INTERNATIONAL COTTON
RESEARCHERS ASSOCIATION

COTTON

INNOVATIONS

VOLUME 4
ISSUE 12

February
2025

www.icra-cotton.org

Content

Cotton Innovations Journal

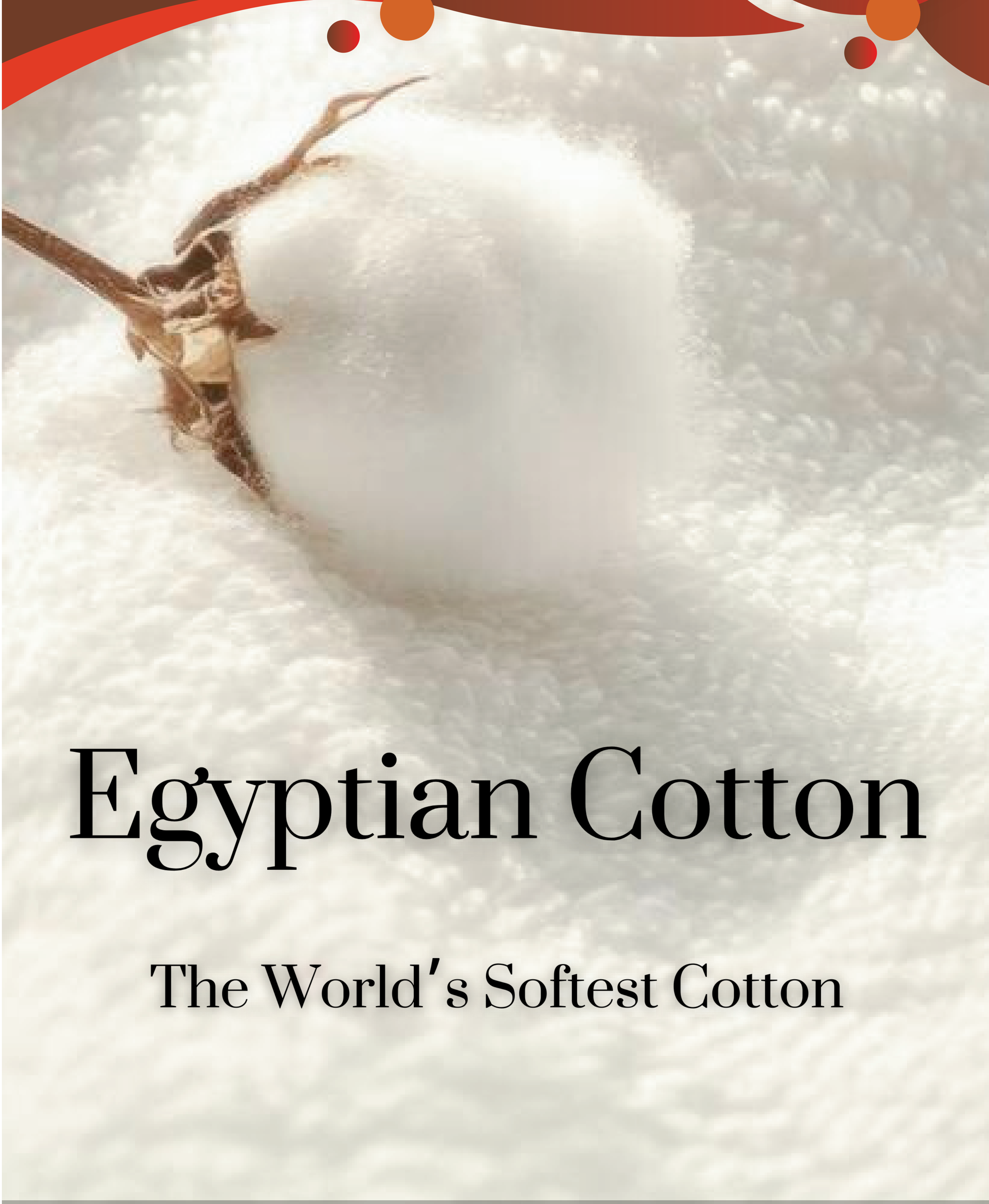
The Cotton Innovations Journal is published twelve times annually. Contributed articles, pictures, cartoons, and feedback are welcome at any time. Please send contributions to the General Editors (see below). The editors reserve the right to edit. The deadline for contributions is one month before the publication date.

Editorial Board

Published by ICRA Secretariat, Pakistan Central Cotton Committee, Multan-Pakistan
http://icra-cotton.org

The newsletter is also available at URL: http://www.icra-cotton.org/page/cotton-innovations

ISSN 2788-6611



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Conservation, Initial Morphological Characterization Fibre and Blue Disease Evaluations of Old National and International Varieties and Discarded Breeding Lines of *Gossypium hirsutum* L. at INTA Sáenz Peña in Chaco Argentina

Royo O, Montenegro A, Bonacic I, Tcach M and Spoljaric M

Instituto Nacional de Tecnología Agropecuaria (INTA) – EEA Sáenz Peña.
Chaco. Argentina.
E-mail: royo.olegario@inta.gob.ar

BRIEF HISTORY AND PASSPORT DATA

A breeding program for the development of new varieties of Gossypium hirsutum L. had been carried out uninterruptedly centred al INTA Sáenz Peña for more than 70 years, especially since the creation of INTA in the 1950s. As with many breeding programs the objective was to obtain varieties well adapted to the different regions of the country with the potential for the growth of cotton and regarding improvements in fibre quality to attend the textile industry and with resistance or tolerance to local diseases. Initially, introductions of foreign varieties were the starting point for the breeding program, and as it proceeded a germplasm bank was kept which was regenerated each 2 years to maintain a good level of germination power in the seeds. By the 1980s a program of conservation of genetic resources for main crops grown in Argentina was put forward at INTA, and refrigerated chambers were established through an agreement with an Italian agency (IAO) at different experimental stations in Argentina. For cotton INTA Sáenz Peña was selected for that purpose.

The accessions were introduced from 21 different origins dating back from the mid-1940s onwards (refer to table 1). The main places of origin are from the United States of America and from Argentina, these latest mainly from crossings of different varieties and lines within the breeding program throughout the second half of the 20th century. Different varieties released by companies like US-based Deltapine, Stoneville, and many Acala and Paymaster-type germplasm make-up part of the collection based at INTA Sáenz Peña. Also, classical Argentinian varieties like Guazuncho-2 INTA, Chaco 510 INTA and Quebracho INTA are there preserved, as well as Reba-type varieties from Paraguay and Africa with other African originated germplasm distributed by the agricultural French agency now named CIRAD. And from Brazil many varieties that were released by the I.A.C. (Instituto Agronómico Campinas) are also kept

Table 1: Number of accessions of Gossypium hirsutum L. by country from the cotton genebank at INTA Sáenz Peña obtained from the database

Number of accessions	COUNTRY
81	unknown
196	Argentina
5	Africa
8	Australia
17	Brasil
3	Bulgaria
2	Cameroun
1	Colombia
12	Ivory Coast
4	China
4	Spain
12	ex-United Soviet Socialist Republics
2	Greece
5	India
13	Paraguay
4	Central African Republic
6	Senegal
1	Sudan
2	Tchad
183	USA
3	Ex_Yugoslavia
TOTAL = 564	From 21 origins

Combined with the medium-term conservation at 0 to 5 °C for around 10 years, duplicates were sent to a base bank located at INTA Castelar in Buenos Aires province for long term preservation at –20°C. Also a database was kept with passport, characterization and evaluation data for the collection that exceeded 550 accessions, divided that amount almost half as old varieties and half as breeding lines that did not reach the stage of a variety in the breeding program but that had a/some relevant character/s worths keeping it/them for a future use in the program, like excellent fibre quality or good resistance or tolerance to certain diseases and pests (Royo 1998).

INITIAL MORPHOMETRICAL CHARACTERIZATION COMBINED WITH FIBRE EVALUATION

Alongside regeneration for the purpose of conservation for both medium and long term timelapse, in the 1990s a morphometric characterization was started to distinguish important descriptors that contributed heavily for the prospect of grouping or clustering. Also, fibre samples were sent to the Fibre Quality Laboratory based at INTA Sáenz Peña where using the HVI (High Volume Instrument) results were obtained concerning data for fibre length, resistance, gin out-turn and micronaire. By 1998 a catalogue with passport and some characterization and evaluation data was published. A matrix with 468 accessions of G. hirsutum L. considering 16 descriptors was analysed with multivariate analysis both cluster (Gower coefficient and average grouping) and principal component. A dendrogram is shown in Figure 1 highlighting 3 main clusters. The most important descriptors for separating the 2 main bigger groups were the seed index, gin out-turn (negative), boll morphology, and fibre length and resistance (see figure 1, figure 2 and table 2 and 3). PC1 explained 15 % of the variability, while PC2 and PC3 explained 12 %; PC4 did it with 9 %. The correlation coefficient was 0,9. PC2 is not shown in a figure because it does not account for any important differentiation of the groups recognized here.

