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Chapter 6

# Cotton Germplasm of Pakistan

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Additional information is available at the end of the chapter

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## 1. Introduction

The economy of Pakistan relies heavily on cotton, which contributes ~60% of total foreign exchange earnings (US$ 15 billion in 2012/13). Cotton is grown on about three million hectares annually with average lint production of 670 kg $ha^{-1}$. Historically the cultivation of cotton can be traced back to 6000 BC with *Gossypium arboreum* L. identified in the ancient remains of Monjadharo (Sindh) [1]. The indigenous cultivated cotton is locally known as Desi cotton, which carries the A-genome [2-3]. Following the industrial revolution in the textile sector, the tetraploid *Gossypium hirsutum* L. gradually replaced *G. arboreum* L., because it generally produces a higher quality lint and has a higher seed cotton yield (SCY) in the Indo-Pak region. These American types originated from New Orleans and Georgia were first introduced in 1818 [4]. This material was primarily a mixture and did not attract the interest of farmers in its initial years of cultivation because of high susceptibility to sucking insects, particularly jassids (*Amarasca devastans* Dist.). Organized selection procedures were adopted to select genotypes suited to the local conditions that laid a concrete foundation for breeding material on the sub-continent.

The four cultivated cotton species can be easily identified based on variations in plant growth habit, leaf shape, boll, flower, seed and fiber features [2-3, 5]. Substantial differences between *G. herbaceum* L. and *G. arboreum* L. have been found based on genetic, cytogenetic, isozyme and genomic data. The two species are easily crossable to produce $F_1$ hybrids that are fertile and vigorous with high pollen fertility (60%). However, in common with other crops species, genetic incompatibility depresses seed viability and affects plant morphology in segregating generations. Consequently resulting plants resemble one of the parents. One reciprocal chromosomal translocation differentiates the two species [6-8]. Recently eight and 13 unique polymorphic loci of *G. arboreum* L. and *G. herbaceum* L., respectively, have been reported [9].

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DNA markers, such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and simple sequence repeat/microsatellite (SSR), have also been utilized to provide genomic polymorphic markers which can distinguish most cotton species [10-12].

Breeders, geneticists, cytogeneticists and biotechnologists have made substantial contribution for the improvement of cotton germplasm conferring resistance and/or tolerance to various stresses including biotic and abiotic, through bridging conventional and genomic tools [13]. Breeding for earliness and photoperiod insensitivity has also been accomplished by introgressing genes from the alien cotton species, which paved the way for not only sustaining cotton production but also provided enough window for cultivating another crop like wheat on the same land, thus laying down a foundation for addressing food security concerns in Pakistan.

## 2. Germplasm history

When *G. arboreum* L. was first domesticated is unclear but it is believed to have occurred in the Indus valley [14]. The indigenous cotton cultivated in Pakistan is *G. arboreum* L. that evolved from the primitive *G. herbaceum* L. In total, six distinct races of *G. arboreum* L. have been reported; "indicum"—primitive perennial form found in Western India, "burmanicum"—North Eastern India and Myanmar, "soudanense"—evolved in Egypt, Sudan and North Africa, "sinense"—evolved in southern part of China, "bengalense"—developed in Northern part of India and "cernuum"—evolved in the Assam and Chittagang hills of India and Bangladesh.

The annual types belonging to the race "cernuum" evolved independently. The cultivars that are belonging to this race are considered a useful genetic resource for producing big bolls, which are cultivated in the Gharo hills [14-16].The cultivated desi cotton belongs to *G. arboreum* L. "bengalense" in Pakistan and to *G. arboreum* L."cernuum" in Bangladesh.

*G. arboreum* L. genotypes/cultivars have been characterized at length based on morphological, physiological and agronomical features which suggest that this species can tolerate drought, and resist diseases and insect pests (such as bollworms and aphids). These features allow the *G. arboreum* L. types to adapt to dry and marginal lands [13, 17-19].

### 2.1. Evolution of *G. arboreum* L. in Pakistan

Historically, farming community of Pakistan has been cultivating *G. arboreum* L. largely on drought prone areas till 1920s which was gradually replaced by the introduction of high yielding Upland cotton varieties. For the last two decades, less than 2% of the total cultivated area of cotton is under *G. arboreum* L. types, which is expected to further decline with the passage of time.

Most cotton varieties/germplasm of *G. arboreum* L. in Pakistan has been bred by selecting variants—resulted due to limited cross pollination or mixing of seeds [20]. Consequently, it

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resulted in narrow genetic base of the cultivars/genotypes developed by selecting from a single population. The study conducted on 30 *G. arboreum* L. genotypes, largely originated in Pakistan demonstrated a narrow genetic base [20-21]. In this study, two major cultivars Ravi and FDH-228, showed 90.1% genetic similarity in RAPD assay [20]. It has been demonstrated that the narrow genetic base, like many other cultivated crop species, can impede the future breeding progress [13].

### 2.2. Breeding history of desi cotton cultivars in Pakistan

The initial breeding program for developing high yielding varieties involved selection from the available mixture of various *G. arboreum* L. types. Two cotton varieties Z. Mollisoni and 278-Mollisoni were developed through selections which gradually replaced the old types. The first cotton research station was established at Lyallpur (currently Faisalabad, located in Pakistan), and breeding for developing improved types by making selections from the available cotton varieties/genotypes was initiated by Mr. T. Trought and later continued by Mr. M. Afzal. In 1927, 15-Mollisone cotton line was tested in national trials which was approved for cultivation in 1930 on account of its high ginning outturn (GOT) 35% compared to 34% for "Mollisoni" and 33% for the mixture cultivated in the farmer's field. Another variety 39-Mollisoni exhibited 36-37% GOT versus 35% for 15-Mollisoni. The highest wrap count 8'S was spun by the lint produced of the varieties 39-Mollisoni and 15-Mollisoni (Table 1).

In 1935, efforts for development of elite desi cotton types from the historically cultivated mixture of *G. arboreum* L. biotypes known as "Multani Kapas" for the South West of Punjab—Multan region [22], were initiated through selection. A high yielding variety 119-Sanguineum (119-S), developed in 1936 and approved for cultivation in 1941, demonstrated relatively higher GOT 36.4% compared to 34% of the mixture of various biotypes. Another candidate line 231-R, bred at Hansi Research Center under the administrative umbrella of Cotton Section Lyallpur, was tested in various trials. Testing continued after 1947, and 231-R was ultimately approved for general cultivation in 1959 [23].

The Cotton Research Institute (CRI), Faisalabad carried out breeding for desi cotton at two research stations. Haroonabad was a drought prone area and the major cash crop of this region was desi cotton (60,712 hectares in the early 1950s). Breeding efforts at the Cotton Research Station Haroonabad started in 1952. Four candidate lines were identified based on leaf morphology (broad or narrow) and flower color (white or yellow). One of the varieties, 73/3, showed a higher GOT (42%) with staple length of 13.7 mm compared to a 37-38% GOT and 16-19 mm staple length of the already cultivated mixture. However, the newly developed varieties could match the yield of the already cultivated mixture of desi cotton. Thus breeding efforts, through selection, were abandoned.

The hybridization work at the Cotton Research Station, Faisalabad, started in 1930 to improve fiber quality, especially the staple length, of the existing cultivated desi cotton varieties. Wide crosses were made between 39-Mollisoni and the Chinese variety Million Dollar, resulting in improved strains (called Jubilee strains-D.C.17, D.C.26, D.C.37, D.C.40 and D.C.41). They had improved staple length and a higher GOT but with a lower yield potential over the control variety Mollisoni. Among these, D.C.40 showed improved quality features (staple length=20.3

| Serial # | Name of variety | Center of release | Year of release | Pedigree/parentage | GOT %¥ | Staple length mm | Fineness µg/inch | Strength tppsi€ |
|---|---|---|---|---|---|---|---|---|
| 1 | S.N.R. | ARI, Tandojam | 1926 (Sindh) | Selection from Sindh desi types | 39.0 | 17.5 | 7.8 | NK |
| 2 | 15-M | AARI,Faisalabad | 1930 | Selection from Local Desi | 35.0 | 17.5 | 8.0 | NK |
| 3 | 39-M | AARI,Faisalabad | 1934 | Selection from local Desi mixture | 36.0 | 17.5 | 8.5 | NK |
| 4 | 119-S | CRS, Multan | 1941 | Selection from local Desi mixture called Multani Kapas | 35.5 | 17.5 | 8.4 | NK |
| 5 | 231-R | AARI, Faisalabad | 1959 | Selection from 39-Mollisoni | 40.0 | 15.9 | 8.4 | NK |
| 6 | TD-1 | ARI, Tandojam | 1963 (Sindh) | Selection from S.N.R. | 41.0 | 15.9 | 9.4 | 80.0 |
| 7 | D-9 | AARI, Faisalabad | 1970 | Bahawalpur Desi – Selection | 41.0 | 14.5 | 8.2 | 80.0 |
| 8 | SKD-10/19 | CRI, Sakrand | 1975 | S.N.R.(single plant selection from S.N.R. G-IV bulked G-4/ NRPT 10 & 19) | 40.6 | 15.5 | 10.1 | 80.0 |
| 9 | Ravi | CRI, AARI, Faisalabad | 1982 | 465 D-selection | 40.3 | 14.9 | 8.0 | 80.0 |
| 10 | Rohi | CRS, Bahawalpur | 1986 | Haroonabad Local x D- 9 | 39.0 | 15.9 | 8.0 | 80.0 |
| 11 | FDH-170 | CRI, Faisalabad | 1995 | D-9 x TD-1 | 40.3 | 14.1 | 8.4 | 80.0 |
| 12 | FDH-228 | CRI,AARI, Faisalabad | 2002 | TD-1 x (Commila x FDH-170) | 43.5 | 13.9 | 7.3 | NK |

**NK:** Not known; ¥=Ginning out turn percentage;**€**=Thousand pounds per square inch

**Source:** Dr. Akhlaq Hussain, Description of cotton varieties of Pakistan 2004 and Cotton Research and development Memoranda till 60's (Ed. Dr. Mahbub Ali).

