**Chapter 21**

**Cotton (*Gossypiumhirsutum* L.)Breeding Strategies**

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**Abstract**This chapteris focused on the achievements and future prospects of cotton breeding and biotechnology. Traditionalplant breeding has been utilized for the development of pure line selectionfor high yielding cotton genotypes in segregating generations through the pedigree method. Selection criteria include boll number plant-1, boll mass, sympodial branches, ginning outturn percentage. Plant breeder efforts have been fruitful in releasing the cotton varieties with high yield potential and superior lint quality traits.Traditional breeding efforts resulted in the increase of seed cotton yield and fiber length. The calculated increase in the yield potential was 1.34 kg ha-1 year-1.However, further genetic gains due to selection for high yield potential had reached a plateau since last two decades and the increase in yield was due to better cotton husbandry techniques in few years. Cotton ideotypes specifically for various agronomic and environmental conditions may be developed. Moreover, utilization of wild relatives for the introgression of disease resistance and abiotic stress tolerance is proposed through traditional plant breeding along with molecular markers to reduce linkage drags due to wild relatives. These high yielding varieties with superior agronomic and adaptability traits may be further used for the development of transgenics. Genome editing technique such as CRISPR/Cas (clustered regularly interspaced short palindomic repeats: associated protein) is one of the emerging technologies to knock out genes or SNP (single nucleotide polymorphism) substitution at specific site with future prospects for the development of disease resistant crop varieties.

**Keywords** CRISPR • Genetic gains •Ideotype • Introgression • Transgenic • Wild relatives

1. **Introduction**

Cotton is an important cash crop,grown in more than 90 countries for its vegetable fiber which is processed by the textile industry. Globally cotton was cultivated on an area of 34.74 million ha which was app. 3% of the total world arable land (FAO 2014). Among the countries, India china, USA and Pakistan are the largest producer of raw cotton. The cotton seed is also consumed by oilseed industry for vegetable fat and cooking oil production while seed cake is consumed by animal and poultry farming. The quality of cotton seed products is influenced by the fatty acid content and chemical composition,which is known to vary among cotton genotypes (Al-Bahrany and Al-Khayri 2000).One important health concern is the gossypol contents and related sesquiterpenealdehydes in cotton seed meal, which function as natural phytoalexins against pathogens and pests (Tian et al. 2018).

Average global cotton production was 73.44 million tons during 2011–2016 (FAO 2016) which was 12% higher than the average world cotton production during 2001–2010, (65.23 million tons) and 36% higher than the average world cotton production during 1991-2000 (54 million tons). There was an increase of 21% in world cotton production when 1990–2000 and 2001-10 were compared while an increase of 13% in global cotton production was seen in present decade (FAO 2016). The world production grew by 1.5% year-1 in last three decades.The global trade of cotton lint was only 21.30 million USD showing that most of the cotton was consumed and processed within cotton producing countries (FAO 2016). The cotton world map of production is shown in Fig. (1).

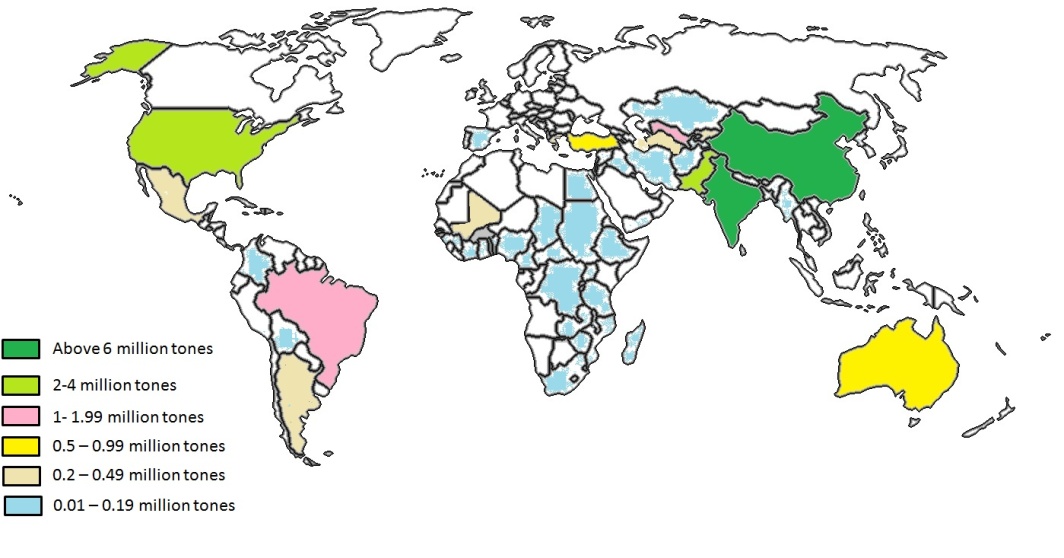


Fig 1. The world map of cotton production. The map prepared as per Food and agriculture statistics, 2016 (www.fao.org)

