 2005). In the embryo apex explants isolated from 2 day-old seedlings, the *In vitro* growing of the tested combinations of 2.0 mg/L BAP and 2.0 mg/L KIN, proved being the best suited for achieving the maximum number of multiple shoots (Pathi & Tuteja, 2013). Also, in a single study performed in two genotypes of *G. barbadense* were researchers used cotyledonary nodes as biological material, 1-2 shoots/ explant were scarcely formed in MS medium supplemented with 0.1 mg/L KIN (Gadir *et al*., 2016). In all these studies conducted in *G. hirsutum* above described as well as in the study reported for *G. barbadense*, cytokinins were fundamental in the proliferation of shoots, although the number of shoots/ explant was not greater than 4. However, in the work of *G. barbadense*, an average of 5.6 shoots/explant was reached in culture medium supplemented with 2.5 mg/L BAP. On the other hand, in none of the studies carried out in *G. hirsutum* the time of permanence of the cultures *In vitro* was indicated, therefore, the effect of the cytokinins in the seedlings was not evaluated beyond the three months of culture, while in the work presented, in *G. barbadense*, the supplement of 2.5 mg/L and 0.5 mg/L BAP with 2.0 mg/L $AgNO_3$ were highly detrimental when the *In vitro* culture was extended up to 6 months.

On the other hand, the supplementation of silver nitrate ($AgNO_3$) in propagation and regeneration culture media has been shown to improve the morphogenic responses. Studies revealed that silver nitrate is a very potent inhibitor of ethylene phytohormone action and is constantly used in plant tissue culture because of silver ion mediated physiological responses that involved polyamines, ethylene and calcium-mediated pathways; also silver nitrate plays a decisive role in physiological process that incluiding morphogenesis (Kumar *et al*., 2009). Likewise researches showed the action of silver nitrate against ethylene symptoms such as epinasty or hyperhidricity, under controlled condition of MS medium supplemented with 2.0 mg/L $AgNO_3$, in two cultivars of *Solanum tuberosum* that showed highest values of leaf area than those cultivars without $AgNO_3$ (Alva & Oropeza, 2013). In black gram (*Vigna mungo*), shoot tip and cotyledonary node explants were cultured on MS medium containing BA, TDZ, AdS and $AgNO_3$ and the best medium composition for multiple shooot induction was with 1.0 mg/L $AgNO_3$ (Mookkan & Andy, 2014). In the propagation studies $AgNO_3$ was responsible for the a high-frequency multiple shoot regeneration from cotyledonary node explants in *G. hirsutum*. $AgNO_3$ inhibit the ethylene production and phenolic secretion (Kumar *et al*., 2016). Recently a study in the *In vitro* development and conservation of passion fruit (*Passiflora gibertii*) during 30, 60 and 90 days used 2.0 mg/L $AgNO_3$ (Faria *et al*., 2017). In the regeneration process the presence of $AgNO_3$ improves shoot regeneration from cotyledon and hypocotyl explants of a several of dicotyledonous species, some of these show recalcitrant in tissue culture. For example, a study reported the effect of $AgNO_3$ and aminoethoxyvinylglycine on *In vitro* shoot and root organogenesis from seedlings explants of recalcitrant *Brassica* genotypes (Chi *et al*., 1990); the effect though organogenesis approach of $AgNO_3$ on the shoot development and plant regeneration of Chili pepper (*Capsicum annum*) (Hyde & Phillips, 1996); and the effect of $AgNO_3$, as ethylene inhibitor, on cucumber *In vitro* shoot regeneration (Mohiuddin *et al*., 1997). Recently a study determined that the best treatment for formation of adventitious shoots from hypocotyl sections of cotton (*G. hirsutum*) was the protocol containing TDZ, NAA and 5.1-10.2 mg/L $AgNO_3$ (Ouma *et al*., 2004). Likewise, $AgNO_3$ (50 µM) added to a MS basal medium supplemented with BAP and NAA it allowed the induction of androgenesis in white cabbage (*Brassica oleraceae*) anthers (Cristea *et al*., 2012).