**Table 1.** List of approved *G. arboretum* L. (desi cotton) varieties

mm; highest wrap count=25 and GOT=38.5%) [24]. Efforts to improve staple length of the existing desi varieties continued by crossing one of the Jubilee strains with *G. anomalum* Wawr. and Peyr. that was introduced from Nigeria. Multiple strains were developed using back-crossing followed by selecting plants with improved fertility. These strains were tested in various yield trials in 1944 and demonstrated a substantial improvement in staple length (21.1 to 22.4 mm) particularly for D.C.94 (staple length=22.35 mm, GOT=38.2% and yield per acre=552 kg). These strains have the potential to compete with *G. hirsutum* L. var 4-F for fiber length. However, none of the strains found favor with the farming community. Interspecific crosses were made at Multan between *G. arboreum* L. and *G. thurberi* Tod. followed by two backcrosses with *G. arboreum* L. A few strains with improved staple length were identified; but these did not out yield the existing cultivars. A few strains with shorter staple length (17

mm) showed higher seed cotton yield (125 g/plant) and GOT (42%) over the control 231-R (90 g/plant). Later this germplasm was used for developing improved desi cotton cultivars.

Sindh, another important cotton growing province of Pakistan, is known for having the earliest traces of cotton cultivation-6000 BC at Monjadharo [1]. Efforts were made to develop desi type in the early 20th Century. Seed of an improved cotton variety "Comilla" was imported from the East Pakistan (now Bangladesh). However, due to lack of concerted efforts, no significantly improved germplasm/variety could be developed. A decade later, few plants were selected from *G. arboreum* var neglectum [25] growing in farmer's field, and a variety 27 W.N. was developed in 1922 [26]. This line was released for general cultivation in 1926 with a different name S.N.R., which had a typical morphology (narrow leaves and white flowers) and lint features (GOT=39% and staple length 17.53 mm). This variety covered more than 40468 hectares in Hyderabad Division. Unlike previous varieties, this variety earned high premium in the internal market because of its improved fiber features. In 1963 another variety, TD-I, was approved for general cultivation (Table 1).

Little efforts were made to improve desi cotton in what is now Bangladesh. Only one short staple cotton variety "Comilla" was developed which was known in the market for its roughness.

### 2.3. Introduction of *Gossypium hirsutum* L.

After the initial introduction of the upland cotton (*Gossypium hirsutum* L.) in Indo-Pak in 1818, the first planned experiments for testing the performance of the upland cotton genotypes were undertaken in 1830 in the Bombay presidency by Dr Lush [23]. It took another almost 50 years for cultivation in farmer's field. Unfortunately, *G. hirsutum* L. varieties could not compete against the indigenous 'desi' cotton (*G. arboreum* L.). These efforts were continued by Benouf and Dobbs under the newly established Agricultural Department in 1906 and later were transferred to Milne in 1908 [22].

Historically, *G. hirsutum* L. was introduced in this region (Subcontinent) ~200 years ago, however, successful cultivation of this species was witnessed only around in 1930s parallel to the revolution witnessed in textile industry in Pakistan [20]. Out of the seven races of *G. hirsutum* L. "latifolium" was extensively used for developing improved cotton cultivars which presently covered ~98% of the total cotton cultivated area in Pakistan. The remaining 2% or even less is under cultivation of diploid cotton species, i.e. *G. arboreum* L. Like Upland cotton, efforts were also made for acclimatizing long staple cotton species (*G. barbadense* L.) but did not capture area in Pakistan because of high photosensitivity resulting in low yields.

Pakistan witnessed a gradual replacement of the *G. arboreum* L. types with the high yielding varieties of *G. hirsutum* L. A number of cotton varieties were also developed through selections but largely by hybridization and a few using mutagens.

## 3. Maintenance and storage

Short (working collection) and medium term storage facilities have been established at Plant Genetic Resources Institute (PGRI), NARC at Islamabad. Almost 60,000 seed samples (500 gm each) can be stored in the bank [27].

Collection of the crop germplasm is done either by undertaking plant collecting expedition trips or collected from researcher in the country or can be obtained from foreign countries. The health status of germplasm is analyzed upon its arrival and germination and moisture content are monitored. Before getting stored drying, packing and sealing of seed are done. These all activities meet the international standards. The current facilities were acquired through collaboration with Japan International Cooperation Agency (JICA), considering the international standards for seed preservation [27].

On the behalf of Federal Seed Certification & Registration Department, seeds of national approved varieties of cotton are stored at PGRI. At that time storage conditions of Pakistan Central Cotton Research Institute, Multan were not up to mark as PGRI recommendations, allowing medium term storage of genetic stock of cotton. Storage facilities for cotton germplasm at CCRI Multan have recently been established through Pak-US cotton productivity enhancement project (ID=1198). In total, ~30,000 accessions can be stored (Muhammad Idrees Khan, personal communication). This facility would help all cotton breeders for preserving their precious cotton germplasm.

In the botanical garden of CCRI, Multan, 28 cotton species of both cultivated and wild are maintained for utilizing in cotton breeding program of Pakistan. These include *G. barbosanum* (B), *G. anomalum* ($B_1$), *G. capitisviridis* ($B_3$), *G. sturtianum* ($C_1$), *G. nandewarense*, *G. robinsonii* ($C_2$), *G. thurberi* ($D_1$), *G. harknessii* ($D_2$-$_2$), *G. aridum* ($D_4$), *G. gossypioides* ($D_6$), *G. lobatum* ($D_7$), *G. trilobum* ($D_8$), *G. laxum* ($D_9$), *G. stocksii* ($E_1$), *G. somalense* ($E_2$), *G. areysianum* ($E_3$), *G.incanum* ($E_4$), *G. longicalyx* ($F_1$), *G. bickii*($G_1$), *G. nelsonii* ($G_3$). All these belong to diploid wild species. Whereas, *G. tomentosum* 2 $(AD)_3$, *G. mustelinum* 2$(AD)_4$, *G. lanceolatum* 2$(AD)_4$ and *G. darwinii* 2$(AD)_5$ are found to belong from tetraploid wild species. Five *G. hirsutum* L. races viz. *latifolium, puncatum, morrilli, palmeri and marie-glante;* one *G. barbadense* L. race *braziliense* (kidney cotton); 13 diploid and 5 tetraploid hybrids; 5 triploid and 2 hexaploid hybrids; 3 pentaploid hybrids and 5 tri and 1 tetra species combinations are also maintained. In total, 62 grafts of out-standing *Gossypium* species and species hybrids were prepared for propagation purpose. These grafts were preserved under green house facility of CCRI Multan.

Though a varietal development procedure in Pakistan ensures enough purity, however, every year variants have been observed in the progenies of a variety developed through single plant selection because of limited natural cross pollination (up to 5%). Sometime, mutation and or mixing of seed during ginning process also contributes very small fraction to exaggerate the problem. In Pakistan, it has been observed that strict rouging is mandatory for maintaining the distinguished features of the variety every year, otherwise after three years the variety would appear like a mixture. In Pakistan, usually the maintenance work has been accomplished so far at the respective breeding center of the variety. Representative plants preferably

in thousands are selected from the progeny row block of the best representative families. Ginning out turn percentage and lint quality parameters are measured through high volume instrument (HVI) or conventional tools. Only the plants meeting the set standards (lint percentage=37.50%; staple length=28.00 mm; micronaire value=3.8-4.9 μg inch-1; fiber strength=>92,000 lb psi) are retained for planting progeny rows.

Seed from representative progenies are harvested followed by planting on bigger blocks (~ 0.5 acre). After comparing the yield and lint quality parameters of each of the progeny, families of the best progeny rows are selected for planting on bigger blocks (~10 hectares, depending upon the availability of seed). The seed harvested from the best blocks are multiplied by planting on large scale (1000s of hectares). The seed harvested from this block will make the foundation seed. It is usually accomplished at Govt Farms (preferably at seed corporation farms) or now on private sector farms under the patronage of private seed companies. This seed is exposed to another round of multiplication to raise certified seed which is disposed to farmers for raising the cotton crop. The area under each aforementioned multiplication step can be increased or reduced depending upon the demand of the seed by the farming community. In Pakistan, 65,000 metric tons of cotton seed is needed every year for sowing on 3.2 million hectare. Of this 40-45% was provided through the formal seed sector (certified seed) until 2008. The informal seed sector, that includes farmers, breeders, and shopkeepers, are the major source of uncertified seed. Farmer-to-farmer sale is very popular among the farming community to provide seed to adjoining farms. Also the cotton growers retained a major portion of the seed produced at their own farms for planting in the next cotton growing season. In Pakistan, Plant Breeder Rights have not yet been enforced and the international seed companies, such as Monsanto, Bayer Crop Science and Biocentury, have major concerns pertaining loosing the legal protection of their products (transgenic events, varieties etc.). These are the major factors which hamper the establishment of a dynamic and robust seed industry in Pakistan.