The yield gains in cotton has been attributed due to traditional plant breeding which developed cotton varieties having high yield potential, superior lint qualities, and tolerance to abiotic and biotic stresses along with early maturity to convert this crop from biannual to an annual growth habit. Cotton breeding is based on the exploitation of genetic variation within cotton germplasm and to select the transgressive plants with superior yield and lint quality. However, breeder selection efficiency was known to dependent on the heritability of the traits. Since most of economical traits were polygenic and are affected by the environment, therefore, the variation among the traits was often masked by the environment. The development of molecular marker system and whole genome sequences analysis has led to the identification of genes and genomic regions associated with traits of interest by which selecting cotton varieties is facilitated inbreeding programs (Fig. 2).

Cotton is among the first crop in which transgenic varieties have been successfully commercialized for large- scale cultivation during mid of 1990s (Zhang 2013). Transgenic cotton provided resistance against the boll worm complex which reduced the pesticide spray by nearly 50% (Traxler et al. 2001;Traxler and Godoy-Avila 2004). It is transformed with *Cry* genes encoding crystal protein δ-endotoxin (Bt toxin) obtained from *Bacillus thuringenesis* which is activated in the insect gut causing the death of the organism. Herbicide resistant genes reduced the yield losses due to weed competition for nutrients, light and soil moisture. The success of transgenic cotton in combating boll worms brought tremendous investment of private sector for the research and development of transgenic cotton in various countries (Qiao 2015). Experimentation and evaluation of other transgenic genes have been carried out with a hope to commercialize them in future for sustainable cotton production. Details are present in *Section 5*. Moreover, cotton regeneration system has been optimized and several methods for development of transgenic cotton have been devised.

**Fig.2** Summary of integrated efforts for the improvement of cotton species through breeding and biotechnology

Establishment of functional genomic database in cotton is another milestone for cotton breeding and biotechnology that could help to develop molecular markers related to traits having importance in cotton breeding programs, mining of valuable genes from genome analyses and helped to understand the key metabolic pathways of commercial importance such as fiber development and oil contents (Arpatet al. 2004).

This chapter reviews theachievements and future prospects of cotton breeding and biotechnology. It covers breeding and biotechnology aspects of cotton for high and sustainable production.

1. **Cotton Sustainable Production**

Cotton yield and production is an outcome of harvested bolls from a unit of population over multiple picks (Constable and Bange 2015). However, production is threatened by various social, economic, environmental, agronomic and biological factors (Gutierrez et al. 2015). Sustained cotton production strategies aim to improve these factors such as limiting degradation of environment, loss of bio-diversity, soil erosion, exhaust and leaching of soil nutrients, evolution of new pathogens and bio-type insectsdue to various practices of agriculture in cotton zone (Gutierrez et al. 2015). A precision agriculture may be implemented to precisely carry out various cultural activities from sowing to harvesting for continues success of the cotton crop with optimum use of farm resources, without affecting the environment and maximization of cotton farm income and profits (Roberts et al. 2002; Torbett et al. 2007).

The biological factors include the genotype performance “the ability to respondsuccessfully to a particular environment”. Varietal performance depends upon its genetic potential, stability over the range of environment and resistance to various yield-limiting factors of a particular area. Cotton yield potential is defined as the harvested yield through current cultivars and crop husbandry practices under ideal conditions in the absence of yield limiting factors (Constable and Bange 2015). Conventional cotton breeding tools such as selection and hybridization have been used to widen the genetic potential of cotton and biotechnological tools such as recombinant DNA technology have been employed to induce resistance against various biological and environmental yield-limiting factors. Better crop stand also play an important role in fully exploiting the benefits of the farm inputs and soil. Good crop stand depends on the optimum plant population per unit area, which is contributed by the percentage of seed germination, speed of germination (equals the sum of n1/d1+n2/d2+n3/d3+…;where, n = number of germinated seeds and d= number of days) and seedling vigor (Gairola et al. 2011). Optimization of these parameters is improved through seed technologies.