The results shown in these works agree with the results obtained in the present study where the 2.0 mg/L $AgNO_3$ supplemented to culture medium was optimal in the development and conservation of several accessions of native cotton germplasm (*G. barbaden*se), observing that the inhibition of the synthesis of ethylene and phenols led to the *In vitro* plants showing little symptoms of hyperhydricity and browning in the culture medium.

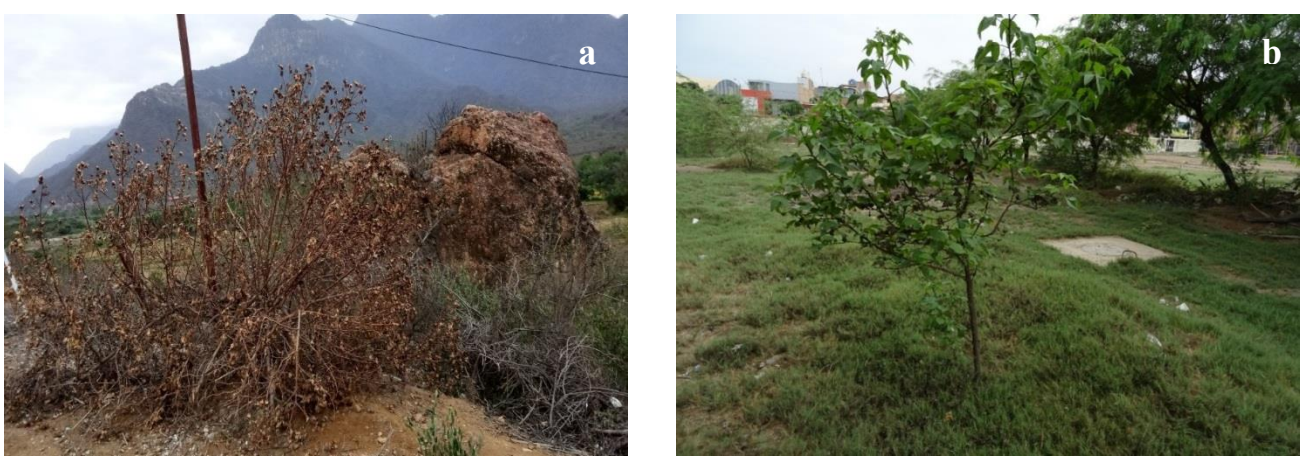

Fig. 2. a. Ancient Peruvian cotton plant (*G. barbadense*), in full fructification, mutilated and abandoned at the edge of the road Chongoyape – El Cumbil (Lambayeque, Perú) and b. *G. barbadense* in its second flowering and fructification, growing in soil with high salinity and salt grass (*Distichlis spicata*) in the University City (UNPRG, Lambayeque, Peru).

Table7. Women weavers (maternal family of the student surveyed) with backstrap loom and ancient Peruvian cotton (*G. barbadense*) in the locality of Morrope (Lambayeque, Peru).

Relationship	Age (years)											
	60 - >			40 - 59			20 - 39			0 - 19		
	+ (No/ %)	- (No/ %)	0 (No/ %)	+ (No/ %)	- (No/ %)	0 (No/ %)	+ (No/ %)	- (No/ %)	0 (No/ %)	+ (No/ %)	- (No/ %)	0 (No/ %)
Great-grandmother (Maternal line)	82/ 65.6	28/ 22.4	15/ 12.0									
Grandmother				80/64.0	37/29.6	8/6.4						
Mothers							39/31.2	80/64.0	6/4.8			
Aunts							120/37.4	194/60.4	7/2.2			
Sisters										18/8.3	162/75.0	36/16.7

+, women weavers;-, non-weaver women; 0, undefined
Great-grandmother, grandmother or mothers = 125
Aunts = 321
Sisters = 216

Table 8. Women weavers (paternal family of the student surveyed) with backstrap loom and ancient Peruvian cotton (*G. barbadense*) in the locality of Morrope (Lambayeque, Peru).