## 4. Funding sources

Under the umbrella of Ministry of Textile Industry, PCCC established in 1948, is considered as the prime research organization working on cotton encompassing economical, technological and agricultural research. Two main research institutes including Central Cotton Research Institute (CCRI) Multan, Punjab and CCRI Sakrand, Sindh are involved in multidisciplinary research encompassing, varietal development, improvement in all kind of agronomic practices, combating insect pest and diseases, farmer's trainings etc. Under PCCC setup, seven other research stations or sub stations are involved in conducting research in specific area—largely on varietal development in collaboration with Provincial Setup. In total, 44 cotton varieties have been evolved by PCCC (Table 2). These varieties fulfill the requirement of spinners for fineness and strength.

| Serial # | Name of variety | Center of release | Year of release | Pedigree/parentage | GOT %¥ | Staple length mm | Fineness µg/inch | Strength tppsi€ |
|---|---|---|---|---|---|---|---|---|
| 1 | M-4 | CRS, ARI, Tandojam | 1942 | Selection from 289-F | 33.0 | 23.8 | 4.5 | 85.0 |
| 2 | M-100 | CRS, ARI, Tandojam | 1963 | (M-4 x Wilds) x M-4 | 34.5 | 27.0 | 4.0 | 85.0 |
| 3 | Qalandri | ARI, Tandojam | 1974 | (M-4 x *G. anomalum*) x Karnak | 34.0 | 28.6 | 3.8 | 92.7 |
| 4 | Sermast | ARI, Tandojam | 1975 | (M-4 x Acala) x M-4 | 34.0 | 28.6 | 3.9 | 92.7 |
| 5 | K-68/9 | CRS, PCCC, Ghotki | 1977 | (124-F x Babdal) x Wilds | 33.0 | 30.1 | 4.2 | 96.0 |
| 6 | Rehmani | ARI, Tandojam | 1985 | *G.hirsutum*21 x McNaire TH14920 | 35.0 | 27.0 | 4.4 | 90.0 |
| 7 | Shaheen | CRS, Ghotki | 1988 | GH 7/72 x (DPL-16 x AC-134–F130kr) | 35.0 | 27.4 | 4.3 | 94.6 |
| 8 | Reshmi-90 | ARI, Tandojam | 1991 | Coker 100A x (DPL-16 x AC-134 | 35.7 | 31.5 | 4.1 | 98.7 |
| 9 | CRIS-9 | CCRI, Sakrand | 1993 | Rajhans x RA-33-47 | 34.4 | 26.3 | 4.4-4.8 | 98.0 |
| 10 | Chandi-95 | NIA, Tandojam | 1996 | (DPL-16 x AC-134-($F_1$ 30kr, 300Gy gamma rays) | 35.0 | 29.0 | 4.2 | 97.0 |
| 11 | CRIS-5A (Marvi) | CCRI, Sakrand | 2001 | {(M-4 x *G. anomalum*) x Karnak} x 9L-34 ICCC | 34.5 | 26.5 | 3.9 | 96.0 |
| 12 | CRIS-134 | CCRI, Sakrand | 2001 | (DPL-16 x AC-134)-$F_1$ Irradiated-30 kr Gamma rays (60 Co) x DPL-70 | 34.8 | 22.5 | 4.0-4.5 | 98.0 |
| 13 | CRIS-467 | CCRI, Sakrand | 2001 | LRA-5166 x CRIS-9 | 37.5 | 27.5 | 4.6 | 98.5 |
| 14 | Shahbaz-95 | ARI, Tandojam | 2001 | {(M-4 x *G. anomalum*) x Karnak} x Acala 1517 | 33.5 | 27.5 | 4.2 | 94.6 |
| 15 | Sohni | NIA, Tandojam | 2002 | NIAB-78 (300 gy) | 37.5 | 27.5 | 4.5 | 98.0 |
| 16 | CRIS-121 | CCRI, Sakrand | 2006 | NIAB-78 x B-909 | 34.8 | 26.1 | 4.6-4.9 | 98.0 |
| 17 | Hari Dost | ARI, Tandojam | 2006 | Sarmast x Deltapine | 38.0 | 27.4 | 4.3 | 97.0 |
| 18 | Sadori | NIA, Tandojam | 2006 | $F_1$[(Shaheen x DPL-14) Gamma rays 250 GY] | 37.2 | 27.8 | 4.4 | 97.0 |
| 19 | Sindh-1 | ARI, Tandojam | 2010 | NIAB-78 x Stoneville | 37.0 | 28.0 | 4.5 | 97.0 |
| 20 | Malmal | ARI, Tandojam | 2010 | CIM-70 x Reshmi | 38.0 | 30.0 | 4.0 | 97.0 |
| 21 | NiaUfaq | NIA, Tandojam | 2010 | DEM-84(R-RAUS 250 GY CO 60 source) | 38 | 28.5 | 4.3 | 97.0 |

¥=Ginning out turn percentage; **€**=Thousand pounds per square inch

**Source:** Dr. Akhlaq Hussain, Description of cotton varieties of Pakistan 2004, Cotton Research and development Memoranda till 60's (Ed. Dr. Mahbub Ali), approval documents of cotton varieties released after 2004 and personal communication with breeders of the cotton varieties.

**Table 2.** List of approved *G. hirsutum* L. (upland cotton) varieties (non-GM) for Sindh

The establishment of Punjab Agricultural Research Board (PARB) as an autonomous body under PARB Act, 1997 for fostering an integrated approach for research planning and efficient allotment of research resource so that the agriculture innovation system of the province can generate appropriate solutions of the issues faced to various stakeholders in the food and fiber chain [28]. The vision of the PARB is to support scientific innovations for the prosperity of Agricultural Stakeholders in Punjab. Ministry of Food, Agriculture and Livestock (MINFAL, desolved after 18th amendment) was also remained actively involved in improving agricultural studies in Pakistan by providing funds.

Presently, a project on cotton productivity enhancement has been initiated by the generous support of the U.S. Department of Agriculture, Agricultural Research Service; under agreement No.58-6402-0-178F (operating through ICARDA Pakistan). Major theme of the project revolves around the characterization of the various viral strains, screening of US cotton germplasm in Pakistan, transferring of new sources of resistance into adapted varieties of Pakistan, etc. [29].

A project "Sustainable Control of the Cotton Bollworm, *Helicoverpa armigera*, in Small-scale Cotton Production systems" was sponsored by the Common Fund for Commodities to be executed by China, India, Pakistan and UK. The overall objective of the project was to develop, apply, and disseminate cropping systems and pest management practices for cost-effective and sustainable control of the cotton bollworm *Helicoverpa armigera*. The project aimed to build on existing knowledge and experiences for the further development of efficient methods, resulting in substantially reduced uses of hazardous pesticides and increased profitability for cotton producers.

## 5. Sharing

In Pakistan, germplasm (conventional) can be shared for utilizing in local cotton breeding programs without imposing any kind of restriction. However, for utilizing in breeding program outside the country, one must get permission from the developer provided the venture is commercially driven. However, two organizations like NIBGE and CEMB are involved in the introduction of alien genes through utilizing genetic engineering approaches. In this regard, for example, CEMB has restricted the utilization of its material through signing MTAs with the private seed companies. Similarly, these two organization also got their novel genes patented (national and or internationally) which itself restrict the use of the genetic material.

Since 1992, Pakistan is signatory to UN convention on biological diversity (CBD), ITPGRA, and International Technical Conference on Plant genetic Resources, Lipzig, Germany. Thus country grant permission for accessing PGR on jointly agreed provisions subjected to pre informed approval of contracting bodies. Also, the contracting bodies are supposed to share the results of research and developments and the benefits that are achieved by exploring such

resources. In order to utilize the germplasm, the access to PGR is a mandatory step. In Pakistan, Biodiversity working group of Ministry of Environment has prepared draft Biodiversity law 2005 and was circulated to all stakeholders for safe sharing of germplasm.

## 6. Characterization, evaluation and utilization

Germplasm characterization and evaluation are the key elements for determining the characteristics of the germplasm. The newly introduced material, if not in sufficient quantity, first its seed quantity is multiplied. In the next normal cotton growing season, the material is planted and data of various characters including plant height, flowering time, number of bolls and their weight, fiber characteristics and yield potential are collected. However, screening to cotton leaf curl disease remains the major focus of all the breeders in the country.