1. **Cotton Genetic Resources and Conservation**

***3.1 Cytogenetics and Evolution***

Cotton has narrow genetic base due to bottleneck after evolving into allo-polyploidy species, domestication of few high yielding genotypes and intense pure line selection (pedigree selection method) by the breeders (Rauf et al. 2010; Kuraparthy and Bowman 2013). The upland (*hirustum*) or pima cotton (*barbadense*) evolved from two highly divergent species native to Africa (*herbaceum* like) carrying A genome and *raimondii* like species carrying D genome which is native to South America (Paterson et al. 2012). Parental species intercrossed about 1-2 million year ago, when species carrier of A genome spread in Mexican region through trans-oceanic dispersal (Paterson et al. 2012). Mating between the species lead to the evolution of several tetraploid species, out of which *Gossypiumhirsutum* and *barbadense* are widely cultivated throughout the tropical and subtropical regions of the world. A number of studies using molecular marker or phenotypic based studies have confirmed the presence of low genetic diversity in cotton elite germplasm (Raufet al. 2010). Therefore, there was a need to broaden genetic diversity within cotton germplasm and to use the wild germplasm resources for the improvement of cultivated species (Shaheenet al. 2012). The genus *Gossypium* comprises more than 50 species, out of which four are cultivated (Fig 3). These cultivated species have2 diploid and 2 tetraploid species. Among 50 species, 7 species areallo-tetraploid and 43 are diploid (Paterson et al. 2012). The A genome is native to the Africa while A1 and A2 genomes are native to Afghanistan, China and Burma. C and G genome evolved in Australia, D native to the America and E genome evolved in Arabian Peninsula(Wendel 2000).

***3.2 World Cotton Collections***

Cotton world collections has been classified into eight major sites including India, Brazil, France (CIRAD), USA, Australia, Uzbekistan, Russia, China (Campbell et al. 2010). Uzbekistan had the highest germplasm collection of *G. hirsutum* L. (13,241 accessions) followed by China (7712 accessions), India (7633 accessions), USA (6302 accessions), Russia (4503 accessions), France (2103 accessions), Brazil (1660 accessions) and Australia (1518 accessions) (Campbell et al. 2010). Uzbekistan (3019 accessions) also hold the world largest collection of *G. barbadense* followed by USA (1584 accessions), Brazil (1509 accessions), Russia (1057 accessions), China (633 accessions), India (534 accessions), France (483 accessions) and Australia (104 accessions) (Campbell 2010).

Germplasm from various sources has been collected and maintained at the Central Cotton Research Institute, Multan, Pakistan (Imran, 2018, Personal Communication). The collection includes 4243 accessions of *G. hirsutum*, 1025 accessions of *G. arboretum*, 556 accessions of *G.herbaceum*, and 109 accessions of *G. barbadense*. Several of classified *Gossypium* species were not maintained in their native sites and cotton germplasm was venerable to diseases and insect infestation and many cotton species were at the verge of extinction (Campbell 2010). A survey was carried out in Mexico during the year 2002-03 to determine the current status of the various diploid species of genus *Gossypium*. Increased population, rapid urbanization and pollution have threatened the local land races of diploid species of cotton (Ulloa et al. 2006). The populations of seven diploid species were collected from door yards, garden plots and as feral plants (Ulloa et al. 2006).Insitu preservation of all 11 diploid Mexican species was under threat.Therefore, collaborative efforts are required to be undertaken to preserve the cotton germplasm resources.The conventional method of in situ conservation was not applicable and exsitu conservation including seed bank for conservation of wild population should be considered (Almeida et al. 2009).

In order to preserve cotton germplasm in situ techniques such as nodal cultures, cryopreservation has been recommended (Altman 1990).

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| E:\Cotton wild species\DSC_0986.JPG  **F**  **E**  **D** | E:\Cotton wild species\DSC_0991.JPG | E:\Cotton wild species\DSC_1001.JPG |
| E:\Cotton wild species\DSC_1006.JPG  **G** | E:\Cotton wild species\DSC_1014.JPG  **H** | E:\Cotton wild species\DSC_1029.JPG  **I** |
| E:\Cotton wild species\DSC_1049.JPG  **J** | E:\Cotton wild species\DSC_1054.JPG  **K**  **A** | |

**Fig. 3**Ex situ wild germplasm collection at Central Cotton Research Institute, Multan, Pakistan. **a**G. *somalense*, **b** *G. laxum*, **c** *G. lobatum*, **d** *G. capitiverdis*, **e** *G. nelsoni*, **f** *G. longicalyx*, **g** *G. gossypioides*, **h** *G. anomolum,* **I** *G. herkensii*, **j** *G. arboreum* red, **k** *G. arboreum* (Photos by H.M. Imran)