Relationship	Age (years)											
	60 - >			40 - 59			20 - 39			0 - 19		
	+ (No/%)	- (No/%)	0 (No/%)	+ (No/%)	- (No/%)	0 (No/%)	+ (No/%)	- (No/%)	0 (No/%)	+ (No/%)	- (No/%)	0 (No/%)
Great-grandmother (Paternal line)	75/60.0	35/28.0	15/12.0									
Grandmother				83/66.4	32/25.6	10/8.0						
Aunts							105/37.0	168/59.2	11/3.9			

+, women weavers; -, non-weaver women; 0, undefined
Great-grandmother or grandmother = 125
Aunts = 284

Seed viability: Table 6 shows that seeds with various fiber colors, collected from January to September 2018, showed germination rates between 50 to 70% and viability between 40 to 70%. The mechanical scarification of the seeds (sanding) only influenced the time of germination since scarified seeds germinated in the period of 5 to 7 days while non scarified seedlings germinated in the period of 12 to 15 days. Likewise, seeds with various fiber colors, collected between the years of 2008 to 2009 and 2012 to 2014 and conserved in plastic bags and plastic containers, respectively, both scarified and not scarified, did not germinate. The germination rate was slightly higher in seeds germinated *In vitro* followed by seeds germinated in Petri dishes and then in pots in greenhouse conditions. The culture medium, supplemented with mineral salts, vitamins and sucrose and the aseptic conditions of incubation, slightly influenced the seed germination on the other culture conditions.

Women weavers with waist loom from the localities of Monsefú, Pacora and Túcume, who are direct descendants of the ancient settlers of the Mochica culture, reported that the native cotton seed retains its viability (more than five years) when it is conserved together with the fibers. However, this does not agree with observations made in the laboratory since seeds with speck, more than five years of collected and stored in plastic bags and containers, did not germinate. A study conducted in rates of germination of *Eriotheca pentaphylla* seeds concluded that preservation of kapok (seem to cotton fibers) during this event determined a higher rates of germination when compare with those without kapok (Fischer, 1997). In opinion of Linares-Palomino & Ponce-Álvarez (2005), kapok is an important accesory of seeds for higher possibilities of survival and germination. A similar case could be made for *Cochlospermum vitifolium* (Cochlospermaceae), with capsules that contain several seeds embedded in kapok (Molau, 1983. Other species of Malvaceae-Bombacoideae such as “palo de balsa” (*Ochroma pyramidale*), “ceibo” (*Ceiba* sp.) and “barrigón” (*Cavanillesia* sp.) they also possess abundant kapok in their seeds, just like *G. barbadense* and *G. raimondii*, subspontaneous and wild species, respectively, in the seasonally dry forest of Lambayeque.

The *ex situ* conservation success of plant germplasm is related with the storage of accesions under appropiated conditions that permit maximum recovering after post-harvest (Berjak & Pammenter, 2013). In the 70’s researchers showed that the seed viability period may be extended by lowering their temperature and moisture content throughout storage. However, investigators also described a group of species whose seeds showed different characteristics, because a decrease in their moisture content tended to decrease the viability period. Then, was divided seeds into two categories: the predictible ones, which was called orthodox, and all the others, called recalcitrant (Roberts, 1973). Recently, a very used classification in this respect is that of desiccation-tolerant seeds (orthodox species), not desiccation-tolerant seeds (recalcitrant species) and exceptional species (species that produce few or no viable seeds) (FAO, 2013; Pence, 2011). However, it is possible that the differences between recalcitrant and orthodox seeds lies only on the maturity stage in which they are detaches from the mother plant, the recalcitran tones in a very immature stage (Barbedo *et al*., 2013). Although there are several studies on seed dormancy, germination and seedling survival made in *G. hirsutum* and *G. barbadense* (Ellis *et al*., 1985; Australian Government, Version 2.1 April 2013), with certainty that these physiological aspects of the native cotton seed need further investigation, therefore the question: ¿Is *Gossypium barbadense* a recalcitrant species? will remain unanswered.