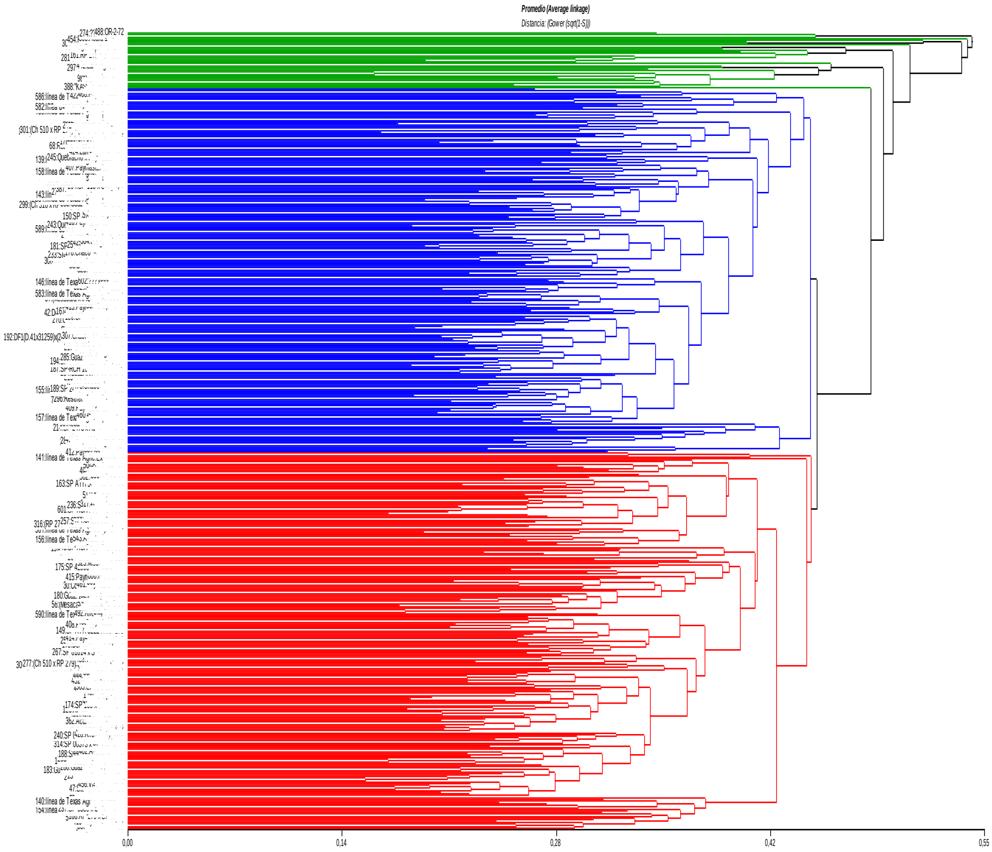

Figure 1: Dendrogram of 468 accessions of G. hirsutum L. considering 16 descriptors highlighting 3 main clusters

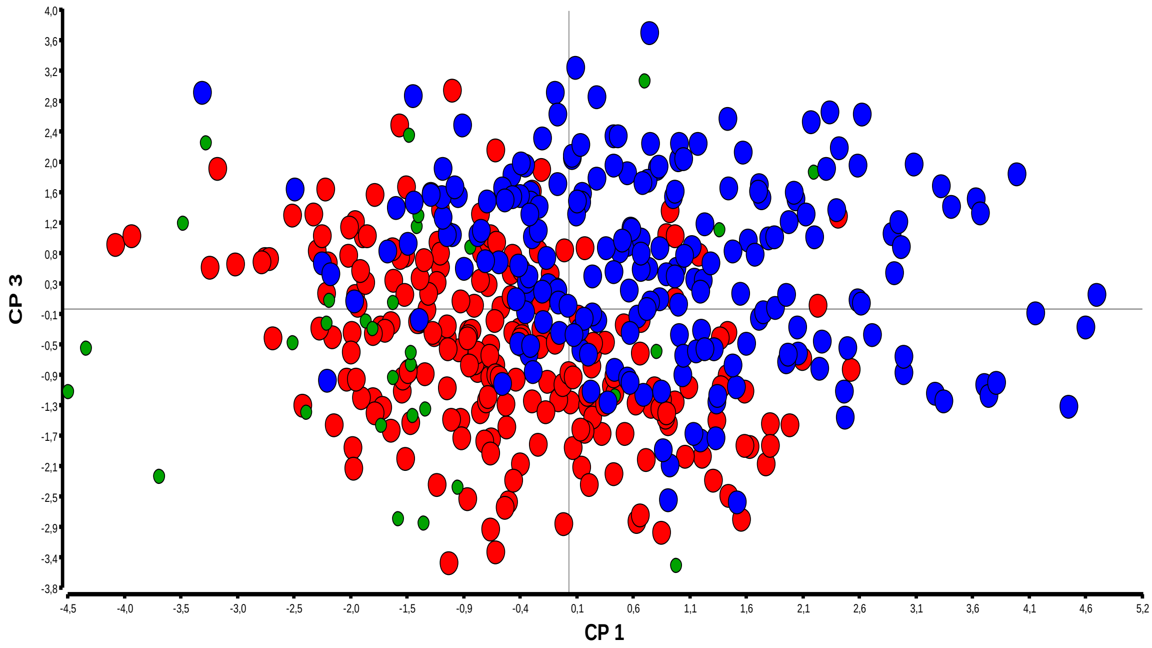

Figure 2: Principal Component Analysis of 468 accessions of G. hirsutum L. considering 16 descriptors showing PC1 and PC3 and highlighting 3 main clusters

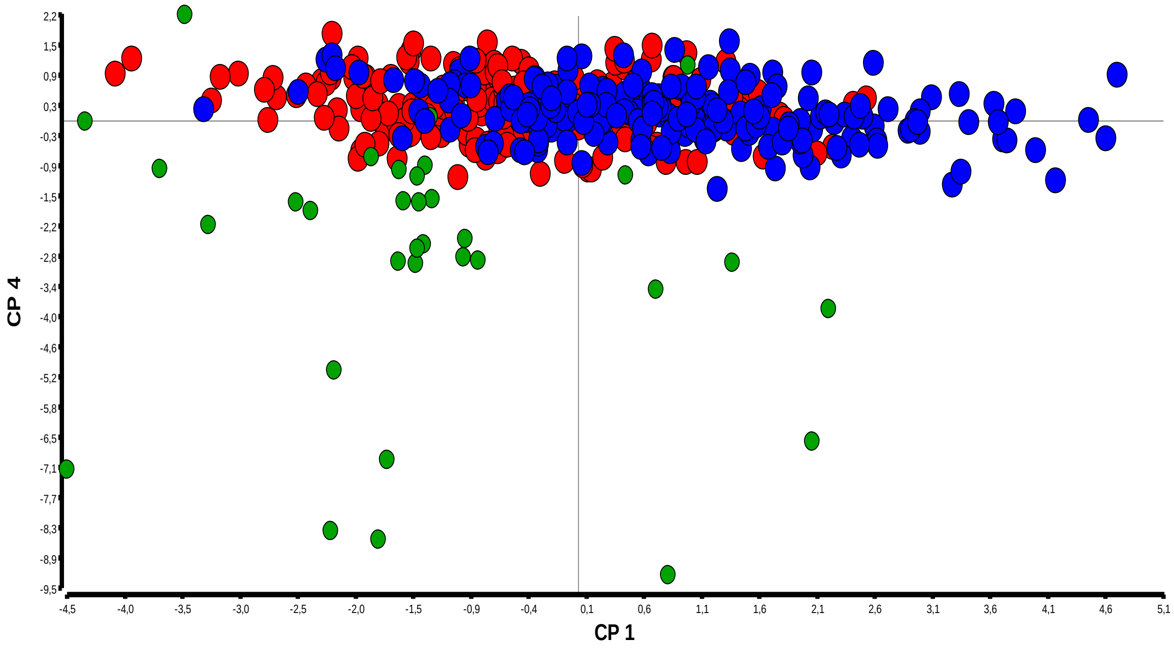

Figure 3: Principal Component Analysis of 468 accessions of G. hirsutum L. considering 16 descriptors showing PC1 and PC4 and highlighting 3 main clusters

Table 2: Descriptors names and states

Leaf morphology	**1=okra; 2=normal**
Leaf hairiness	**1=hairless; 2=low; 3=medium; 4=high**
Bract morphology	**1=frego; 2=slightly frego; 3=normal**
Boll morphology	**1=rounded; 2=rounded to elliptical; 3=rounded to cone-shaped; 4=elliptical; 5=elliptical to cone-shaped; 6=cone-shaped**
Presence/abscence of nectaries on leaves	**1=absent; 2=present**
Number of boll-carrying branches	**number**
Hight to first branch	**centimeter**
Internode length	**centimeter**
Gin out-turn	**percentage**
Seed index	**weight of 100 seeds**
Boll weight	**grams**
Fibre strength	**g/tex**
Fibre length	**milimeter**
Micronaire	**index**
Plant height	**centimeter**
Bract width	**1=narrow; 2=intermediate; 3=wide**