***3.3 Utilization of Wild Germplasm***

The wild germplasm could be utilized for introgression of cytoplasmic male sterility, diseases and abiotic stress resistance, and to widen the genetic diversity of the cultivated species (Shaheenet al.2012; Table 1). Synthetic species can be reconstituted from donor species i.e. *herbaceum* and *raimondii*. Linkage drag from wild types i.e. introgression of undesirable alleles along with gene of interest is major limitation for use of wild species in cotton improvement breeding program. The deterioration of yield and quality, divergent gene regulatory system, chromosome structural differences, hybrid break down and genome assortment without introgression were the major limitation in utilization of wild germplasm for cotton breeding (Dioufet al.2014; Zhang et al. 2014). Molecular markers were used to reduce the linkage drags in back cross and transgressive breeding populations or development of substitution lines (Wang et al. 2011). Introgression of useful genes such as diseases or insect resistance especially from the wild relative also introduced non-targeted orundesirable genes (such as photoperiod sensitivity, late maturity,poor fiber and yield traits) which reduce the overall performance of introgessed populations and thus these breeding lines were not directly commercialized. Use of molecular markers in foreground and background selection could help to minimize the introgression of non-targeted loci and maximize the genome of recipient species along with gene of interest. This could also help to reduce the number of back crosses require to achieve the degree of homozygosity.

Chromosome substitution lines from wide crossing between *barbadense* × *hirsutum* were developed with the objective to combine the high yield, lint percentage with better lint quality traits (Sahaet al. 2010). The chromosome by chromosome approach was exploited to narrow down the search for the genotypes with high yield and premium quality fiber (Sahaet al. 2010). Breeding potential of introgression lines (*barbadense* × *hirsutum*) showed that some of the breeding lines had predominance of additive variance and were positive contributors of alleles related to yield and quality (Zhang et al. 2016). Developed introgression lines may be used for the development of hybrids and transgressive lines (Zhang et al. 2016).

**Table 1** Development of breeding material from wild species of cotton

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| Interspecific crosses | Breeding material | Reference |
| *hirsutum* Cultivar 86-1× G. armourianum | Resistant to sucking pest such as jassid, white fly due to intense hairiness | Junqiet al. (1995) |
| *hirsutum* × *arboreum* | Sterile triploid hybrids which may be amphiploid to transfer cotton leaf curl virus resistance | Tahir et al. (2002) |
| *hirsutum* × *arboreum* and | Eight varieties were developed after introgression with lint yield. High yielding cotton variety Shiyuan 321 (Jimian 24) occupied the highest area in yellow river valley | Zhenglanet al. (2002) |
| *longicalyx* × amourianum × *hirsutum*  *longicalyx* × *herbaceum* × *hirsutum* | Tri species hybrids were resistant to nematodes infestation | Bell and Robinson (2004) |
| *hirsutum*Acala 44 × *barbadense*Pima S-7 | Three QTL CM12, STS1, 314-7 had large effect over verticillium wilt resistance | Boleket al. (2005) |
| G. barbadense,  G. arboreum and  G. thurberi | Developed breeding lines after introgression with *hirsutum* had superior fiber quality, expanding genetic diversity and resistant to Fusarium wilt and Verticilium wilt | Pang et al. (2006) |
| *G. anamolum* | Genes for fiber fineness and strength and resistance to insect and disease | Mehetreet al. (2010) |
| *hirsutum*× *barbadense* | Salt tolerant back cross inbred lines surpassing both parents due to transgressive breeding | Tiwariet al. (2013) |
| G. *gossypioides* | Resistant to cotton leaf curl virus disease and may be exploited for introgression | Azharet al. (2013) |
| *hirsutum*× *arboretum* (followed by back crossing to *hirsutum* | Interspecific F1 hybrid was completely resistant to cotton leaf curl virus, resistance break down with back crossing scheme due to lack of introgression from *arborem* | Nazeeret al. (2014) |
| Synthetic amphiploidhirsutum× arboreum (AADDAA) | Amphiploid was resistant to verticillium and drought resistance | Chen et al. (2015) |
| Gossypiumcapitis-viridis×(*G. hirsutum× G. australe*) | Tri-species hybrid was intermediate in canopy characteristics and resistant to insect | Chen et al. (2015) |
| *herbaceum* (A1) × *raimondii* (D5) | Sterile hybrid with characteristics intermediate between the species. Synthetic species was used to understand speciation, genome interaction and evolution of tetraploid species | Wu et al. (2017) |

* 1. ***Colored Cotton***

Cotton produces white lint color. However, there is great variability within tetraploid cotton for the lint color. The lint is available from various shades of brown color to the light green (Fig 4). Colored cotton provide great potential for the textile industry to produce cloth without use of synthetic dyes and chemicals which causes allergies to the consumers and their effluent are toxic for the environment. It is also major component of the organic cotton, i.e. cotton produced with less damage to the environment without use of synthetic chemicals. However, colored cotton fiber qualities (fiber length, strength and micronaire) are inferior to the white cotton and cotton color is affected by the environment upon maturity. Therefore, there is need for the selections of colored cotton with stabilize the pigmentation and superior fiber quality for its acceptability in the textile industry.