Survey to women weavers with waist loom system: Among the 125 surveyed students from the Educational Institution Inca Garcilazo de la Vega of Morrope (Lambayeque), on the mother’s line, 65.6% of great-grandmothers (≥ 60 years old), 64.0% of grandmothers (40-59 years old) and 31.2% of mothers (20-39 years old), while of 321 maternal aunts (20-39 years old) and 216 sisters (0-19 years old), only 37.4 and 8.3%, respectively, developed the ability to weave with a waist loom using native cotton (Table 7). In the paternal line, 60.0% of great-grandmothers (≥ 60 years old) and 66.4% of grandmothers (40-59 years old), while of 284 paternal aunts (20-39 years old) only 37.0% developed the ability to weave with a waist loom using native cotton (Table 8).

These results showed that in Morrope, a locality of very traditional customs in the Lambayeque region, due to its close anthropological and cultural links with the pre-Columbian Mochica culture of ancient Peru, the ability of women to weave with waist loom using native cotton it is being lost from generation to generation. This loss is around 50% since between great-grandmothers and grandmothers (40 to more years of age) there are about 65% of women with such ability, in the generation of mothers and aunts (20-39 years of age) this value decreases to around 35% and in the case of sisters (0-19 years of age) it reaches around 8.0%. In a similar study conducted in the coastal strip (Chavimichic area) of the La Libertad region, among 1 024 respondents, it was determined that 38.5% knew how to spin, 78.0% knew how to weave with a waist loom and 57.0% said their daughters were interested in spinning and knitting, and in another survey conducted in the area of Chancay, La Leche, Motupe and Olmos of the Lambayeque region, among 1154 respondents, it was determined that 55.4% knew how to spin and 42.8% knew how to weave with a waist loom (Vreeland Jr., 1985). In these surveys the ages of the respondents were not included.

Vulnerability: Among the species of the genus *Gossypium* of Peru only *G. raimondii* Ulbr. it is considered endemic with a very restricted distribution in the regions of Amazonas, Cajamarca, La Libertad and Lambayeque. Few years ago INRENA (Instituto Nacional de Recursos Naturales) declared it as a species in a "critical" state (Chanco *et al*., 2006). With the native cotton (*G. barbadense*) this situation does not occur

although probably originated from west Peruvian Andes, *G. barbadense* is distributed in several countries of South America, Mesoamerica and the Caribbean (Percy & Wendel, 1990). There are even germplasm Banks of germplasm of *G. barbadense* in several countries of the world such as GRIN (Germplasm Resources Information Network)/USA and Cotton Collection of the USDA-ARS, College Station, Texas (USA); Bank of germplasm of EMBRAPA/Brasil, Institute of Genetics and Plant Experimental Biology (IG & PEB), Academy of Sciences of Uzbekistan, and others; however, Rodrigues *et al*., (2016) has recognized that since its introduction in Brazil, *G. barbadense* populations have reduced its occurrence and genetic variability and a similar situation may be occurring in Peru even when it is recognized as primary center.

On the other hand, the criteria established in the IUCN (2012) do not allow to classify *G. barbadense* in the category of "threatened" although it is possible that there is no adequate evaluation or the data is insufficient. However, during germplasm collection activities, if the name of a farmer was consigned in the Accesion Data, it was not necessarily because the farmer was the owner of the farm but because he was closest to the plant sampled, that is, they were plants that nobody had planted. In personal conversations with farmers and local population, they all agreed that the native cotton plants were disappearing at an accelerated rate, they also agree that no one planted the specimens spite they have a sample plant in their gardens. Gradually in the time, country people noted that specimens lost until disappear their very beautiful color of fibers. Among the reasons that this is happening is that they considered the native cotton plants as reservoirs of pests and diseases and even more so when they were very close to commercial cotton (*G. hirsutum*) so the depredation and extinction of *G. barbadense* is and will be a fact (INIPA, 1985). If we add to this the possible recalcitrance of the seeds and the decrease of women weaving with waist looms, they would contribute to the vulnerability of the species, although the loss of vocation of women who weave with a waist loom is more an ethnobotanical and anthropological problem.