There are two categories for germplasm evaluation. The first category comprises systematic collection of descriptors that is chiefly conducted by the guardian of the working collection of the National Collection of *Gossypium* Germplasm, largely by PCCC. These second evaluations usually involve germplasm collection in varying sizes subsets and often are neither systematic nor exhaustive in their approach. Such investigation is being leaned towards goal. University and federal investigators often undertake evaluations for studying the various aspects of cotton plant biochemistry and physiology especially after exposing to various abiotic stresses. Following research institutes are involved in taking notes of various cotton plant characters:

1. Morphological and agronomic trait evaluations: Agronomy section AARI Faisalabad, CRI AARI Faisalabad (including its stations), NIBGE Faisalabad, NIAB Faisalabad, NIA Tandojam and institutes of PCCC.
2. Cytogenetic: CCRI Multan, CRS Multan and NIAB Faisalabad.
3. Biochemical (gossypol): NIAB Faisalabad
4. Quantification of Bt toxin: CEMB Lahore, NIBGE Faisalabad, ABRI Faisalabad, NIGAB Islamabad.
5. Seed Quality: FSC&RD Islamabad
6. Disease resistance: PCCC, NIBGE Faisalabad, AARI Faisalabad
7. Stress evaluation: NIBGE Faisalabad, UAF Faisalabad, CCRI Multan and AARI Faisalabad.
8. Fiber properties: CCRI Multan, NIBGE Faisalabad, CRS Multan and CRI AARI Faisalabad.

The accessions are hybridized with the adaptive cotton variety. After, fixing all the traits of interest, breeder of the line develops a descriptor. This line is then submitted for registration to FSC&RD and also for testing in the National Coordinated Varietal Trials (NCVTs). Federal Seed Certification and Registration Department conducts these CVRTs for two successive

years. The data of various characters of the advanced line is compared with the data given in the descriptor. Salient features of the cotton varieties released till present are documented by the FSC&RD in a book "Cotton Varieties of Pakistan" which provides information about the descriptions of the varieties that is primarily based on stability, uniformity and distinctness, and also on the studies conducted for two successive years under field and laboratory conditions [30].

Recent challenge for evaluation of the newly released cotton varieties is narrow genetic base that is limiting future breeding progress against various stresses. Mainly selection and crossing of well adapted cotton parent genotypes for developing new varieties are the main causes of yield stagnation in the country. It can be partly overcome by involving genetically diverse parent genotypes in the genealogy of a new variety. For example, genes conferring resistance to abiotic stresses especially drought, and biotic stresses especially resistance to the CLCuD can be introgressed from *G. arboretum* L. and or *G. herbaceum* L. into the cultivated *G. hirsutum* L. cotton species. For undertaking this process on massive scale, tissue culturing tools may help in overcoming the phyletic barriers. Preliminary steps have already been taken for introgressing useful genes into the cultivated cotton varieties at CRS Multan and CCRI Multan. Similarly, QTLs/genes conferring high quality traits have been transferred into the cultivated cotton species using DNA markers at NIBGE Faisalabad. All these experiments would help in widening the genetic base of the cultivated cotton varieties in the field. Another strategy for creation of genetic variability is the deployment of various mutagens (radiations and chemicals). In this regard, leading genotypes of *G. hirsutum* L. and *G. arboreum* L. have been treated with EMS for developing TILLING populations that would help in understanding the genes involved in conferring various traits of interest. The preliminary genomic information from model species such as Arabidopsis and cacao genome can be instrumental in exploring the conserved but complex pathways in least possible time.

DNA fingerprints by deploying SSRs of all the leading cotton cultivars including germplasm and also the extent of genetic divergence among the genotypes should be made available to the cotton breeders. This information can be used in planning crosses. Secondly, involving of more than two parent genotypes preferably conical crosses should be made which may help in increasing the genetic window among the newly developed cotton varieties [31].

### 6.1. Utilization of germplasm for the development of Upland cotton varieties in Pakistan

The variety 268-F, bred at research sub-station Jhang, was early maturing and was approved for cultivation in 1948 because of the superior physical properties of its fiber (could spin up to 41 counts) over the existing strains/varieties (4-F, L.S.S., 289-F/43 etc.). However, ultimately 268-F was banned because of its poor germination rate [32].

The cotton variety L.S.S. was extensively used in hybridization in Pakistan. A cotton variety 362-F, developed by selection from the population of L.S.S. was approved for general cultivation in 1958 because of its earliness trait exhibited in the Lyallpur region. However, this variety showed adaptability only in the 'Thal' region (a sandy, rain fed area). The variety was of the bushy type but was not adopted in other cotton growing districts (Table 3).

| Serial # | Name of variety | Center of release | Year of release | Pedigree/parentage | GOT %¥ | Staple length mm | Fineness µg/inch | Strength tppsi€ |
|---|---|---|---|---|---|---|---|---|
| 1 | 3-F | CRI, Faisalabad | 1913 | Selection from varieties introduced from USA by East India Company | 33.0 | 20.6 | 4.9 | 85.0 |
| 2 | 4-F | CRI, Faisalabad | 1914 | Selection from stray plants of American Cotton | 32.0 | 20.6 | 5.0 | 85.0 |
| 3 | 289-F | CRI, Faisalabad | 1921 | 4-F-Selection, Natural hybrid-an off type plant found in the 4 F field | 32.0 | 25.0 | 4.5 | 95.0 |
| 4 | 289 -F/K25 | BCGA, Khanewal | 1930 | 289-F bulk selection | 33.5 | 23.8 | 4.5 | 95.0 |
| 5 | L.S.S. | CRI, Faisalabad | 1934 | Selection from 4-F-a single plant variant (natural hybrid) in the 4-F field | 32.2 | 23 | 5.0 | 85.0 |
| 6 | 289-F/43 | CRS, AARI, Faisalabad | 1934 | Selection from 4-F-natural hybrid, an off-type plant in the 4-F field | 31.0 | 23.8 | 4.5 | 95.0 |
| 7 | 124-F | CRI, AARI, Faisalabad | 1945 | Selection from 289-F/43 | 33.0 | 24.6 | 4.8 | 96.0 |
| 8 | 216 -F | CRI, AARI, Faisalabad | 1946 | Selection from 4-F | 33.0 | 23.8 | 4.5 | 90.0 |
| 9 | 199-F | CRI, AARI, Multan. | 1946 | Selection from 4-F-98 (material from Sakrand) | 35.0 | 24.6 | 4.5 | 90.0 |
| 10 | 238-F | CRI, AARI, Faisalabad | 1948 | Selection from 289-F/43 | 31.5 | 23.8 | 4.5 | 88.0 |
| 11 | Lasani-11 | CRS,AARI, Faisalabad | 1959 | Selection from 181-F | 34.5 | 28.6 | 4.0 | 90.0 |
| 12 | AC-134 | CRI, AARI, Faisalabad | 1959 | 148-F x 199-F | 34.5 | 26.5 | 4.5 | 93.5 |
| 13 | 362-F | CRI, AARI, Faisalabad | 1959 | Selection from 289-F | 33.0 | 23.8 | 4.5 | 93.0 |
| 14 | BS-1(13/26) | CRS, AARI, Khanpur | 1962 | Selection from M-4 | 33.8 | 26.0 | 4.2 | 94.2 |

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| Serial # | Name of variety | Center of release | Year of release | Pedigree/parentage | GOT %¥ | Staple length mm | Fineness µg/inch | Strength tppsi€ |
|---|---|---|---|---|---|---|---|---|
| 15 | MS-40 | CRS, AARI, Multan | 1970 | (124-F x 181-F), a single variant plant (natural hybrid) selected from AC-252 field | 34.0 | 31.3 | 4.0 | 89.4 |
| 16 | MS-39 | CRS, AARI, Multan | 1970 | Natural hybrid in L-11 field | 33.5 | 31.8 | 3.6 | 87.5 |
| 17 | 149-F | CRS, AARI, Multan | 1971 | 124-F x Babdal | 34.5 | 28.0 | 4.0 | 97.0 |
| 18 | B-557 | CRI, AARI, Faisalabad | 1975 | 268-F x (45-F x L.S.S) | 35.9 | 28.1 | 4.5 | 93.0 |
| 19 | MNH-93 | CRS, AARI, Multan | 1980 | (124-F x Babdal) x (MS-39 x Mex 12) | 37.5 | 28.6 | 4.5 | 94.2 |
| 20 | NIAB-78 | NIAB, Faisalabad | 1983 | DPL-16 x AC-134)-$F_1$ Irradiated-30 kr Gamma rays (60 Co) | 37.0 | 27.0 | 4.6 | 92.0 |
| 21 | MS-84 | CRS, AARI, Multan | 1983 | (124-F x 181-F) x DPL-16 | 34.0 | 33.3 | 3.9 | 91.3 |
| 22 | SLH-41 | CRS, PCCC, Sahiwal | 1984 | (289-F x Mysor American) x (124-F x Babdal) x Mex 68) | 36.7 | 27.8 | 4.4 | 95.8 |
| 23 | Rehmani | CRS, AARI, Tandojam | 1985 | *G. hirsutum* 21 x McNaire TH-14920 | 35.0 | 27.0 | 4.4 | 90.0 |
| 24 | MNH-129 | CRS,AARI, Multan | 1986 | {(124-F x Babdal) x (MS-39 x Mex 12)} x DPL-16 | 38.5 | 28.7 | 4.4 | 95.0 |
| 25 | CIM-70 | CCRI, PCCC, Multan | 1986 | Coker 8314 x (124-F x Babdal) x Coker 100 WA) | 31.1 | 28.6 | 4.2 | 92.5 |
| 26 | S-12 | CRS, AARI, Multan | 1988 | {(124-F x Babdal) x (MS-39 x Mex 12)} x 7203-14-4-Arizona | 41.3 | 28.0 | 4.6 | 93.0 |
| 27 | FH-87 | CRI, AARI, Faisalabad | 1988 | AC-134 x Paymaster | 36.8 | 27.8 | 4.2 | 96.0 |
| 28 | RH-1 | CRS, AARI, R.Y. Khan | 1990 | LH-62 x W-1104 | 31.8 | 29.8 | 3.9 | 103.7 |
| 29 | NIAB-86 | NIAB, Faisalabad | 1990 | (DPL-16 x AC-134-F1 30kr) x Stoneville-213 | 34.5 | 29.0 | 4.3 | 95.0 |