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| **E:\picture\1 (10).jpg**  **A** | **E:\picture\1 (7).jpg**  **B** | **E:\picture\1 (1).jpg**  **C** |
| **E:\picture\1 (3).jpg**  **D** | **C:\Users\Acer\AppData\Local\Temp\Rar$DR97.822\DSC_2517.JPG**  **E** | |

**Fig. 4** Genetic diversity in cotton lint color. **a** Light brown,**b** Khaki, **c** Light green, **d** Dark brown, **e** White (Photos by H.M. Imran)

Colored lint develops as a result of accumulation of natural pigmentation in the fiber after exposure to the natural sunlight. All colors are genetically controlled having monogenic inheritance. The green color lint is controlled by the allele Lg (de Carvalho et al. 2014). There were about six alleles (Lc1-Lc6) for the brown color (Kohel 1985). Lc1-Lc2 produced brown color, Lc3 produce dark brown color and Lc4- Lc6 produces light brown pigmentation in lint (Kohel1985). Lc1 was assigned to chromosome 7 and Lc2 on chromosome 6 (Wang et al. 2014).

1. **Traditional Cotton Breeding Programs**

Traditional breeding based on basic principles and selection methods has been successfully employed in selecting cotton varieties with greater yield potential along with acceptable fiber quality traits. It involves developing crosses between good × good, or good × poor, which were selected through pedigree or recurrent selection in segregating population to establish pure lines. The selection pressure for early type varieties resulted in improvement of harvest index per unit area, and switching the cultivars from perennial to annual growth habit reduction in the monopodial branches with subsequent increase in the direct fruiting branches. A positive impact of breeding has been noted over the fiber quality traits and genetic gains for fiber quality traits (Kuraparthy and Bowman 2013). However, it is known that cotton yield has reached a plateau and further genetic gain in lint yield was not possible due to drain of genetic variation within elite cotton germplasm (Raufet al. 2012). Several types of cotton ideotypes have been proposed to develop cotton varieties with high yield potential or sustainable yield under various agro-ecological conditions, biotic and abiotic stresses (Table 2).

**Table 2** Various ideotype of cotton to full fill ambitious breeding objectives of cotton

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| Breeding objective | Plant ideotypes | References |
| High yield potential | Compact canopy, high number of boll per meter2, multiple bolls per fruiting point (boll borne in cluster), medium size boll, good boll opening, earlier anthesis, long reproductive duration, increase photosynthetic rate | Seklokaet al. (2008);  Loisonet al. (2017) |
| Mechanized farming | Uniform opening, small to medium compact canopy, direct fruiting branches, early maturing, good boll opening, normal broad shaped leaf at the base and okra leaf at the canopy | Karathikeyanet al. (2015) |
| Early maturity | Effective flowering time, plant height at harvest, length of fruiting branch, height to node ratio, average boll retention at first fruiting branch | Seklokaet al. (2008) |
| Insect resistant | Long frego bract, okra leaves, hairiness, small leaf area, red colored leaves, glandless leaves, nectariless | Taggar and Arora (2017) |
| Drought resistance | Small leaf area, early maturing, intense hairiness, high cuticular waxes, longer root length, greater stem reserve mobilization | Raufet al. (2016) |
| Heat resistance | Cell membrane stability, ability to bear flower and retain boll at lower nodes, leaf and stem angle, lower leaf senescence, canopy architecture, leaf thickness, leaf angle | Loison et al (2017)  Pauli et al.(2017) |

Rapid genetic gain in the seed cotton yield potential of Pakistani cultivar was noted until 1980s (Fig 5). The yield potential expanded from 600 kg ha-1 to 3500 kg ha-1 from 1916 to 1988; afterward there was no change in the yield potential of the released cultivars. The increase in the yield potential was about 4.4% per year which was about 1.34 kg ha-1 year-1. There was also steady increase in the ginning out turn and staple length of the Pakistani cultivar; both traits increased by 0.5% and 0.6 per year respectively (Figs.5‒6).

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**Fig. 5** Change in yield potential and staple length of cotton cultivars release in various periodicals

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**Fig. 6** Change in ginning out turn and yield gaps yield potential and staple length of cotton cultivars release in various periodicals

Correlation analyses showed that there was high significant (P ≤ 0.05) and positive relationship between the actual and potential yield, showing that release of high yielding cultivars had profound effects over the actual cotton seed yield in Pakistan (Table 3). Correlation between the potential seed cotton yield of cultivars with ginning out turn and staple length was also positive and significant (P ≤ 0.05) showing that high yielding cultivar also had superior fiber quality and increase in yield potential was not at the expense of fiber quality (Table 3). There was no improvement in yield potential later in 1990s – 2005. However, there was some increase in actual yield which may due to better crop husbandry managements, early sowing and management of insect infestation. Studies have also shownthat increase in lint yield occurred at the expense of seed yield (Loisonet al. 2017). Estimated genetic gain in lint yield at Cameroon was about 3.3 kg ha-1 year-1 due to increased ginning out turn. However, no genetic gains were noted for physiological traits such as radiation use efficiency, aerial biomass, harvest index, leaf area index and seed cotton yield (Loisonet al. 2017).