Conclusions

The present study reports the germplasm collection of native cotton (*Gossypium barbadense*) in Lambayeque region and other adjacent regions such as Piura, Cajamarca and La Libertad. Likewise, also reports on the development of a highly efficiente protocol based on using cotyledonary nodes as explants for the propagation and conservation of several accesions of native cotton. The viability of the seed diminished with the storage time, observing that seeds of more than five years of storage, even covered with the speck, did not germinate, concluding that it is possible that native cotton is a recalcitrant species. The vulnerability of the species would be related to the loss of alleles that determine the color and tonality of the fiber as well as the loss of the ancestral tradition of weaving with a waist loom.

Acknowledgements

The authors thank the Professor Ernesto E. Hashimoto Moncayo, Vicerrector de Investigación of the UNPRG (Lambayeque) for the unconditional support in the realization of the present study. Likewise, to Lic. Luis Vílchez Ezcurra for his contribution in the elaboration of the surveys.

References

Abdullaev, A.A., I.B. Salakhutdinov, S.S. Egamberdiev, E.E. Khurshut, S.M. Rizaeva, M. Ulloa and Y. Abdurakhmonov. 2017. Genetic diversity, linkage desequilibrium, and association mapping analyses of *Gossypium barbadense* L. germplasm. *PLOS ONE* 14 1/30.

Agrawal, D.C., R.R. Banerjee, A.B. Kolala, A.V. Dhage, A.V. Kulkarni, S.H. Nalawade and K.V. Krishnamurthy. 1997. *In vitro* induction of multiple shoots and plant regeneration in cotton (*Gossypium hirsutum* L.). *Plant Cell Rep*., 16(9): 647-652.

Ahsan, M.Z. and M.S. Majidano. 2014. Regeneration of cotton (*Gossypium hirsutum* L.) through asexual methods, a review. *Amer. Eura. J. Agric. & Environ. Sci*., 14(12): 1478-1486.

Alva, S. and M. Oropeza. 2013. Effect of culture medium consistence and silver nitrate on micropropagation of two potato (*Solanum tuberosum*) cultivars. *Rev. Colomb. Biotecnol.*, 15(2): 55-62.

APG IV (Angiosperm Phylogenic Group). 2016. An update of angiosperm phylogeny group classification for the orders and families of flowering plants. *Bot. J. Linn. Soc*., 181(1): 1-20.

Australian Government, Version 2.1 April 2013. The biology of *Gossypium hirsutum* L. and *Gossypium barbadense* L. Office of the Gen Technology Regulator. Australian Government. httpp://www.ogtr.gov.au

Bandyopadhyay, N. and S.K. Sen. 2016. Development of a suitable plant regeneration protocol of cotton cultivar variety in comparison to non cultivar Coker. *IOSR J. Biotechnol. & Biochem.,* 2(5): 14-20.

Barbedo, C.J., D. da Cruz Centeno and R. de C.L.F Ribeiro. 2013. Do recalcitrant seeds really exist? *Hoehnea,* 40(4): 583-593.

Berjak, P. and N.W. Pammenter. 2013. Implications of the lack of desiccation tolerance in recalcitrant. *Front. Plant Sci.,* 4(478): 1-9.

Blackstaffe, L., M.D. Shelley and R.G. Fish. 1997. Cytotoxicity of gossypol enantiomers and its quinone metabolite gossypolone in melanoma cell line. *Mel. Res.,* 7(5): 364-372.

Chanco, M., B. León and I. Sánchez. 2006. Malvaceae endémicas del Perú. *Revista Peruana de Biología.* Número especial 13: 413s–425c.