Table 3: Autovectors values of 5 principal components with the original descriptors weights

Autovectors					
Descriptors	PC1	PC2	PC3	PC4	PC5
Leaf morphology	0,03	0,15	-0,09	0,53	0,37
Leaf hairiness	-0,13	0,2	0,31	0,12	0,05
Bract morphology	0,08	0,13	0,04	0,63	0,25
Boll morphology	0,34	0,09	0,32	-0,01	-0,08
Presence/abscence of nectaries on leaves	0,15	0,07	0,11	0,27	-0,25
Number of boll-carrying branches	-0,02	-0,36	0,14	-0,09	0,52
Hight to first branch	0,02	0,5	-0,03	-0,17	0,12
Internode length	3,70E-03	0,62	-0,22	-0,1	-0,08
Gin out-turn	-0,38	0,07	0,16	0,07	-0,17
Seed index	0,51	-0,06	-0,28	0,02	0,06

Autovectors					
Boll weight	0,33	-0,03	-0,44	0,04	-0,07
Fibre resistance	0,36	0,13	0,42	-3,50E-04	-0,15
Fibre length	0,27	0,08	0,46	-0,12	0,02
Micronaire	-0,25	0,07	-0,09	0,24	-0,38
Plant height	-0,1	0,32	-0,03	-0,31	0,48
Bract width	0,21	-3,10E-03	-0,13	-0,14	-0,1

The group in blue had medium to big seed sizes (seed index), medium to low gin out-turn, medium to high fibre length and resistance, with medium to high leaf hairiness, and most bolls conic-shaped; while the group in red, to the contrary, had medium to small seed sizes, medium to higher gin out-turn, medium to low fibre length and resistance, medium to low leaf hairiness, and the bolls mainly round-shaped. In both main groups the origin of accessions is diverse. The smaller group in green (see figure 3) had accessions carrying distinct characters like either okra-shaped leaves or frego-shaped bracts or lack of nectaries on their leaves. Thus, a simple initial classification for the upland cotton gene bank is readily available for its use in breeding or for other purposes. Further screenings are currently being carried out to broaden the number of descriptors and with the aim of possibly establishing a more precise classification for the accessions.

EVALUATION FOR BLUE DISEASE

In figure 4 the mean percentage of plants with symptoms of Blue Disease is shown, a virus-caused disease transmitted by aphids, from two different years and at two locations in the Argentinian cotton growing area, from BGSP-1 to BGSP-601. The right-half of the graph, which were accessions from the breeding program of INTA Sáenz Peña, showed many accessions with no symptoms, in contrast with the left-half with mostly old introduced foreign varieties. It is believed that the lines are resistant due to genes introduced to them from the triple hybrid with a wild relative, the HAR germplasm, where the R stands for Gossypium raimondii. Lines high in percentage of plants with symptoms in the right-half of figure 4 probably did not acquire genes for resistance or tolerance to Blue Disease from HAR line or from other sources.

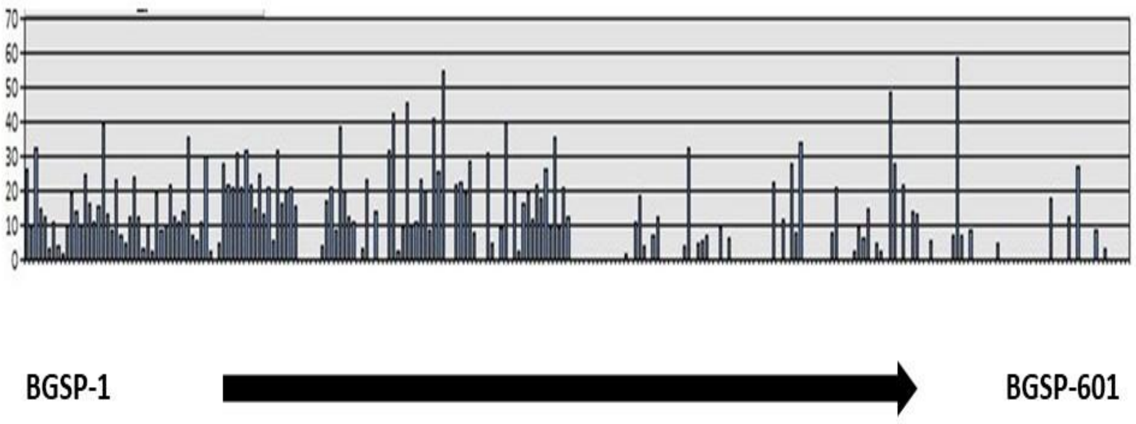

Figure 4: Percentage of plants with symptoms of Blue Disease in accessions of the cotton gene bank at INTA Sáenz Peña

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Bioindicators: A Valuable Tool for Selecting Mutagenized Cotton Varieties

Winkler, H. Martín1; Cereijo, Antonela E.1; Landau, Alejandra M.3; Scarpin, Gonzalo J.1; Dileo, Pablo N.1; Muchut, Robertino J.1; Roeschlin, Roxana A.1,2; Lorenzini, Fernando G.1,2; Paytas, Marcelo J.1

1 Instituto Nacional de Tecnología Agropecuaria (INTA) Reconquista Santa Fe. Ruta 11 Km 773 (3560). Argentina.
2 Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.
3 Instituto de Genética “Ewald A. Favret” (IGEAF) CICVyA-CNIA-INTA. Dr. Nicolás Repetto y De Los Reseros s/n (1686) Hurlingham, Buenos Aires, Argentina.
E-mail: winkler.horacio@inta.gob.ar

BACKGROUND

A high genetic variability is crucial in a plant breeding program, as it increases the chances of achieving desirable productive traits in crops. In this context, induced mutations techniques have been effectively used to obtain new variability in crop plants. Mutation breeding was initially focused on increasing the yield and quality of crops, however, today it also targets on improving adaptability and/or tolerance to environmental conditions. The induced mutation technique includes physical and/or chemical mutagenesis, followed by a selection process until a certain number of plants with the desired traits of interest are obtained to be individually evaluated. This selection process can include different approaches, ranging from molecular to physiological, morphological or productive analysis.

Biomarkers are biological indicators used to identify specific genetic, biochemical or physiological traits in plant, that are associated with desirable characteristics, such as disease resistance, tolerance to abiotic stress, productivity, or crop quality. The biomarkers can be molecular (such as genetic or DNA markers) or biochemical and are used to select plants with desirable traits without waiting for those traits to be fully expressed in the phenotype, thus accelerating the breeding process.

The cotton breeding program at INTA Reconquista includes induced mutations techniques to increase genetic variability and to generate new parental lines with improved characteristics. In this case, the selection process is specifically focused on obtaining materials with greater capacity to tolerate abiotic stresses such as drought and soil salinity. Hence, diverse biochemical and physiological biomarkers were identified, analyzed and validated as suitable for a reliable selection of plants with interesting characteristics under stress conditions.

RESULTS AND DISCUSSION

The analysis of the partial correlations between the measured variables is shown in Figure 1. As can be seen, both positive and negative correlations were found between the different variables, with varying magnitudes.