**Table 3** Correlation coefficients between yield and quality traits

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| --- | --- | --- | --- | --- |
| Traits | Yield potential | Actual yield | Yield gap | G.O.T. |
| Actual yield (Kg ha-1) | 0.72\* |  |  |  |
| Yield gap (kg ha-1) | 0.66\* | -0.06NS |  |  |
| Ginning out turn (%) | 0.76\* | 0.61\* | 0.22NS |  |
| Staple length (mm) | 0.62\* | -0.21NS | -0.02NS | 0.47\* |

\* Significant at P ≤ 0.05; \* ginning out turn

Plant breeders also developed the hybrid cotton with significant economical heterosis for fiber yield and quality traits (Lian-gen 2011). It is principally grown in India, China and Vietnam, while India pioneered the hybrid cotton. In India 40% of the cotton cultivation was done through hybrid seed while in Vietnam about 70% of the cotton area was occupied by the hybrids. Development of high yielding hybrids could expand the yield potential of the varieties. The yield advantage of hybrid cotton in China was more than 20% over the open pollinated varieties (Dong et al. 2004). Bthybrid cotton was considered as an example of successful integration of traditional cotton breeding with biotechnology, as hybrid Bt cotton was based on the manifestation ofheterosis between non-Bt and Bt breeding lines (Dong et al. 2004). Recently several cotton hybrid varieties with high yield, lint percentage, multiple resistance and wide adaptability has been released for general cultivation in various parts of the world (Table 4). New technologies such as molecular markers have been employed to study the genetic purity of hybrids (Dongreet al. 2011). Hybrid varieties also exploited the heterosis for the development of long or extra-longstaple cotton through interspecific hybridization between the *hirsutum* × *barbadense* species.

**Table 4** Cotton hybrid performance under various agro-environment conditions

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| Crosses | Heterosis | Reference |
| Hybrid transplantation technology | Transplanting BT hybrid could help to escape the chilling stress under controlled condition and provided additional one week growth period. | Dong et al. (2005) |
| Hybrid performance over organic vs. conventional cultivation | 37–71 % more bolls under organic condition and superior fiber traits | Blaiseet al. (2008) |
| Hybrid Ji-FRH3018  CMS-3096 × Ref 866 | High resistance to bollworm, Fusarium wilt and Verticillium wilt and high yield and lint quality | Guoet al. (2010) |
| Cytoplasmic male sterility source from *Gossypiumharknsii* | Negative impact of CMS source over number of bolls per plant and fiber quality traits | Tutejaet al. (2011) |
| Cotton hybrids CRI-28, CRI-29, XZM 2 and Jimian18. | Gene differential expression in hybrids when compared to parents which changed over various phonological stages. | Zhu et al. (2011) |
| Commercial Hybrid Xiangzamian 3 | Hybrid had better canopy cooling ability. Maximum heterosis for net photo synthesis rate was observed during post noon when temperature exceeded 45⁰C | Zenget al. (2012) |
| *hirsutum* × *barbadense*chromosome segment introgression lines | Stable heterotic loci (hLP-A4-3) was detected in all three years | Guoet al. (2013) |
| Okra leaf hybrid F1s, Crossed two sterile near isolines and three restorer near isolines | Lint percent 7–12%, boll weight (6–11%), Canopy light intensity (2–147%), 1–10% in net photosynthetic rate (Pn), and −3 to 3% in lint yield over check hybrid (Zhongza 29) | Zhu et al. (2013) |
| Recombinant inbred lines | 29 QTLs were linked to mid parent heterosis. Genetic basis of heterosis in cotton was due to dominance, partial dominance or epistasis | Shang et al. (2016) |