Chi, G.L., D.G. Barfield, G.E. Sim and E.C Pua. 1990. Effect of AgNO3 and Aminoethoxyvinylglycine on *In vitro* shoot and root organogenesis from seedling explants of recalcitrant Brassica genotypes. *Plant Cell Rep*., 9(4): 195-198.

Coutinho, E.M. 2002. Gossypol: a contraceptive for men. *Contraception*, 65(4): 259-263.

Cristea, T.O., C. Leonte, C. Brezeanu, M. Brezeanu, S. Ambarus, M. Calin and M. Prisecaru. 2012. Effect of $AgNO_3$ on androgenesis of *Brassica oleraceae* L. anthers cultivated *In vitro*. *Afri. J. Biotechnol.,* 11(73): 13788-13795.

Efe, L. 2005. Callus formation and plant regeneration from two cotton species (*Gossypium hirsutum* L., and *G. barbadense* L.). *Pak. J. Bot.,* 37(2):227-236.

Ellis, R.H., T.D. Hong and E.H. Roberts. 1985. Handbook of Seed Technology for Genebanks. Vol. II. Compendium of Specific Germination Information and Test Recomendations. *Int. Board. Plant Gen. Reso.,* (IBPGR), Rome. pp. 494-496.

FAO (Food and Agriculture Organization). 2013. Genebank Standards for Plant Genetic Resources for Food and Agriculture. Rome, FAO.

Faria, G., L. Felizardo, A. Ferreira, P. Rocha, A. Suzuki, A. Souza, T. Junghans, M. Costa, A. Peixoto, A. Morais, B. Lopes and T. Oliveira. 2017. Concentrations of silver nitrate in the In vitro development and conservation of *Passiflora gibertii* N.E. Brown. *Amer. J. Plant Sci*., 8(12): 2944-2955.

Firoozabady, E. and D.L. DeBoer. 1993. Plant regeneration via somatic embryogenesis in many cultivars of cotton (*Gossypium hirsutum* L.). *In vitro Cell Dev. Biol.-Plant,* 29(4):166-173.

Fischer, E.A. 1997. The role of plumes in *Eriotheca penthaphylla* (Bombacaceae) seed survival in south-eastern Brazil. *J. Trop. Ecol*., 13: 133-138.

Gadir, I.K.A.A., M.A. El Siddig, H.H.A. Ibrahim and A.A. El Hussein. 2016. In vitro direct regeneration of cotton (*Gossypium* spp.) cultivars of Sudan. *Brit. Biotechnol. J.,* 11(4):1-9.

Gonzalez-Garza, M.T., B.D. Mata-Cárdenas and S. Said-Fernández. 1989. High susceptibilitic of five axenic *Entamoeba histolytica* strains to gossypol. *Transact. Royal Soc. Trop. Med. & Hyg.,* 83(4): 522-524.

Hemphil, J.K., C.G.A. Maier and K.D. Chapman. 1998. Rapid *In vitro* regeneration of cotton (*Gossypium hirsutum* L.). *Plant Cell Rep.,* 17(4): 273-278.

Hutchinson, J. and H. Manning. 1945. The Sea Island cotton. *Mem. Cotton Res. Station Trinidad Ser Genet*., 25: 80-92.

Hyde, C. and G. Phillips. 1996. Silver nitrate promotes shoot development and plant regeneration of chile pepper (*Capsicum annum* L.) via organogenesis. *In vitro Cell & Develop. Biol. Plant.,* 32(2):72-80.

INIPA (Instituto Nacional de Investigación y Promoción). 1985. Algodón "Del País" un cultivo milenario norteño. Serie Informe Especial. 127 p.

IUCN (International Union for Conservation of Nature) Red List Categories and Criteria: Version 3.1 (2012). IUCN Species Survival Commission (SSC).

Khan, T., A.K. Singh and R.C. Pant. 2006. Regeneration via somatic embryogenesis and organogenesis in different cultivars of cotton (*Gossypium* spp.). *In vitro Cell & Develop. Biol. Plant,* 42(6): 498-501.