| Serial # | Name of variety | Center of release | Year of release | Pedigree/parentage | GOT %¥ | Staple length mm | Fineness µg/inch | Strength tppsi€ |
|---|---|---|---|---|---|---|---|---|
| 30 | Gohar-87 | CRS, PCCC, Bahawalpur | 1990 | (124-F x Babdal) x B-557 | 36.0 | 28.0 | 4.5 | 98.6 |
| 31 | CIM-109 | CCRI, Multan | 1990 | (DPL-16 x AC-134 – F1 30kr) x A89/FM | 35.0 | 27.3 | 4.4 | 91 |
| 32 | Reshmi -90 | CRS, ARI, Tandojam | 1991 | Coker 100A x (DPL-16 x AC-134 – F1 30kr) | 35.7 | 31.5 | 4.1 | 98.7 |
| 33 | NIAB -26N | NIAB, Faisalabad | 1992 | (DPL-16 x AC-134-$F_1$ irradiated 30kr) x DPL-NSL | 37.5 | 28.0 | 4.4 | 95 |
| 34 | MNH -147 | CRS, AARI, Multan | 1992 | [{(124-F x Babdal) x (L -11 x Lankart 57)} x {(124-F x Babdal)} x Mex Pollen) x MS-64)] x {B-557 x (124-F x Babdal) x DPL-16)} | 41.3 | 28.5 | 4.2 | 95.5 |
| 35 | FH -682 | CRI, AARI, Faisalabad | 1992 | (B-557 x Ala (68)1) x Lankart-57 | 37.0 | 28.5 | 4.3 | 95.7 |
| 36 | CIM-240 | CCRI, PCCC Multan | 1992 | Coker 8314 x (124-F x Babdal) x Coker 100 WA) x W 1104 | 36.5 | 27.8 | 4.7 | 94.0 |
| 37 | BH-36 | CRS, PCCC, Bahawalpur | 1992 | M-4 x T x Bonham-76C | 38.7 | 27.8 | 4.3 | 100.5 |
| 38 | Gomal -93 | CRS, PCCC, D.I. Khan | 1993 | 387-F x AC-134 | 34.5 | 26.5 | 4.5 | 93.0 |
| 39 | SLS-1 | CRS, PCCC, Sahiwal | 1995 | SLH-19 x SLH-19 x ( DPL-16 xAC-134 – F1 30kr) | 35.0 | 27.4 | 4.5 | 95.3 |
| 40 | S-14 | CRS, AARI, Multan | 1995 | {(124-F x Babdal) x (AC-252 x DPL-16) x DPL-16) x Lankart 4789 A} x {(124-F x Babdal) x (AC-252 x DPL-16) x Coker. ($F_1$ x $F_1$)} | 43.0 | 29.5 | 4.2 | 93.0 |
| 41 | RH-112 | CRS, AARI, R.Y. Khan | 1996 | (124 -F x Babdal) x Delfoss) x (AC-134 x C.T.) | 34.3 | 27.6 | 4.6 | 95.0 |
| 42 | MNH-329 | CRS, AARI, Multan | 1996 | {(124-F x Babdal) x (MS-39 x Mex 12)} x {B-557 x (124-F x Babdal) x DPL-16)} | 41.0 | 28.5 | 4.2 | 96.0 |

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| Serial # | Name of variety | Center of release | Year of release | Pedigree/parentage | GOT %¥ | Staple length mm | Fineness µg/inch | Strength tppsi€ |
|---|---|---|---|---|---|---|---|---|
| 43 | NIAB-Karishma | NIAB, Faisalabad | 1996 | {(DPL-16 x AC-134-$F_1$ 30kr) x Stoneville 213)} x W 83-29 Mex | 37.4 | 28.6 | 5.0 | 93.3 |
| 44 | FH-634 | CRI, AARI, Faisalabad | 1996 | CEDEX x B-557 | 36.3 | 28.5 | 4.1 | 95.1 |
| 45 | CIM-1100 | CCRI, PCCC, Multan. | 1996 | (W-1104 x {(124-F x Babdal) x (MS-39 x Mex 12)} x 7203-14-4-Arizona) x CP 15/2 | 38.0 | 29 | 4.0 | 94 |
| 46 | CIM-448 | CCRI, PCCC, Multan | 1996 | {(124-F x Babdal) x (MS-39 x Mex 12)} x 7203-14-4-Arizona (sister line CIM-1100) | 38.0 | 28.5 | 4.5 | 93.8 |
| 47 | FVH-53 | CRS, AARI, Vehari | 1998 | KIVI 1021 x {(124-F x Babdal) x (MS-39 x Mex 12)} x 7203-14-4-Arizona | 38.4 | 28.6 | 5.2 | 98.5 |
| 48 | CIM-446 | CCRI, PCCC, Multan | 1998 | CP-15/2 x {(124-F x Babdal) x (MS-39 x Mex 12)} x 7203-14-4-Arizona | 36.1 | 27 | 4.7 | 97.4 |
| 49 | CIM-443 | CCRI, PCCC, Multan | 1998 | (DPL-16 x AC -134 – $F_1$ 30kr) x A-89/FM x LRA-5166 | 36.5 | 27.6 | 4.9 | 96.1 |
| 50 | MNH -554 | CRS, AARI, Multan | 2000 | {(124-F x Babdal) x (L-11 x Lankart-57) x 4-C} x (C-603 x Mex 3) x LRA -5166 | 41.3 | 28.0 | 4.2 | 94.0 |
| 51 | MNH -552 | CRS, AARI, Multan | 2000 | (124-F x Babdal) x LRA -5166 | 40.0 | 27.5 | 4.9.0 | 95.0 |
| 52 | FH-901 | CRI, AARI, Faisalabad | 2000 | {Coker 8314 x (124 -F x Babdal) x Coker 100 WA) x W 1106} x {(W 1104 x {(124-F x Babdal) x (MS-39 x Mex } | 38.0 | 27.5 | 5.2 | 92.0 |
| 53 | FH-900 | CRI, AARI, Faisalabad | 2000 | (FH-672 x AET-5) x (B- 557 x LRA- 5166) | 38.0 | 28.5 | 4.3 | 95.1 |
| 54 | CIM-482 | CCRI, Multan | 2000 | {(DPL -16 x AC -134 – $F_1$ 30kr) x ALS 15(CIM -39 x ALS -15} x CP-15/2 | 39.2 | 29.0 | 4.5 | 98.0 |

| Serial # | Name of variety | Center of release | Year of release | Pedigree/parentage | GOT %¥ | Staple length mm | Fineness µg/inch | Strength tppsi€ |
|---|---|---|---|---|---|---|---|---|
| 55 | BH-118 | CRS, Bahawalpur | 2000 | (T x 339 x ST-7A) x (ST-7A x AET- 5) | 38.5 | 28.0 | 4.6 | 98.0 |
| 56 | CIM-473 | CCRI, Multan | 2002 | [{(Coker 8314 x (124 -F x Babdal)} x {Coker 100 WA) x (CIM- 46 x (AC -134 x (DPL- 16 x AC- 134-$F_1$ 30kr)}] x LRA -5166 | 39.7 | 29.5 | 4.3 | 95.0 |
| 57 | NIAB-999 | NIAB, Faisalabad | 2003 | (DPL -16 x AC -134-$F_1$ 30kr) x LRA- 5166 | 36.5 | 28.7 | 4.6 | 98.0 |
| 58 | FH-1000 | CRS, AARI, Faisalabad | 2003 | [{(124 -F x Babdal) x (MS- 39 x Mex 12)} x 7203-14-4-Arizona] x [{(124 -F x Babdal) x (MS- 39 x Mex 12)} x 7203-14-4-Arizona] | 38.8 | 29.5 | 4.6 | 96.9 |
| 59 | CIM-499 | CCRI, Multan | 2003 | CIM-433 x 755-6/93 | 40.0 | 29.6 | 4.4 | 97.3 |
| 60 | CIM-506 | CCRI, Multan | 2004 | CIM-360 x CP-15/2 | 38.6 | 28.7 | 4.5 | 98.9 |
| 61 | CIM-707 | CCRI, Multan | 2004 | CIM-243 x 738-6/93 | 39.0 | 32.2 | 4.2 | 97.5 |
| 62 | NIAB-111 | NIAB, Faisalabad | 2004 | $F_1$seed 300 Gy gamma radiation 0R (NIAB-313/12 x CIM-100) $F_1$ 300 Gy | 37.5 | 30.5 | 4.4 | 218.8 |
| 63 | BH-160 | CRS, Bahawalpur | 2004 | Cedix FDW 946 x 673/93 | 39.0 | 29.5 | 4.2 | 95.1 |
| 64 | CIM-496 | CCRI, Multan | 2005 | CIM-425 x 755-6/93 (1993) | 41.1 | 29.7 | 4.6 | 93.5 |
| 65 | CIM-534 | CCRI, Multan | 2006 | Hybridization of local line 5-4/94 with locally developed variety CIM-1100 | 40.32 | 27.9 | 4.5 | 97.2 |
| 66 | MNH-786 | CRS, Multan | 2006 | (S-14 x CIM-448) x (MNH-564 x MNH-516) | 38.7 | 27.2 | 5.1 | 95.0 |
| 67 | NIBGE-2 | NIBGE, Faisalabad | 2006 | S-12 x LRA- 5166 | 36.2 | 28.6 | 5.0 | 100.0 |
| 68 | NIAB-846 | NIAB, Faisalabad | 2008 | NIAB-78 x REBA-288 Pollen irradiated (10Gy) with gamma rays | 38.5 | 29.8 | 4.7 | 96.0 |
| 69 | CIM-554 | CCRI, Multan | 2009 | 2579-4/97 x W-1103 | 41.5 | 28.5 | 4.7 | 96.8 |