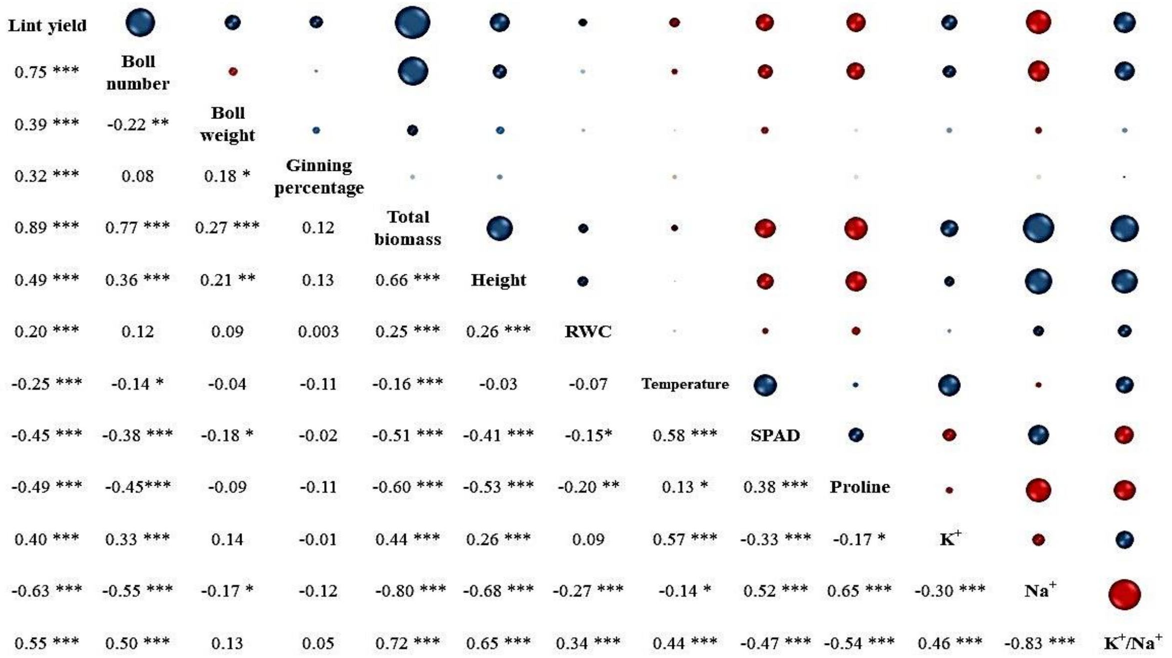

Figure 1: Partial correlations among the different morphophysiological, biochemical and agronomic measured variables. The size of the circles represents the coefficients of correlation between variables. The blue and red colors represent positive and negative correlations, respectively. The intensity of the color corresponds to the significance level. References: *<0.05, **<0.01, *<0.001.**

In order to identify the most desirable physiological traits as selection criteria, it was necessary to explore through regression analysis the associations and their magnitude between lint yields (LY) evaluated at the end of the plant cycle, based on the morphophysiological and biochemical variables measured at vegetative stages. Results show (Figure 2) that there are positive correlations between the LY obtained and total biomass, relative water content (RWC) and water potential, while this correlation was negative with SPAD values, foliar temperature and proline.

Total biomass was the variable that showed the strongest relationship with LY and the bolls number generated. On the other hand, the RWC is one of the variables that exhibit a direct and significant relationship with LY and its components. This difference in RWC in leaf samples provides a quantitative measure of their water status, which would allow for a rapid discrimination of genotypes that maintain high RWC values under water deficit stress. Regarding SPAD values, the results showed that it increased significantly under both water and salt stress conditions, and also presented significant negative correlations with LY. This indicates that an increase in the SPAD index reflects more stressed individuals, which are associated with greater decrease in LY and their components. Finally, proline could be considered an osmoprotective osmolyte and an appropriate variable to measure for selection of individuals, as significant increases in response to water deficit and high salinity have been demonstrated in cotton. However, a positive correlation between proline content and the relative tolerance or susceptibility of plants is not always found.

In this study, ions Na+ and K+ were also measured. Although they did not show a direct and significant correlation with LY, the obtained results indicate that K+ levels should be considered as a variable in the selection of individuals with better performance under water stress, while Na+ levels could be used as a variable in the identification of mutated individuals with a better response to salt stress treatment.

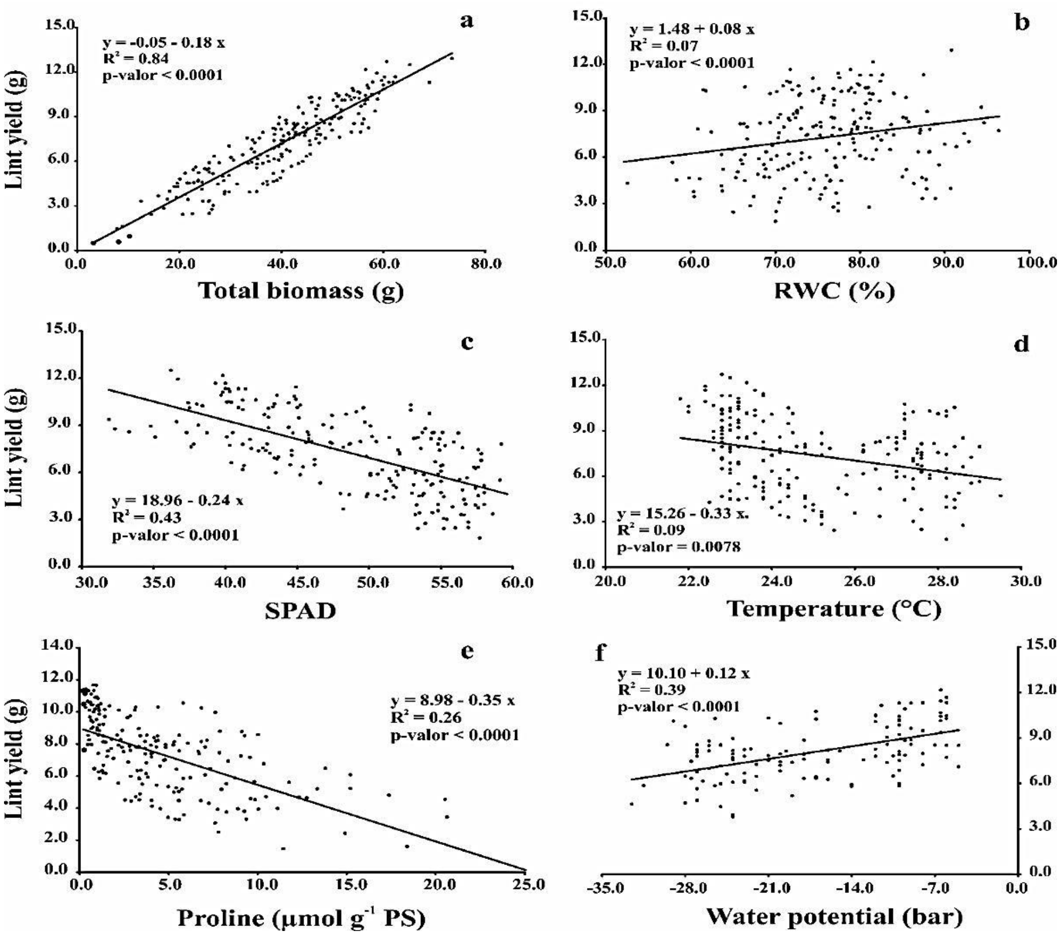

Figure 2: Linear regression analysis for the different morphophysiological and biochemical variables associated with lint yield. Relationship between lint yield and total biomass (a), relative water content (b), SPAD (c), foliar temperature (d), proline (e) and water potential (f).

CONCLUSIONS

The studies conducted allowed for the analysis of the relationship between different morphophysiological and biochemical parameters under severe stress (water or salt) at an early stage of the growing cycle, and the various yield parameters measured at the end of the cycle. Through this, suitable biomarkers were identified for the selection of mutated individuals based on their response to these types of abiotic stresses.

Enhancing Cotton for Narrow Row Management Systems

Dileo, Pablo1; Muchut, Robertino1; Cereijo, Antonela1; Lorenzini, Fernando1,2; Roeschlin, Roxana1,2; Winkler, H. Martín1; Scarpín, Gonzalo1; Acosta, Mileva1; Paytas, Marcelo1

1 Instituto Nacional de Tecnología Agropecuaria (INTA), EEA Reconquista, Santa Fe, Argentina.
2 Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET).
E-mail: dileo.pablo@inta.gob.ar

BACKGROUND

In Argentina, cotton production has considerable regional importance due to the added value it generates and the employment it provides, but it also plays an important role in foreign trade and connections to the industrial sector. During the growing season 2023/24, the cotton planted area in Argentina was about 598,000 hectares, being Santiago del Estero, Chaco and Santa Fe, the largest cotton growing provinces, located in the north of the country. Specifically, the planted area in Santa Fe province (where INTA Reconquista is located) in the season 2023/24 increased by 17% compared to the previous season, reaching 180,500 hectares.

There are various cotton production and harvesting systems worldwide. The mechanized harvesting of cotton in the world has traditionally been related to the crop design commonly used: cotton planted with a row spacing greater than 0.52 m is preferably harvested using the picker system, while narrow-row cotton (0.52 m or less spacing) is harvested using the stripper system.

In terms of proper crop management, in addition to production and harvesting system choice, genetics also plays an important role in determining the final fiber quality of harvested cotton. The optimal plant structure for the stripper harvesting system involves a compact plant with few or no vegetative branches, and with reproductive structures close to the main stem, thus creating a columnar plant structure. This type of plant allows the obtention of cleaner fiber, with fewer impurities that could decrease the final quality.

In the above-mentioned cotton production region from Argentina, farmers employ mainly the stripper harvesting system, thus the crop production management involves a narrow row distribution between plants (0.52 m row spacing).

At INTA Reconquista Santa Fe, there is a traditional breeding program for the crop, which focuses on developing cotton varieties adapted to the environmental conditions and management practices of cotton plating region of Santa Fe province. For this reason, the goal of this program is to obtain adapted varieties with improved lint yield, good fiber quality, appropriate health characteristics, and additionally, focusing the selection on plant structure and architecture. The cotton breeding program in our team began in 2019 with the first crossings and selection assisted by molecular markers.