Kumar, G.P., S. Sivakumar, G. Siva, M. Vigneswaran, T.S. Kumar and N. Jayabalan. 2016. Silver nitrate promotes high-frequency multiple shoot regeneration in cotton (*Gossypium hirsutum* L.) by inhibiting ethylene production and phenolic secretion. *In vitro Cell. & Develop. Biol. Plant.,* 52(4): 408-418.

Kumar, V., G. Parvatam and G.A. Ravishankar. 2009. $AgNO_3$ - a potential regulator of ethylene activity and plant growth modulator. *Electr. J. Biotechnol.,* 12(2): 1-15.

Linares-Palomino, R. and S.I. Ponce-Álvarez. 2005. Tree community patterns in seasonally dry tropical forests in the Cerros de Amotape Cordillera, Tumbes, Peru. *Forest Ecol. & Manag*., 209(3): 261-272.

Mansour, M.H., N.M. Zohdy, S.E. El-Genhaihi and A.E. Amr. 1997. The relationship between tannins concentration in some cotton varieties and susceptibility to piercing sucking insects. *J. Appl. Entom*., 121(1-5): 321-325.

MINAM (Ministerio del Ambiente). 2013. Distribución y concentración de las razas locales de algodón nativo en la costa norte del Perú. Ministerio del Ambiente, República del Perú. 57 p.

Mohiuddin, A.K.M., M.K.U. Choudhury, Z.C. Abdullah and S. Napis. 1997. Influence of silver nitrate (ethylene inhibitor) on cucumber *In vitro* shoot regeneration. *Plant Cell*, *Tiss. & Organ Cult.,* 51(1): 73-78.

Molau, U. 1983. 127. Bixaceae, 128. Cochlospermaceae. - In: G. Harling, B. Sparre (eds). Flora of Ecuador 20, Swedish Natural Science Research Council, Stockholm, pp. 3-15.

Montamat, E.E., C. Burgos, N.M.G. Burgos, I.E. Rovai, A. Blanco and E.L. Segura. 1982. Inhibitory action of gossypol on enzymes and growth of *Trypan. Cruzi*. *Sci.,* 218(4569): 288-289.

Mookkan, M. and G. Andy. 2014. $AgNO_3$ boosted high-frequency shoot regeneration in *Vigna mungo* (L.) Hepper. *Plant Signal. & Behav.,* 9(10): e972284.

Morre, J.L., H.R. Permingeat, M.V. Romagnoli, C.M. Heisterborg and R.H. Vallejos. 1998. Multiple shoot induction and plant regeneration from embryonic axes of cotton. *Plant Cell, Tiss. & Organ Culture,* 54: 131-136.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant*., 15: 473-497.

Ouma, J.P., M.M. Young and N.A. Reichert. 2004. Optimization of *In vitro* regeneration of multiple shoots from hypocotyl sections of cotton (*Gossypium hirsutum* L.). *Afri. J. Biotechnol.,* 3(3): 169-173.

Pathi, K.M. and N. Tuteja. 2013. High-frequency regeneration via multiple shoot induction of an elite recalcitrant cotton (*Gossypium hirsutum* L. cv. Narashima) by using embryo apex. *Plant Sugnal. & Behav.,* 8(1): e22763.

Pence, V.C. 2011. Evaluating costs for the *In vitro* propagation and preservation of endangered plants. *In vitro Cell. & Develop. Biol. Plant.,* 47(1):176-187.

Percy, R.G. and J.F. Wendel. 1990. Allozyme evidence for the origin and diversification of *Gossypium barbadense* L. *Theor. & Appl. Gen.,* 79(4): 529-542.

Qin, Y.H. and J.Y. Liu. 2006. Cotton tissue culture and plant regeneration. *Mol. Plant Breed.,* 4: 583-592.

Rashid, B., T. Husnain and S. Riazuddin. 2004. *In vitro* shoot tip culture of cotton (*Gossypium hirsutum*). *Pak. J. Bot.,* 36(4): 817-823.