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| Serial # | Name of variety | Center of release | Year of release | Pedigree/parentage | GOT %¥ | Staple length mm | Fineness µg/inch | Strength tppsi€ |
|---|---|---|---|---|---|---|---|---|
| 70 | NIAB-777 | NIAB,Faisalabad | 2009 | NIAB-78 x Reba-288 | 38.8 | 28.9 | 4.4 | 93.0 |
| 71 | CRSM-38 | CRS, Multan | 2009 | 583-85/99 x FH900 583-85/99 = LRA5166 x BJA592 | 39.5 | 29.0 | 4.5 | 95.0 |
| 72 | NIBGE-115 | NIBGE, Faisalabad | 2012 | S-12 x LRA-5166 | 38.15 | 29.51 | 4.93 | 93.1 |
| 73 | BH-167 | CRS, Bahawalpur | 2012 | VH-53 x BH-142 (Hybridization) | 41.2 | 29.1 | 4.8 | 92.7 |
| 74 | FH-942 | CRI, AARI, Faisalabad | 2012 | FH-900(S) x CIM-121(hybridization) | 38.01 | 29.63 | 4.28 | 95.1 |
| 75 | NIAB-852 | NIAB, Faisalabad | 2012 | NIAB-78 x REBA-288 Pollen irradiated (10Gy) with gamma rays | 37.8 | 31.6 | 4.5 | 91.2 |
| 76 | CIM-573 | CCRI, Multan | 2012 | H-2118 x H-2119 (cross in 2000-01) | 39.34 | 31.61 | 4.64 | 90.2 |
| 77 | SLH-317 | CRS, Sahiwal | 2012 | {LRA-5166 x (SLH-205 x LRA-5166)} | 38.0 | 29.8 | 4.4 | 96.7 |
| 78 | NN-3 | NIBGE, Faisalabad | 2013 | S-12 x LRA-5166 | 38.14 | 30.17 | 4.63 | 93.6 |
| 79 | NIAB-Kiran | NIAB, Faisalabad | 2013 | NIAB-98 x NIAB-11I | 38.96 | 30.41 | 4.61 | 93.8 |
| 80 | NIAB-112 | NIAB, Faisalabad | 2013 | NIAB-111 x NIAB-999 | 38.3 | 28.6 | 4 | 90.0 |
| 81 | CIM-608 | CCRI, Multan | 2013 | 2(*G. hirsutum*/*G.anomalum*)x $G^3$-*hirutum* | 40.3. | 29.88 | 4.78 | 95.4 |
| 82 | GS-14 | Gohar Seed Corporation, Multan | 2013 | CIM-448 x exotic variety Acala SJ-2 (USA) | 40.82 | 28.04 | 5.88 | 96.0 |

¥=Ginning out turn percentage; **€**=Thousand pounds per square inch

**Source:** Dr. Akhlaq Hussain, Description of cotton varieties of Pakistan 2004, Cotton Research and development Memoranda till 60's (Ed. Dr. Mahbub Ali), approval documents of cotton varieties released after 2004 and personal communication with breeders of the cotton varieties.

**Table 3.** List of approved *G. hirsutum* L.(upland cotton) varieties (non-GM) for Punjab

In the mid-1960s, efforts were made to grow cotton varieties previously recommended for cultivation in various countries especially in the USA. Deltapine, a smooth leaf variety, was introduced in Multan and Sheikhupura. In total, 16-17 insecticides sprays were applied on these newly introduced varieties, and 922 kg/hectare seed cotton yield was harvested, demonstrating a limited scope of the introduced cotton varieties in this region. In the same normal cotton growing season, a number of exotic cotton varieties like Tide Water, Stoneville-213, Stoneville 7-A, Acala P-5, Carolina Queen, Dixie King, Express H3-P1, Defos 44, Deltapine Smooth Leaf and Coker Wild along with AC-134 and L-11 (local controls) were planted at the CRS Multan. The yield of some of these varieties was comparable with the control AC-134. Breeding efforts for overcoming the menace of insect pests infestation were made through selection, but went fruitless because of a limited genetic diversity available in the exotic germplasm. Hybridization of the exotic germplasm with the locally adapted cultivars/germplasm was remained the only strategy for improving the local cotton varieties by introducing high yielding genes of the exotic material into local cultivated cotton varieties [33]. Big boll trait was transferred from Lankert-57, and compactness and earliness from Babdale. The major limitation of big boll variety was susceptibility to insects. The newly developed strains derived from these crosses out yielded the standard AC-134. The other advantages over the indigenous cultivar AC-134 were drought tolerance and earliness in maturity. The upland cotton varieties developed till 1990s were dominantly of open type. Boll size was relatively smaller than the present day varieties. Emphasis was given to improve boll size especially after the introduction of Bt cotton varieties in Pakistan. Earlier, all successful varieties till the evolution of S-12, bred for large number of bolls rather than boll size for compensating the boll damage done by bollworms infestation. NIAB-78, proved to be the most successful variety, bears large number of bolls with small to medium sizeed boll. Later, the best extension services provided by public sector organizations and especially the private sectors, dominantly pesticide companies, educated farmers for eradicating pest population through chemical means.

In Pakistan, one of the main objectives is to develop a variety that matures early than that of the varieties released before 1980s. Such early maturing varieties help farmers to sow wheat-a major staple food crop in Pakistan. However, very early maturing varieties are not suitable because high temperature early in the cotton growing season may affect boll opening which ultimately may cause significant reduction in seed cotton yield [26]. Best suitable time for cotton maturity in Pakistan is between November 15-30—enough time to harvest high yield and good quality lint without compromising the cultivation of wheat crop. For addressing this issue, breeders have been successful in releasing cotton varieties like NIAB-78, S-12, CIM-496, IR-NIBGE-3701 and MNH-886, etc. which allows farmers to plant wheat in time. Further efforts for releasing varieties which mature in mid of Nov. through exploiting the available germplasm, resulted in the development of an advance line IR-NIBGE-5, flowers five and seven days earlier than IR-NIBGE-3 and IR-NIBGE-3701, respectively. However, such kind of genetic material requires unusually much more water and nutrients. Thus, a comprehensive breeding approach by bridging molecular and conventional tools is needed for releasing highly adaptive cotton varieties.

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*6.1.1. Leading G. hirsutm L. cultivars of Post-CLCuD Era*

Leaf curl disease on cotton was first time reported in 1912 from Nigeria, and then it spread in many other cotton growing countries such as Pakistan, India and China. This disease is of viral origin and transmitted by a vector whitefly (*Bemisia tabaci* Gennadius), which may cause 30-70% or even more depression in seed cotton yield. It was first time appeared on few plants in 1967 in Pakistan. Typical symptoms of the disease are small and large veins thickening and upward or downward curling of the leaf. Under high infection, a small leaf like structure—called enation underneath of the leaf has been observed (Figure 1).

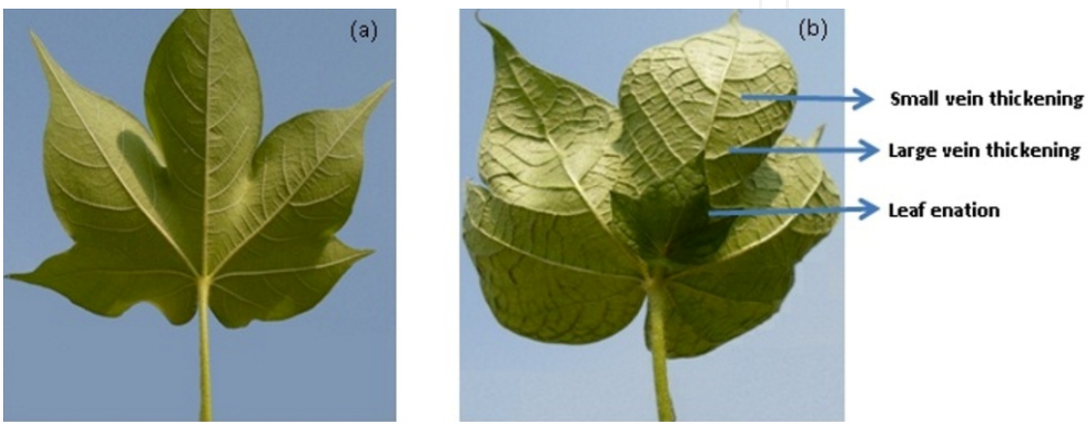



**Figure 1.** Comparison of healthy (a) versus infected cotton leaves showing symptoms of cotton leaf curl virus disease

Efforts were made for combating the disease by finding resistance sources from the available cotton germplasm. In this regard, more than 1000 cotton lines available in the gene pool of CCRI Multan were screened under natural conditions (Muhammad Afzal, CCRI Multan, personnel communication). Three genotypes LRA-5166, CP-15/2 and Cedix were identified. However, LRA-5166 and CP-15/2 were used extensively for deriving resistance into the cultivated susceptible cotton cultivars through various hybridization breeding procedures. In this regard, CIM-1100 was the first resistant cotton varieties released from CCRI Multan in 1997 followed by a series of resistant cotton varieties by CCRI Mutlan and few from other cotton breeding research institutes (Table 3). Deploying of the two sources of resistance in breeding program has created a major genetic bottleneck in evolution.