Currently, there are advanced and selected lines that are being evaluated in different locations in the north of Santa Fe province, conducting comparative yield trials with the commercial cotton varieties available in the country. Particularly, during 2023/24 season an experiment was carried out in three different regions of Santa Fe province. In each site, 11 cotton materials were evaluated, including 4 Argentine cotton commercial cultivars and 7 advanced lines from INTA Reconquista cotton breeding program. All genotypes were shown in a narrow row spacing system (0.52 m between rows). Statistical design consisted of Completely random Blocks, with 4 replicates per genotype in each location. At the end of the cycle, different yield and fiber quality parameters were measured. Statistical analysis consisted of Lineal Mix Models with Genotypes as fixed effects and locations defined as random effects. Media comparisons were carried out using DGC test with a significance of 95%.

YIELD PARAMETERS INCREASED IN ADVANCED LINES COMPARED WITH COMMERCIAL GENOTYPES

Selected lines presented significant statistical differences in terms of Seed Cotton Yield (SCY) and Lint Percentage (LP) (p-value<0.05), which is shown in Figure 1. In average, all advanced lines evaluated presented 5.4% superior SCY values compared to the media value of SCY for the commercial varieties evaluated. In addition, the SCY average value for the advanced lines where 15.7% higher than the value of SCY obtained for the commercial variety DP 1238, which is the most popular sown cultivar in cotton regions of Argentina. In terms of LP, similar situation to SCY was present: average value for LP in advanced lines was higher (3.4%) than LP medium value in commercial varieties. And, as well as with SCY, in terms of LP, the average value for Advanced lines was 7,5% higher than the LP value estimated for NuOpal commercial variety.

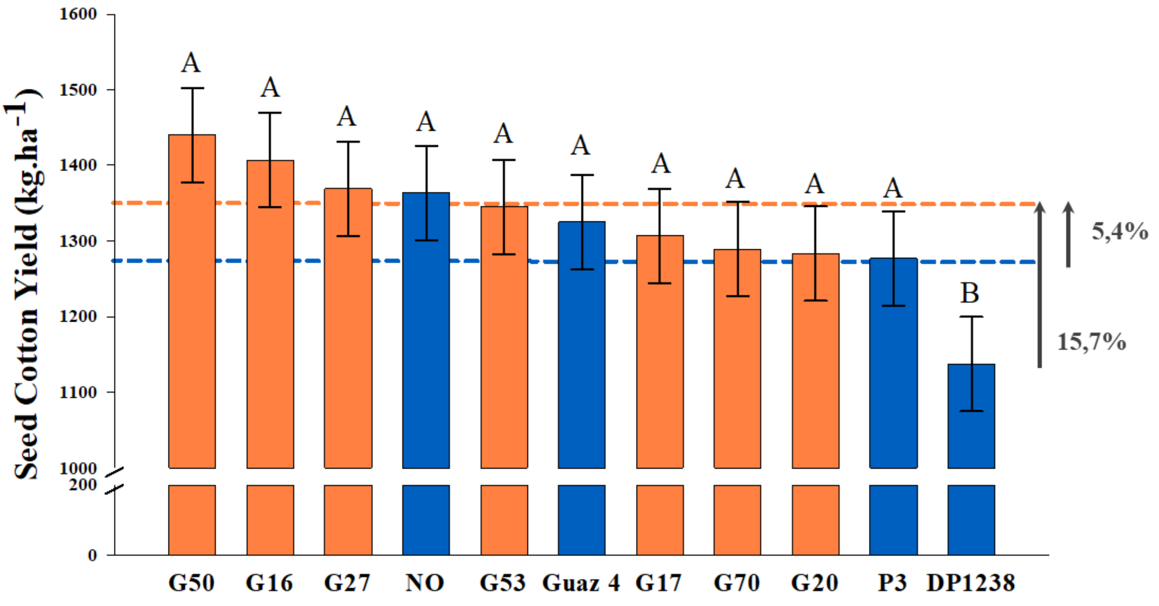

Figure 1. Seed Cotton Yield (kg.ha-1) for all genotypes evaluated. Orange bars show values for advanced lines from breeding programme, NO: NuOpal; Guaz 4: Guazuncho 4 INTA; P3: Porá 3 INTA. Blue bars show values for commercial lines. Orange doted line represents media Seed Cotton Yield for all advanced lines evaluated, Blue doted line shows media values for commercial cultivars evaluated. Different letters show significant statistical differences (p-value<0,05; DGC test).

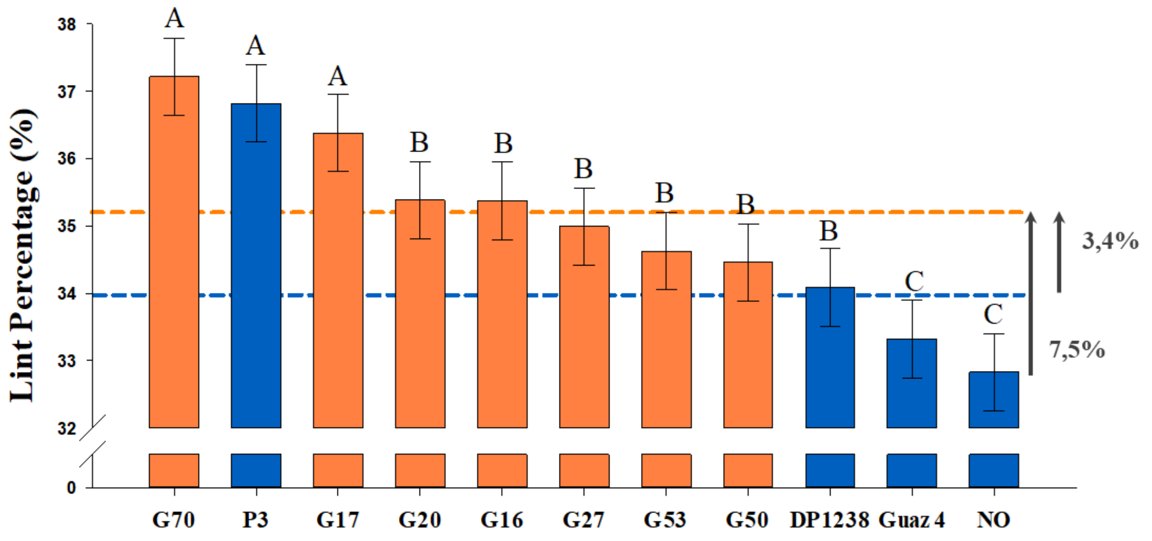

Figure 2. Lint Percentage (%) for all genotypes evaluated. Orange bars show values for advanced lines from breeding programme, NO: NuOpal; Guaz 4: Guazuncho 4 INTA; P3: Porá 3 INTA. Blue bars show values for commercial lines. Orange doted line represents media Seed Cotton Yield for all advanced lines evaluated, Blue doted line shows media values for commercial cultivars evaluated. Different letters show significant statistical differences (p-value<0,05; DGC test).

The higher values for SCY in advanced lines in comparison with commercial varieties could be associated with an increase in the number of bolls per area (NB) and the boll weight (BW). The results showed increased both in NB and BW parameters (Table 1), thus considering that advanced varieties retained or developed a higher number of bolls and with an increment in their weights, compared with current commercial varieties.

Table 1. Proportional increment in yield parameters of Boll number per area and Boll Weight when comparing advanced lines average values and commercial varieties average values.

Yield Parameter	**Increment (advanced lines vs. Commercial cultivars)**
Boll number per area (bolls.m-2)	**4.22%**
Boll Weight (g.boll-1)	**5,92%**

Increased in terms of quality in advanced lines compared with commercial cultivars of cotton

In terms of fiber quality parameters, the results showed improvements in fiber quality when comparing average values of different parameters between advanced lines and commercial varieties (Table 2).

The results indicate that breeding advancements have led to notable improvements across several key fiber quality parameters, enhancing overall cotton fiber characteristics. Below there is a summary of the observed increments in fiber quality parameters.

Table 2. Proportional increment in fiber quality parameters when comparing advanced lines and commercial varieties average values.

Fiber Quality Parameter	**Increment (advanced lines vs. Commercial cultivars)**
Spinning Consistency Index	3.07%
Micronaire	4.65%
Maturity	4.54%
UHML (Upper High Medium Length) (mm)	3.15%
UI (Uniformity Index)	0.65%
SFI (Short Fiber Index)	-8.17%
Strength (g.tex-1)	3.31%

COMPACT AND COLUMNAR STRUCTURE AS DESIRABLE CHARACTERISTICS IN PLANTS FOR NARROW ROW SYSTEMS

As previously mentioned, not only yield and quality are the desired characteristics on the selected lines, but also plant structure. As shown on Figure 3, the advanced lines evaluated in the present work exhibit a low number of vegetative branches, with reproductive structures mainly fixed at first positions in reproductive branches, and close to the main stem, generating the characteristically columnar structure.