1. **Genomic Database**

The presence of large amount of sequence data set of cotton has led to have an integrated cotton functional data set (Zhu et al. 2017). Cotton functional data set (CottonFGD, <https://cottonfgd.org>) has been established which allow the easy, quick and user friendly access for the cotton functional genomic data set including all the*Gossypium species*genome data published. Cotton FGD was exploited to access genes for leaf shape and arginase (Zhu et al. 2017). The comparison of data sets from various upland and sea-island identified various polymorphic sites for the development of molecular markers for these traits. These sequences were used for marker- assisted -selection (MAS) within F2 population to identify the genomic regions associated with these traits. Marker- assisted- selection along with Cotton FGD tools narrowed down the genomic region associated with traits of interest resulting in the identification of 81 genes. Among the identified genes, *ATHB-51* (*Gh\_D01G2042*), a homeobox-leucine zipper protein was homologue to leaf shape gene in *Arabidopsis thaliana*. The draft genome sequence of *raimondii* species has been published (Wang et al. 2012) which showed that *raimondii* genome contained more than 40,976 protein-coding genes. However, species genome had under tremendous recombination with more than 40% gene synteny (Wang et al. 2012). Some key genes related to the fiber elongation were explored through expressed sequence tags (Arpatet al. 2014). More than 80 genes related to various developmental stages of fiber development were identified (Arpatet al. 2014). Several fiber related genes such as*GhTTG1-GhTTG4*, *GhGa20ox1-3*, *iaaM*,*GbPDF1*, *GhJAZ2* (fiber initiation), *GhSusA1, PHYA1* (fiber length)*GbPDF1, E6*, *GhExp1*,*RLK* (fiber strength), *GbTCP*, *WLIM1a*, *PAG1*,*GhCaM7*, *ACO*,*GhHOX3*, *PIP2s* (fiber elongation) has been reviewed and were considered as primary genes expressed at various stages of fiber development (Ashraf et al. 2018).The genes were unknown to regulate the fiber development pathways. Susy gene was found to be positively involved while GhPRP5 was negatively involved in the same fiber elongation pathways. The list of biotic and abiotic resistant genes and their putative function has been described in Table5andTable 6.

**Table 5** Development of transgenic for protection against various types of biotic stresses

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| Trans-genes | Resistance | Reference |
| Hen egg white lysozyme, GAFP4, *FreB* gene (VDAG\_06616)  Gastrodia antifungal proteins | Resistant to Verticilium wilt | Wang et al. (2016);  Wenfang et al. (2017);  Rehman et al. (2018) |
| *GR79 EPSPS* and *N-acetyltransferase* (*GAT*) genes | Resistant to glyphosate | Liang et al. (2017) |
| *cry1Ac* | BT toxin induction in *hirsutum* | Anayol et al. (2016) |
| *Galanthusnivali* agglutinin (GNA) and *Amaranthuscaudatus*agglutinin | Genes with anti aphid function having 75% control over the pest. | Yang et al. (2017) |
| Pyramiding RNAi and BT technology | PyramdingRNAi and BT technology was effective to control resistant *Helicoverpaarmigera* types | Ni et al. (2017) |

**Table 6** Characterization of various trans-genes under abiotic stresses

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| Trans-genes | Tolerance | Reference |
| Isopentenyltransferase gene | Increased salt tolerance due to higher chlorophyll and cytokinin contents which delayed leaf senescence | Liu et al. (2012) |
| LOS5/ABA3 (LOS5) encodes a molybdenum co-factor | Drought resistance due to lower transpiration losses and over accumulation abscisicacid | Yueet al. (2012) |
| Annexin gene, GhAnn1 | Enhance drought and salt tolerance | Zhang et al. (2015) |
| PeDREB2a and HhERF2 from eighty-six AP2/ERF | Transgenic plants containing PeDREB2a and KcERF showed tolerance to salt and drought stresses | Bo Li et al. 2017 |

**6 Mutation Breeding**

Mutation breeding program has been initiated for the improvement of cotton in various parts of the world during 19060s. High fruiting, diseases resistance, early maturity, lower monopodial growth habits were the main targets of these breeding programs (Saeed Iqbal 1994; Muthusamy et al. 2005).The mutants were generated with exposure of plant tissues such as seed (30KR), pollen (10 gyGy gamma rays), shoot tip (5-50 Gy gamma rays) ovule (50 Gy gamma rays) or with chemical mutagen 1-5 mM of ethyl methanesulfonate(Saeed Iqbal, 1994;Muthusamy et al. 2005; Muthusamy and Jayabalan 2011;Muthusamy and Jayabalan 2014; Aslam et al. 2018). Lower doses and concentration generated higher genetic variation for traits such as early flowering, plant height, number of bolls, yield of seed cotton, ginning percent, seed index, harvest index and fiber traits (Muthusamy and Jayabalan 2011; Muthusamy and Jayabalan 2014).Cell lines of varieties (Acala SJ2 and B1654) were developed by exposure of 2.13 µmol to sulfonyl urea. The embryogenic resistant cell had L50 several 100 times greater than unselected lines (Rajeskaran et al. 1996). Regenerated plants were resistant to primsulfuron (0.06 to 0.21 μM), and were cross tolerant to two imidazolinone herbicides, imazethapyr and imazaquin (Rajeskaran et al. 1996). The Coker-2312 cell lines were exposed to the progressive higher doses of glyphosate (20 mM) (Tong et al. 2010). Regenerated calli line “R1098” can tolerate about 1.48 kg acid ha-1 glyphosate (Tong et al. 2010).