Resistance to the Multan strain of virus was controlled by two genes [33]. This resistance was overcome in within five years because of the evolution of new strain of virus called Burewala strain. Till today, none of the variety was found completely asymptomatic. However, high tolerance or field resistance was observed in few cotton genotypes, viz. NIBGE-2472, NIBGE-3661, NIBGE-115 [34], FH-142, and NN-3[35]. Cotton germplasm (3000 accessions of *G. hirsutum* L. and introgressed lines) received through the United States Department for Agriculture (USDA) has also been screened. Initial studies have shown that Mac-07 and approximately 95 lines are resistant to the disease. These newly identified sources can be used extensively in improving the cotton germplasm/varieties resistant to the CLCuD.

Introgression breeding procedures have been deployed to introgress important traits from *G. arboreum* L. like, resistance to CLCuD, tolerance to drought etc. into the cultivated *G. hirsutum* L. cotton varieties. In these experiments, chromosome of *G. arboreum* L. was doubled by applying colchicine followed by hybridization with the allotetraploid *G. hirsutum* L. under natural conditions. Exogenous treatment of hormones 50 mg/L gibberellic acid and 100 mg/L naphthalene acetic acid were applied for overcoming the problem of boll shedding. It has been demonstrated that the percentage of pollen viability in $F_1$s was 1.90% in 2(*G. arboreum*) x *G. hirsutum* versus 2.38% in *G. hirsutum* x 2(*G. arboreum*). Further confirmations were made through cytological studies; found that all $F_{1s}$ were sterile. All the $F_1$ plants exhibited resistance to the CLCuD after exposing through grafting of the infected buds, indicating the chances of success for transferring resistance into the cultivated tetraploids [36]. Currently the progenies/advance generations are being screened against the disease at CCRI Multan (Project PI Mr. Zahid Mehmood, Pr Scientist) and CRS Multan (Project PI Dr. Saghir Ahmed, Botanist). Similarly, emphasis was also made for improving quality parameters of the local cultivated species var FH-1000 by crossing it with the *G. barbadense* L. The introgressions of the DNA fragments from *G. barbadense* L. were monitored through SSR markers [37]. The resultant hybrids or progenies had two types of leaves, narrow versus broad lobed types. It was reported that bolls of the narrow lobed types did not mature due to bad opening. However, the broad lobed types were found comparatively high yielder but poor in lint quality. Efforts on various fronts are going on for evolving useful germplasm or varieties.

## 7. Databases

In early seventies, activities related to the collection as well as conservation of germplasm have been started in Pakistan. In this regard, cotton germplasm have been collected from various countries largely of upland cotton. Recently, more than 3000 accessions have been imported from US under the Pak-US cotton productivity enhancement project. Before this, cotton material (accessions of *G. hirsutum*) was imported from different countries including USA, Uzbekistan, and France, etc. Most of the accessions are available with CCRI Multan, and maintained descriptions of each accession both hard as well as soft copy which can be obtained upon the request. However, no website is available showing the description of the cotton germplasm in Pakistan.

Efforts for conservation of genetic material are also under way at PGRI, Islamabad. Passport data of around 75% of the accessions of different crops has been entered in the form of dBase files. Users can get information in the form of computer print outs on request. However, local scientists on a limited scale can get direct on-line access to the files. Consultation of data books that are maintained by the institute can be done at any time by local scientists. Linkage of documentation section with all PGRI laboratories is made sure by availability of a local network that is not in working condition yet. However, currently there are no options available for networking with other gene banks for the data exchange on crop basis as well as regional basis. The process of data base and information system establishment is on the move. PGRI has plans for its connection to other gene banks in future.

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## 8. Novel trends and perspectives

Exotic Bt cotton strains were first cultivated in Sindh in 2000. This introduced material showed high susceptibility to sucking insect pests and CLCuD. Breeding to introgress the *Bt* gene (*Cry1Ac*) by backcross hybridization was initiated in 2000 by various public and private sector organizations of Pakistan but resulting lines could not be tested in the field due to the suspension of biosafety rules in Pakistan. The National Institute for Biotechnology & Genetic engineering (NIBGE) initiated development of Bt cotton and field testing under the "voluntary code of conduct" issued by the Ministry of Environment. A huge quantity of data for the safe release of Bt cotton in the field was generated using rabbit as an experimental animal, and also the impact of Bt cotton residues on weed and soil microbial populations. It was demonstrated that the cultivation of Bt cotton is safe to wild as well as domesticated animals, and its impact, if any, will be low or negligible. This approach for characterizing risk is consistent with the accepted risk assessment procedures and shared similarities with the previous assessments over a wide range of situations (Zaman & Co-workers, unpublished).

The area under Bt cotton cultivation has been increased dramatically. Around 40,000 kg of seed of the Bt cotton strains IR-FH-901 (later approved as IR-NIBGE-901), IR-NIBGE-2 (later approved as IR-NIBGE-1524 in 2010), IR-CIM-448 (later approved as IR-NIBGE-3701) and IR-CIM-443, was provided to farmers and was grown on over 3,238 ha (hectares) in 2005-2006 [38]. IR-NIBGE-1524 was approved for general cultivation in 2010 and 2011 for Punjab and Sindh (Table 4). This variety was drought tolerant, with an open canopy and bears small bolls. It was planted on a large area (more than 5%) in 2007 and retained ~2% of the area, particularly in the drought prone, each year until 2012 in Punjab. In Sindh, it was planted on ~10% of the area in 2012 (Director General Agriculture Sindh). The NIBGE Bt cotton strains were used extensively in breeding programs as a source for developing Bt cotton varieties by various research organizations, and established the foundation of Bt cotton cultivation in Pakistan.

In post-Bt era, preference for cultivating compact to semi-compact varieties has been given for sowing in normal season. Earlier, semi-compact to compact type cotton varieties like CIM-448, CIM-497, NIAB-111 and BH-160 were released for general cultivation but could not capture significant area. There were two major reasons. Firstly, it is difficult to control insect pests especially bollworms in compact shaped plant versus open type plant. Secondly, compact shaped plant does not compensate for low population density compared to the open shaped plant. Before Bt cotton cultivation, major area >10% covered by open type varieties, viz. B-557, NIAB-78, MNH-93, S-12, CIM-240, NIAB-Karishma, CIM-473 and CIM-496 etc. Bt cotton varieties offered inbuilt resistance to *Heliothus*, spotted and marginally to pink bollworm. Thus one of the disadvantages of cultivating compact shaped varieties has been addressed. First Bt cotton variety, IR-NIBGE-3701—semi-compact shaped variety, tested for yield in National Coordinated Bt Trials (NCBT) in 2009, out yielded all candidate lines and standard cotton variety CIM-496. IR-NIBGE-3701 formed the basis for cultivation of compact shaped variety among the farming community. Later on, CIM-886 dominantly a compact shaped variety covered a significant area in 2012.

| Serial # | Name of variety | Center of release | Year of release | Pedigree/parentage | GOT %¥ | Staple length mm | Fineness µg/inch | Strength tppsi€ |
|---|---|---|---|---|---|---|---|---|
| 1 | IR-NIBGE-3701$ | NIBGE, Faisalabad | 2010 | Selection from IR-CIM-448 | 43.23 | 27.52 | 5.43 | 90.2 |
| 2 | IR-NIBGE-1524$ | NIBGE, Faisalabad | 2010 | Transgenic line as a donor parent for Bt gene.NIBGE-2 as an adapted parent | 38.55 | 30.15 | 4.73 | 92.5 |
| 3 | Neelum-121 | Neelum Seeds Corporation | 2010 | A-92 x exotic variety | 41.87 | 28.70 | 4.81 | 29.5 |
| 4 | FH-113 | CRI, AARI, Faisalabad | 2010 | FH-925 x Bollgard | 38.13 | 28.61 | 5.00 | 24.85 |
| 5 | AA-802 | Ali Akbar Seeds, Multan | 2010 | [{(FH-1000 x HK-303) x LRA-5166} x Linea-100] | 43.26 | 29.49 | 4.77 | 92.8 |
| 6 | AA-703 | Ali Akbar Seeds, Multan | 2010 | CIM-482 x Exotic Line | 38.8 | 29.8 | 4.45 | 99.98 |
| 7 | MG-06 | ThattaGurmani Research Center, KotAdu, Muzafarghar | 2010 | CIM-443 x IR-448 | 38.0 | 29.32 | 4.7 | 28.7 |
| 8 | Sitara-008 | Agri Farm Research Center, Multan | 2010 | NIAB-III x IR-448 | 40.0 | 27.3 | 4.6 | 95.9 |
| 9 | IR-NIBGE-901£ | NIBGE, Faisalabad | 2011 | Transgenic line as a donor parent for Bt gene. FH-901as an adapted parent, used in backcrossing | 38.86 | 27.06 | 5.38 | 90.8 |
| 9 | MNH-886 | CRS, Multan | 2012 | FH-207 x MNH-770 x Bollguard-1 | 41.01 | 28.21 | 4.95 | 99.5 |
| 10 | Bt. CIM-598 | CCRI, Multan | 2012 | CIM-446 x IR-CIM-448 | 41.82 | 29.03 | 4.38 | 94.8 |
| 11 | Tarzen-1 | 4-Brothers Seed Corporation, Multan | 2012 | {(CIM-496 x hk 303) x Linea-100} | 42.6 | 29.15 | 4.96 | 95.0 |
| 12 | Neelum-141 | Neelum Seeds Corporation | 2012 | IR-448 x C-2-2 | 41.05 | 29.0 | 4.9 | 101.5 |