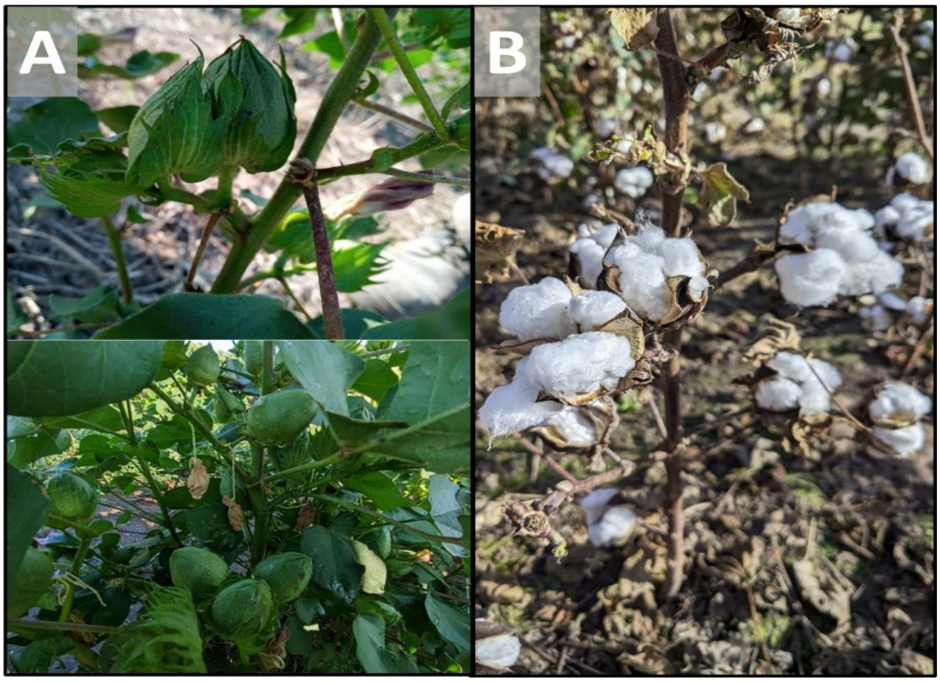

Figure 3. Advanced lines of INTA Reconquista cotton breeding program showing architectural desirable characteristics: bolls retained in the first positions and close to the main stem of plants, demonstrating columnar like structure. A: green bolls during maturation stage. B: cotton plant ready for harvest with mature bolls.

Until now, the results of the cotton breeding program at INTA Reconquista show favorable outcomes related to the generation of new materials adapted to the environmental conditions and management systems of the cotton-growing regions of the country, with an emphasis on Santa Fe province. This will provide new cotton varieties to the national market, as well as to the rest of the world that requires them.

Advances and Future Challenges in the Use of Molecular Markers Applied to Cotton Cultivation

Klein, Lorena; Tcach, Nydia; González, Ariela; García, Carolina; Spoljaric, Mónica
Instituto Nacional de Tecnología Agropecuaria (INTA) – EEA Sáenz Peña, Chaco. Argentina.
E-mail: klein.lorena@inta.gob.ar

INTRODUCTION

Genus Gossypium includes approximately 45 diploid species (2n = 2× = 26) and five tetraploid species (2n = 4× = 52). Currently, two new allotepraploid species have been described: G. ekmanianum Wittmack (Grover et al. 2015) and G. stephensii (Gallagher et al. 2017; Chen et al. 2020). Cultivated cotton is considered the most important natural fiber worldwide. Its use throughout history has its origins in the domestication and manipulation of four different cotton species with "spinnable" fibers. These species are represented by two diploids G. herbaceum L. (A1) and G. arboreum L. (A2), which are found naturally in Asia and Africa, and two allotetraploids G. barbadense L. (AD2), "Pima or Egyptian" cotton and G. hirsutum L. (AD1) or Upland cotton whose center of origin is in Central America.

The domestication processes of the latter two species began in approximately 2500 BC with the first agricultural societies in South and North America. With the appearance of the cotton gin in 1793 and the advent of cheaper industrial dyes, G. hirsutum became more relevant for its agronomic and industrial characteristics and became the most popular cotton variety due to its hardiness, which allowed higher production at a lower cost (Street, 1957), a trend that continues today with more than 90% adoption in most cotton producing countries. On the other hand, G. barbadense represents between 3 and 5% of total world production (Fang, 2018).

The continuous conventional improvement processes of Upland cotton, focused specifically on commercially important traits, allowed obtaining better varieties with higher productivity. However, from the 1990s onwards, a clear stagnation in yield was demonstrated (Mendoza, 2000), associated, according to various authors, with low genetic diversity due to intensive artificial selection (Abdalla et al., 2001; Klein, et al. 2022; Wendel et al., 1992).

In addition, the constant fluctuations in climatic factors over the last 50 years have led to a greater exposure of the crop to various stresses (biotic and abiotic), which translates into significant losses. In cotton cultivation, it is estimated that these losses may have a highly negative impact in the future, associated with increased drought and heat (Vinocur & Altman, 2005). In this sense, several authors establish that the growth and productivity of cotton (G. hirsutum L.) decreases by up to 50% in the event of water stress, depending on its duration and intensity (Brubaker & Wendel, 1994; Brubaker et al., 1999; Boman & Lemon, 2006; Edmisten et al., 2007), as well as reducing genetic variability, i.e. the expression of the phenotype (Spoljaric et al., 2018b). Furthermore, biotic stress caused by pests and diseases contributes to annual losses worldwide of between 10 and 30%, as well as a significant increase in global production costs for their control (Tarazi et al., 2019).

In Argentina, cotton is a key regional crop in the north of the country. It extends over a wide agro-ecological zone between 25° and 31° south latitude and includes the provinces of Catamarca, Chaco, Córdoba, Corrientes, Entre Ríos, Formosa; Salta, Santa Fe and Santiago del Estero (Mondino, 2006). According to data provided by the National Secretary of Agriculture, approximately 600 thousand hectares were planted in the last 23/24 season. The limitations and problems of the crop are in line with global problems.

In this context, advances in modern biotechnology, especially molecular biology techniques, have become powerful tools within genetic improvement programs to address all these limitations, allowing for example the identification of cultivars and species, the establishment of evolutionary relationships between different groups of plants, the evaluation of genetic variability between populations, genetic mapping and assisted selection (Ahmar et al., 2020).

The Biotechnology Laboratory of INTA Sáenz Peña works on different lines of research to obtain varieties with different characteristics, where the Germplasm Bank represents the genetic basis of the improvement program. To do this, genetic materials are conserved and molecularly evaluated to determine the available variability, as well as the productive and/or adaptive characteristics of agronomic interest.

BIOTECHNOLOGY: STUDIES WITH THE MOLECULAR MARKERS TECHNIQUE.

The loss of genetic diversity due to extensive manipulation of cultivated species has created a limitation in cotton, so the search for variability is essential to ensure the continuity of improvement programs. INTA Sáenz Peña has a germplasm bank where more than 700 Gossypium spp. accessions (improved, obsolete, wild, landrace, among others) are conserved, originating from various countries through the exchange of materials. In this context, to find new alleles useful for the improvement program, genetic variability was studied in 18 accessions from three different origins (United States of America, People's Republic of China and Argentina). The results showed a grouping by geographic origin, indicating the local adaptation of the cotton varieties exerted by selection pressure, and finding at the molecular level, greater genetic distances between the germplasm of geographic origin from Argentina and that from China, which is of interest to evaluate a possible hybrid vigor in production traits (Klein et al. 2022).

On the other hand, genetic introgression is one of the evolutionary processes that generate heritable variation in domesticated populations. However, despite its great evolutionary, ecological and economic importance, genetic introgression between domesticated species and their wild relatives has been little studied (Martínez Castillo & Dzul Tejero, 2017). Therefore, work is currently being done on collecting germplasm to evaluate the genetic variability and possible introgression between G. barbadence and G. hirsutum.

Molecular markers allow not only an exhaustive study in search of variability, but also to identify lines and varieties that are better adapted to a given environment in order to minimize the negative effects of exposure to prolonged stress on the plant. Therefore, a total of 15 microsatellite markers (SSR) associated with high temperature stress genes are being evaluated in 21 cotton accessions belonging to the germplasm bank, with the aim of their validation and subsequent use as assisted selection markers (MAS) for the identification of varieties or lines with tolerance to high temperature stress.

On the other hand, it is very important to adopt new technologies to generate varieties with agronomically interesting characteristics that are not present in cotton and that allow adaptation to new environmental conditions, reducing the use of agrochemicals and insecticides and minimizing losses due to pest and/or disease damage.