Rauf, S., M. Usman, B. Fatima and I.A. Khan. 2005. *In vitro* regeneration and multiple shoot induction in upland cotton (*Gossypium hirsutum* L.). *Plant Tissue Cult.,* 15(1): 75-81.

Roberts, E.H. 1973. Predicting the storage life of seeds. *Seed Sci., & Techn.,* 1: 499-514.

Rodrigues, J.S.D., L.P. Carvalho and F.J.C. Farias. 2016. Comparison of wild accesions of *Gossypium barbadense* L. from Peru and Brazil via microsatellite markers. *Bio-Sci., J.*, Uberlândia 32(5):1352-1363.

Rodríguez, V.A. 1985. El algodón "del país" y el artesanato textil en la sociedad "Chavimochic". En: Informe Especial. Algodón "del País" un Cultivo Milenario Norteño. Instituto Nacional de Investigación y Promoción Agropecuaria (INIPA). Pp. 39-58, Nº 33. Chiclayo, Perú.

Sanghera, G.S., M.S. Gill, G. Singh and S.S. Gosal. 2012. *In vitro* plant regeneration through multiple shoot induction in cotton (*Gossypium* spp.). *Elixir Appl. Bot.,* 43: 6870-6876.

Smith, C.W. 1995. Cotton (*Gossypium hirsutum* L.). Chapter 6. In: Crop Production: Evolution, History, and Technology. John Wiley and Sons, In. New York. pp. 287-349.

Tropilab Inc 2007. *Gossypium* tincture for Amazon herbs. httpp://www.tropilab.com/gossypiumtincture.htlw. Accesed 02-jul-2019.

Vreeland Jr., J.M. 1985. El proyecto de investigación del algodón "del país": Un estudio de la tecnología tradicional en el ámbito rural norteño. En: Informe Especial. Algodón "del País" un Cultivo Milenario Norteño. Instituto Nacional de Investigación y Promoción Agropecuaria (INIPA). Pp. 1-39, Nº 33. Chiclayo, Perú.

Wang, P., Y. Zhu, X. Song, Z. Cao, Y. Ding, B. Liu and T. Zhang. 2012. Inheritance of long staple fiber quality traits of *Gossypium barbadense* in *G. hirsutum* background using CSILs. *Theor. & Appl. Gen.,* 124(8): 1415-1428.

Wendel, J.F. and C.E. Grover. 2015. Taxonomy and evolution of the cotton genus, *Gossypium*. D.D. Fang and R.G. Percy (edit.). Cotton. 2nd. Ed. Agronomy Monograph 57. ASA, CSSA, 5585 Guilford Road, Madison, WI 53711, USA.

Wendel, J.F., C.L. Brubaker and T. Seelanan. 2010. The origin and evolution of *Gossypium.* J.McD. Stewart, D. Oosterhuis and J.J. Heitholt (eds.). Physiology of Cotton. Springer – Netherlands, pp. 1-18.

Westengen, O.T. 2004. Genetic diversity and geographic pattern in early South American cotton domestication. Master Thesis in Tropical Ecology and Management of Natural Resources. *Dept. Ecol & Nat. Resou. Manag. Agricult. Univer., Norway*. Pp. 12-18.

Westengen, O.T., Z. Huamán and M. Heun. 2005. Genetic diversity and geographic pattern in early South American cotton domestication. *Theor. & Appl. Gen.,* 110(2): 392-402.

Yang, X.Y., X.L. Zhang, L.L. Fu, L. Min and G.Z. Liu. 2010. Multiple shoots induction and wild cotton (*Gossypium bickii*) through organogénesis and the analysis of genetic homogeneity of the regenerated plants. *Biologia,* 65(3): 496-503.

Yasmin, A., S. Lochi, N. Memon, S. Yasin, S. Khaskhali, R. Abro and S.H. Abro. 2016. *In vitro* studies on organic potential of some important cotton varieties. *Sci. Int.,* 28: 4029-4034.

(Received for publication 5 August 2019)