| Serial # | Name of variety | Center of release | Year of release | Pedigree/parentage | GOT %¥ | Staple length mm | Fineness µg/inch | Strength tppsi€ |
|---|---|---|---|---|---|---|---|---|
| 13 | FH-114 | CRI, Faisalabad | 2012 | Non-*Bt* early maturing cotton lines FH-925 with Australian *Bt* variety Bollgard-1(*Cry1Ac*) | 39.64 | 28.12 | 4.85 | 95.5 |
| 14 | IR-NIBGE-3 | NIBGE, Faisalabad | 2012 | Developed through selection from IR-NIBGE-2381);Bt version of FH-1000 | 38.68 | 28.3 | 4.96 | 97.6 |
| 15 | Sitara-009 | Agri Farm Research Center, Multan | 2012 | {(CIM-496 x Sitara-008) x MNH-786} | 39.8 | 25.7 | 4.87 | 97.6 |
| 16 | A-One | Weal Ag Corporation, Multan | 2012 | {FVH-53 x Exotic *Bt*} | 38.01 | 29.91 | 4.56 | 96.6 |

$=Approved for Punjab and Sindh provinces while rest of the varieties are only approved for Punjab; £=Approved for Sindh province only; ¥=Ginning out turn percentage; €=Thousand pounds per square inch

**Source:** Minutes of 42nd meeting of Punjab Seed Council at Lahore dated Feb 16, 2012, approval documents of cotton varieties and personal communication with breeders of the cotton varieties.

**Table 4.** List of approved Bt-cotton varieties

Now the emphasis is on releasing varieties with a high boll count and a low shedding rate. It has dramatically been changed after the introduction of Bt cotton as it offers inbuilt resistance to the cotton plant; otherwise this trend has not been observed in varieties released before the Bt-era. Also, spring cultivation is gaining popularity in the Punjab province. Around 5-10% of the area is sown early (Feb-March) because of the Bt varieties are not prone to early infestation by bollworms.

## 9. Conclusions

In Pakistan the provision of high quality seed has been a major issue that emerged after the first epidemic of CLCuD. The informal seed sector (growers/breeders/private seed companies) profited by selling unapproved seed of advanced resistant lines resulting in the release of unstable cotton lines in early 1990s and onward. This situation was further exaggerated after

the introduction of Bt cotton varieties. Thus a number of varieties, not properly bred, have been released in a very short time period, which accelerated the varietal replacement rate. All these issues hampered the process of production of certified seed.

The low germination of most cotton varieties, particularly in the post-Bt cotton era, is another area of concern for growers, regulators and policy makers. The germination rate can be improved by avoiding the use of early opened bolls and seed cotton exposed to excessive rain. Similarly, proper control of the moisture content of seed and proper storage conditions can also ensure the good health of cotton seed.

For Bt-cotton, the mixing of various types, mixing of non-Bt seed with the Bt variety, and the expression level of Bt genes in different varieties are the major issues which need to be addressed. The marketing of earlier released cotton varieties/strains under different names in the market is another area of concern which has affected the reputation of the cotton seed industry. In this regard, FSC&RD must ensure the distinctness of each of the newly developed varieties and or advanced strains with authenticated pedigree that may be verified by the use of DNA fingerprinting.

The deterioration of a cotton variety leads to reduced seed cotton yield. One of the major causes of this is a high natural cross pollination rate, largely by honeybees, in the Bt cotton era due to a reduction in the number of insecticide applications. Most of the breeding centers are located near urban areas where farmers also grow fodder and vegetable crops which provide alternative hosts for pollinators. Under such circumstances, selfing of plants is recommended on the representative plants of the variety/genotype which would help in maintaining the typical features of the variety.

Cultivation of hybrid cotton showing heterosis for seed cotton yield has remains a major challenge in Pakistan. Conventional methods of hybrid seed production (manual emasculation of floral buds), low seed setting, high cost of production resulting in high cost of seed and purity of seed are the major issues for cultivating hybrid cotton on significant area. Though limited efforts by the private sector (Mr Siddique Akbar Bukhari spent ~30 years; Guard and Four Brothers Seed Corporation Pakistan.) and public sector organizations (CCRI Multan, CRI Faisalabad, NARC Islamabad etc.) have been made, but are unable to provide seed which can cover even one percent of the total cotton growing area of Pakistan. In this regard, the development of male sterile and restorer lines, deployment of new genomic tools (such as RNAi technology), and also chemical emasculation, are the most plausible approaches for overcoming the issue surrounding the widespread adoption of hybrid seed.

Cultivated cotton has a narrow genetic base which limits future breeding progress. The selection and crossing of well adapted cotton varieties for developing new varieties are the main causes of the narrow genetic base. This problem can be partly overcome by involving genetically diverse parent genotypes in the genealogy of a new variety. For example, genes conferring resistance to abiotic stresses, particularly drought, and biotic stresses, particularly resistance to CLCuD, can be introgressed into *G. hirsutum* L. from *G. arboreum* L. and or *G. herbaceum*L. Tissue culture tools may help in overcoming the phyletic barriers. Preliminary steps have already been taken for introgressing useful genes into the cultivated cotton varieties

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at CRS Multan and CCRI Multan. Similarly, QTLs/genes conferring high quality traits have been transferred into *G. hirsutum* L. using DNA markers at NIBGE, Faisalabad. Another strategy for the creation of genetic variability is the deployment of various mutagens (radiation and chemical). In this regard, leading genotypes of *G. hirsutum* L. and G. *arboreum* L. have been treated with EMS to develop TILLING populations—would help in understanding the genes involved in conferring various traits of interest.

The introduction of new genes from distantly related species using gene cloning and transformation approaches has emerged as a revolutionary genomic tool worldwide. In common with many other major cotton growing countries, the public sector in Pakistan has made substantial investment in developing GM-cotton conferring resistance to biotic and abiotic stresses. Bt cotton containing *Cry1Ac* gene is cultivated on 82% of the area of Pakistan. The first step towards the introduction of two genes (*Cry1Ac* and *Cry2Ab*) has been taken at CEMB Lahore and NIBGE Faisalabad. The material is being tested in multiple trials. For commercialization of advanced cotton lines containing these two genes, proposals for approval from the National Biosafety Committee have been submitted. Secondly, the expressing transgenes in different genetic backgrounds should be quantified to identifying genotype (s) best suited for commercial cultivation. This practice will reduce the possibility of resistance against the target pest developing—will help in formulating IPM strategies. Thirdly, Bt toxins are lethal to insects belonging to different orders. Hence, proper characterization of Bt gene cultivars is imperative before their release into the environment. To evaluate the possible impact of transgene containing cotton, or their byproducts, it is important to establish dedicated biosafety labs, which are lacking at the moment in the country, Ethyl methanesulfonate to ensure the safe release of GM crops and their products.

## Abbreviations

| | |
|---|---|
| AARI | Ayub Agricultural Research Institute |
| AFLP | Amplified Fragment Length Polymorphism |
| ARI | Agricultural Research Institute |
| CBD | Convention on Biological Diversity |
| CCRI | Central Cotton Research Institute |
| CEMB | Centre of Excellence in Molecular Biology |
| CLCuD | Cotton Leaf Curl Disease |
| CRI | Cotton Research Institute |
| CRS | Cotton Research Station |
| DAS | Days After Sowing |
| EMS | |
| GM | Genetically Modified |

| | |
|---|---|
| GOT | Ginning Outturn |
| HVI | High Volume Instrument |
| IR | Insect Resistance |
| ITPGRA | The international Treaty on Plant Genetic resources for food and Agriculture |
| NARC | National Agriculture Research Centre |
| NARS | National Agricultural Research System |
| NIAB | Nuclear Institute for Agriculture & Biology |
| NIBGE | Nuclear Institute for Biotechnology and Genetic engineering |
| PAEC | Pakistan Atomic Energy Commission |
| PARB | Punjab Agricultural Research Board |
| PARC | Pakistan Agricultural Research Council |
| PCCC | Pakistan Central Cotton Committee |
| PGR | Plant Genetic Resources |
| RAPD | Random Amplified Polymorphic DNA |
| RFLP | Restriction Fragment Length Polymorphism |
| SSR | Simple Sequence Repeat/Microsatellite |
| TILLING | Targeting Induced Local Lesions in Genome |

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breeding cotton varieties and unraveling the genetics of various traits of cotton using conventional approaches over the last six decades.

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