The access of boll weevil (Anthonomus grandis) into Argentina in 1993 caused enormous losses in the cotton region, causing a negative impact not only economically, but also socially and environmentally. Economically, due to the decrease in yield and quality; socially, due to the abandonment of cotton activity by small producers, family agriculture and other productive strata; and environmentally, since it involved greater use of agrochemicals, according to the Plant Health Committee of the Southern Cone (COSAVE, 2011). In response to this problem, through a Technological Linkage Agreement between INTA and the cotton-producing provinces (Chaco, Formosa, Santa Fe and Santiago del Estero), the first transgenic plants with tolerance to the cotton boll weevil (Anthonomus grandis) were obtained (Lewi et al., 2019). In this sense, using Molecular Marker techniques, the generations of genetically transformed plants (genotype Coker 312), which are planted in Biosafety greenhouses and in field trials, are monitored. The objective is to evaluate the genotypes by using a specific molecular marker (alpha amylase), making it possible to discard segregating plants and minimizing selection times for those with a greater probability of maintaining the gene of interest stabilized in the genome (Figura1). Likewise, knowing and characterizing the weevil populations existing in the main cotton provinces allows for better future decision making when adjusting Integrated Pest Management tactics. For this, in collaboration with the Entomology laboratory of INTA Sáenz Peña, in the framework of the doctoral thesis: Characterization of the genetic variability of the populations of the cotton boll weevil Anthonomus grandis Boheman (Coleoptera: Curculionidae) present in the main cotton region of Argentina (CONICET-INTA), the technique of genomic DNA extraction in adult weevil insects was developed, using the Ctab method, in order to be able to carry out a genomic analysis of the weevil populations in the main cotton region of Argentina. In this same context, and due to the recent damage caused by pink caterpillar larvae (Pectinophora gossypiella Saunders) in some cotton-producing areas under irrigation of the Río Dulce in Santiago del Estero (Argentina), the question arose about the possible development of resistance to the Bt proteins possessed by most of the commercial cultivars currently used in Argentina (Mondino, 2024).

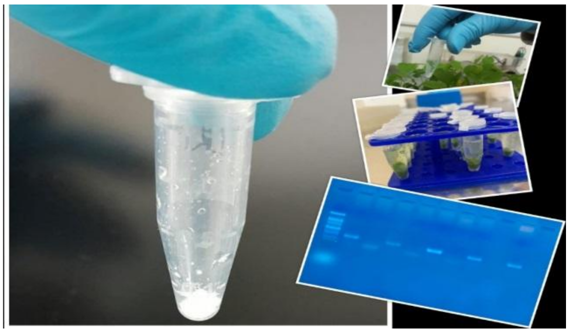

Figure 1: DNA extraction and agarose gel electrophoresis of amplification products of the specific alpha amylase marker.

Due to the high impact in terms of economic losses generated by this pest, in collaboration with the Entomology laboratory, the DNA extraction protocol was successfully tested (using the Ctab method) from larvae found in cotton reproductive structures present in the province of Santiago del Estero. The samples obtained, were sent to the laboratory of the Instituto de Microbiología y Zoología Agrícola CICVyA, CNIA, INTA Castelar for subsequent analysis using molecular markers seeking to detect resistance genes in insect populations.

There are enormous challenges ahead for breeding to break the stagnation in performance and allow for advances in line with current changing times to provide rapid responses to present and future demands. Markers are an essential tool to optimize breeding programs and crop adaptability to different productive ecosystems.

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Prospecting for *Gossypium barbadense* L. in Northern Argentina: Background and Current Status

Spoljaric Mónica, Goytia Yanina, Klein Lorena, González Ariela,
Vallejos Claudio Tcach, Nydia, Dileo Pablo Tcach, Mauricio, Royo Olegario

Instituto Nacional de Tecnología Agropecuaria (INTA). EEA Sáenz Peña, EEA Reconquista. Argentina.
E-mail: spoljaric.monica@inta.gob.ar

INTRODUCTION

Conservation of genetic resources is a strategy used by most countries to ensure the current and potential availability of seeds. Cotton genetic resources are the basis for the development of more productive and resilient varieties in the face of adverse environmental stresses and the emergence of diseases and insect pests. The genetic base is enriched with the acquisition of germplasm through prospecting, collecting and exchange, ranging from wild relatives of crops, native, local and Creole plant genetic resources, and varieties obtained by plant breeding (Atencio et al., 2023). The concept of prospecting refers to the process of searching and evaluating background information on the presence of the species in the region of interest.

In Argentina, cotton species Gossypium hirsutum L. has been cultivated for a century in the northern part of Argentina as the traditional productive area. Gossypium barbadense L. was excluded, as in many countries, because it was less productive than G. hirsutum L. However, their southernmost distribution has been reported, which extends in the Northwest and Northeast, possibly coming from Bolivia and Paraguay where they entered with Spanish colonization and migratory movements. The reported materials were identified in a state of wild conservation and in some cases natural (Carnevali, 1970).

The use of non-elite germplasm for genetic improvement has been increasing in recent years, partly because the genetic base of G. hirsutum L. is increasingly limited to provide novel alleles and, on the other hand, with the advent of biotechnology the use of molecular markers and sequence polymorphisms facilitate various types of breeding-assisted selection (Hinze et. al, 2017). In this sense, G. barbadense L. is recognized for its fiber quality attributes, particularly in improved extra-long fiber cultivars such as Sea Island from the Antilles and the United States, coarse fiber Tangüis from Peru, and commercial cottons from Egypt and Sudan. Although wild and naturalized species, compared to improved ones, do not present the same technological properties of fibers, they do have the potential to develop genotypes with superior fiber quality. On the other hand, the importance of conservation and improvement is not only for its fiber but also for its attributes of local adaptation to adverse environmental contexts of abiotic and biotic nature that can mitigate disease conditions and emerging insect pests. However, it is necessary to know the presence of the species in the region of interest, investigate its past history, as well as its current history. The objective of this report is to describe the surveys currently being carried out and in the past in G. barbadense L. to identify the presence of the species in the region.

BACKGROUND AND CURRENT STATUS

The oldest reports date back between the years 1882 and 1885, when the Argentine army carried out various expeditions in the construction of a new plan of the Chaco Territories, for which in their itinerary they recorded the presence of cotton south of the Pilcomayo River (between the 60° and 62° meridians). These were named “Indigenous Gossypium”, being their state of conservation as wild growing in the heights surrounding water bodies in the most humid and permeable areas of the current province of Formosa (61 1/2 degrees of longitude and 23" 38' south latitude). Cotton plants with heights similar to trees and good boll production were also identified on the eastern margins of the Bermejo, Paraje Dragones and Lavalle area. (Bladrich, 1889) (Figure 1).

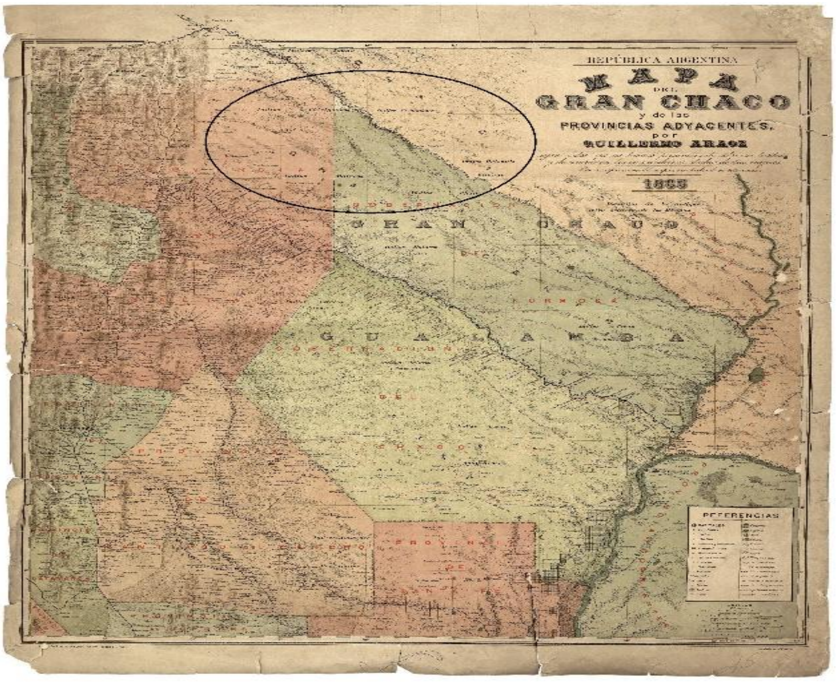

Figure 1. Demarcation of the first recorded survey (1882-1885) on a historical map of the area.

Subsequently, (between 1936 and 1937) research expeditions were carried out in the Northwest of the province of Formosa in the Ramon Lista department that borders Paraguay to the north (Figure 2). Wild cotton with free seeds, typical in G. barbadense L., and kidney-seeded cotton, known as G. barbadense var. brasiliensis. In addition to these characteristics, seeds were found with linter, intense fawn-colored cotton, spotted fawn and white. At the time of carrying out the fiber analysis, samples were 25 years old, however, interesting results were obtained, for example G. barbadense var. brasiliensis had a fiber length of 26 mm of white color, followed by the same species, but of spotted fawn color (23-24 mm) and shorter length (between 20 and 21 mm) presented by the other samples collected (Gutiérrez at al., 1960).

The last prospecting record carried out by INTA dates back to between 1960 and 1963 (Figure 2). The members of the cotton team made several exploratory and collecting trips in the provinces of Catamarca, La Rioja, Salta, Formosa and Corrientes as well as the neighboring countries of Bolivia and Paraguay; however, in this report we will only review the activities carried out in the Argentina. The provinces with the highest number of specimens collected were Corrientes and Salta, followed by Formosa, Catamarca, and La Rioja. Typical forms of G. barbadense and G. barbadense var brasiliensis were found with white and fawn-colored fiber; free seeds without linter, welded kidney-shaped seeds. In relation to the leaves, flowers and capsules, morphology was identified as shown in Figure 3, F. The collected specimens were found on the banks of the rivers and near the houses (Figure 3, D) (Gutiérrez at al., 1960). Finally, a collection of 150 accessions was formed, where many of the materials presented problems in obtaining viable flowers since they were sensitive to the photoperiod, so this variability was lost, remaining as historical passport data of the INTA germplasm bank. (Arriel et al., 2022).

INTA's cotton team resumes prospecting and collecting activities in northern Argentina after 60 years. The search for records of surveys and collections began in 2019. From this compilation, the exploration sites were identified, and a communication strategy was carried out using the INTA channels for the identification of the species in specific places. This strategy and exploratory trips were documented in a video about the testimonies of the people who conserve the resource (https://www.youtube.com/watch?v=td8nDwgbV_M).

The presence of the species was identified in the provinces of Chaco, Santa Fe, Corrientes, La Rioja and Jujuy. The provinces of Formosa and Salta will be explored in the coming years. In relation to Catamarca, the presence of the species cannot be identified through the strategies proposed. To date, a collection of 50 specimens has been formed with characteristics typical of G. barbadense and G. barbadense var. brasiliensis (Figure 3 A), the majority being of geographical origin from Chaco and Corrientes. The conservation of the species in the prospected territories is with human intervention, that is, with a degree of dependence for survival because they are part of their gardens. To date, the collection formed is investigated to determine transgenic introgression according to González et al. (2020); and DNA extraction activities among other previously reported activities (Spoljaric et al., 2022). The conservation of the collected specimens is in vivo in a greenhouse under semi-controlled conditions and in a cold chamber where the seeds are kept in a trilaminated aluminum envelope under controlled temperature conditions (0-5°C).

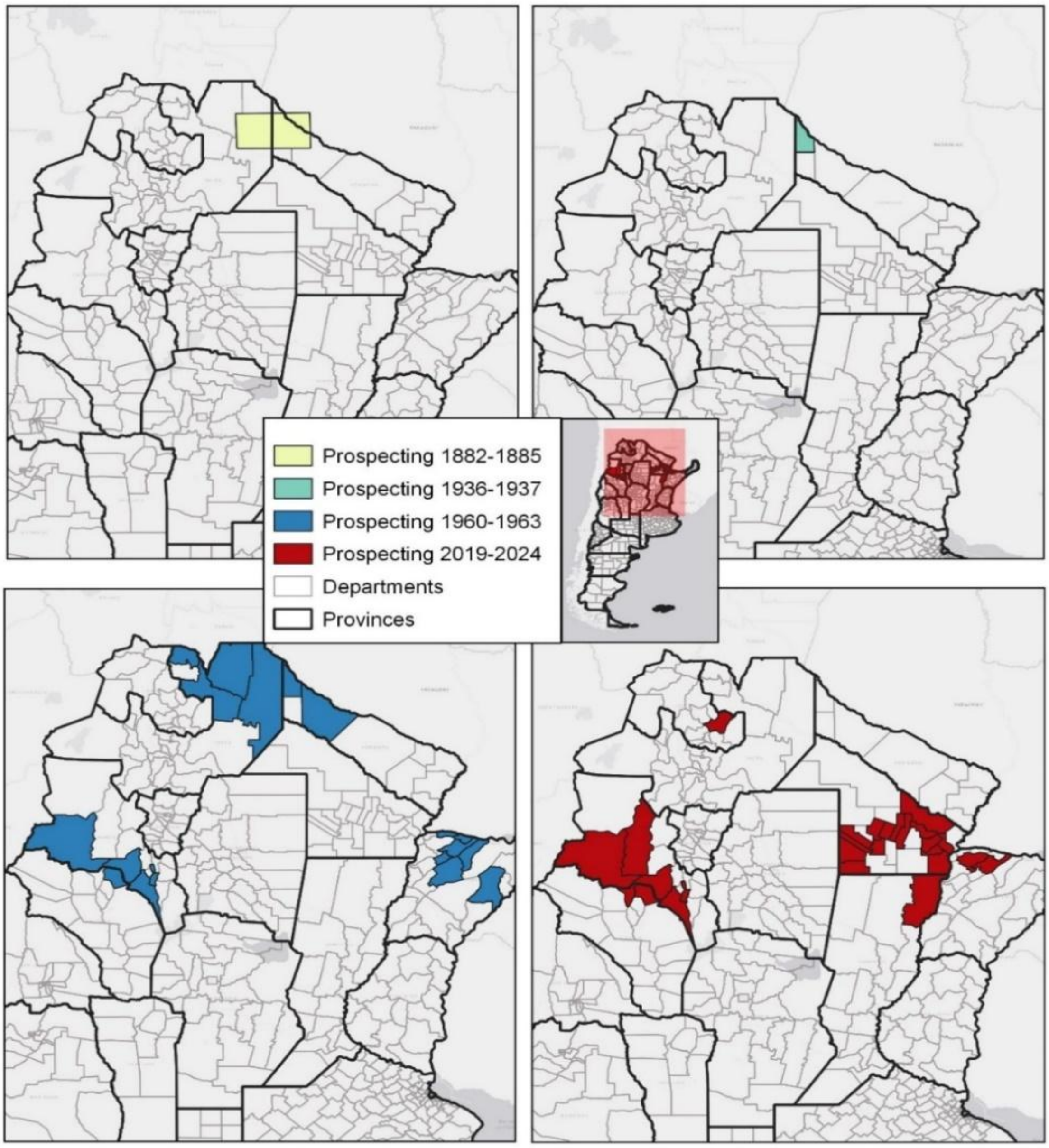

Figure 2. Geographic distribution of prospecting areas according to prospecting periods and cotton germplasm collections

Figure 3. (A) Flower collection period 2019-2024. (B) Cotton plant in the city, period 2019-2024 (C) Leaves and flower, period 2019-2024 (D) INTA technician and lost cotton plant, 1960-1963 (E) INTA technician and lost cotton plants, 1960-1963 (F) Herbarium, period 1960-1963.

LEGISLATION ON GENETIC RESOURCES

Prospecting and collecting activities in the past did not have international regulations on genetic resources. However, Argentina is currently a signatory to the Convention on Biological Diversity (CBD) (UN, 1992), the Nagoya Protocol and the International Treaty on Plant Genetic Resources for Food and Agriculture (TIRFAA).). The CBD was ratified by National Law 24,375 in 1994, the ITPGRFA was ratified by National Law 27,182 in 2016 and the Nagoya Protocol was ratified by National Law 27,246 in 2017. Also, in the National Constitution it delegates to the Provincial state the original domain of its natural resources. Its article 41 establishes "it is up to the Nation to dictate minimum budgetary standards for protection and to the provinces to complement them"; also, Art. 124 says, "the original domain of the natural resources existing in its territory corresponds to the provinces." For this reason, each province has different instruments to guarantee the conservation and access to its genetic resources. In this sense, a prospecting and collecting activity is the presentation of documentation to the provinces to comply with the legal instruments on access and distribution of benefits of genetic resources.

FINAL CONSIDERATION

The new survey stage allowed us to form a conceptual framework following the surveys of previous stages as a baseline to partially understand the geographical distribution and conservation status of the specimens identified in northern Argentina. The conservation of the species is mostly found in the private gardens in urban areas of people who experience feelings or emotions of nostalgia linked to cotton. The largest number of specimens identified was in the province of Chaco, followed by the province of Corrientes. In relation to the characteristics of the species, typical forms of G. barbadense, G. barbadense var. brasiliensis and segregating phenotypes (G. barbadense x G. hirsutum) were found; however, no specimens of fawn-coloured fibre could be found, that is, the brown colour identified in past collections. As a future perspective, prospecting and collection permits will continue to be requested from the missing provinces to cover the map and obtain an updated geographical distribution. The collection formed will be studied at a morphological and molecular level to identify alleles of importance for genetic improvement and conservation of variability.

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