A team at Nuclear Institute of Agriculture Biology, Faisalabad, Pakistan led by the lateRana Saeed IqbalKhan evolved a revolutionary cultivar NIAB-78 obtained by irradiating the F1 cross of (Deltapine × AC-134). The Govt. of Pakistan awarded him the highest presidential award for the evolution of this cultivar and his services for the cotton community (Personal communication: Hafiz M. HassanMumtaz, 2019). The developed mutant was early maturing and high yield potential which increase the overall cotton production of Pakistan to several folds and was able to fit in double cropping system (Wheat-Cotton) of Pakistan. It occupied about 90% of the area during early 80s and total Pakistan cotton production increased from 3 million bales to about12.8 million bales (www.niab.org.pk). The cultivar was abounded due to its susceptibility to CLCuVD during mid 1990s(Personal communication: Hafiz M. HassanMumtaz, 2019). A high yielding mutant NIAB-92 was developed by irradiating seed of Stoneville-231 with gamma rays at 30KR from 60CO. It has semi hairy, compact canopy wih 0-2 monopodial branches as compared to its parental cultivar (Saeed Iqbal, 1994). Later on, two new mutants (NIAB-999, NIAB-111) were released for cultivation which were high yielding, heat tolerant and CLCuVD resistant. NIAB-777 was developed by crossing the NIAB-78 with REBA-288. The pollen of REBA-288 were irradiated with 10 Gray of gamma rays before pollinating NIAB-78 (Aslam et al. 2018). Plants were selected for high yield, better fiber quality and disease resistance during segregating which resulted in the evolution of NIAB-777 cultivar (Aslam et al. 2018).Mutant with high fiber quality (fiber strength = 40.5) was isolated from segregating populations generated after exposure of “MD15” to 3.2% v/v ethylmethanesulfonate (Bechere et al. 2013).

**7 In Vitro Plant Regeneration and Tissue Culture Applications**

A prerequisite for genetic transformation is the development of a reliable in vitro regeneration system. Cotton is a recalcitrant species for tissue culture and regeneration via somatic embryogenesis is only confined to few varieties i.e. cocker lines (Trolinder and Goodin 1987). Cocker line such as 312 had high regenerable response and heart shaped globular embryo were cultured on semi solid media which germinated into plantlets (Trolinder and Goodin 1987). Research efforts were being carried out for the optimization of genotype independent protocols for regeneration and genetic transformation. Protocol for the invitro regeneration of cocker 312 is as follows: “Seeds of cotton genotype Coker-312 were surface sterilized with 0.1% SDS, 0.1% mercuric chloride and one drop of Tween-20 for 20 minutes in shaking. The seeds were then thoroughly washed three times with double distilled deionized autoclaved water and placed on ½ MS under dark conditions for germination. Hypocotyl portion of the germinated seedlings was aseptically excised and placed on callogenesis media having 4.33 gL-1MS salts, B5 vitamins, 0.1 m gL-12,4-D, 0.5 mgL-1kinetin, and 30 gL-1glucose (Fig. 7, Kumar et al. 2004). The embryogenic calli was selected and further induced for somatic embryo genesis. Heart shaped embryos were selected for shoot regeneration and finally healthy shoot were rooted on growth media (Kumar et al. 2004).

Cotton regeneration protocol have been claimed to be optimized in several cultivars around the globe (Pathi and Tutega 2017). For instance, Chinese cultivar YZ-1 showed higher regeneration potential (81.9%) when compared with coker lines 312 and 212 (Jin et al. 2006). A selection toward regeneration potential in elite cultivar was carried out for two cycles and selected cell lines within elite cultivar were designated as “Max-R” (Mishra et al. 2003). The regeneration response was also known to be dependent over the explants, in addition to the growth media such as vitamins and plant growth regulators (Pathi and Tutega 2017). Explant such as embryo axis showed higher regeneration response due to direct regeneration in contrast to the leaf disc segments. Shoot tip explants have been used for transformation and transgenic mersitematic cell of Indian cotton cultivars were regenerated into plantlet (Satyavathi et al. 2002). Invitro regeneration in cotton was shown to be genetically controlled traits and showed moderate heritability when local genotypes were crossed with cocker lines (Rauf and ur Rahman 2005). Over dominance to additive type of gene action was involved in the expression of traits such as callus induction, embryogenic calli and germinating embryo (Rauf and ur Rahman 2005).

Regeneration 1.tifregeneration 2.tifRegenration 3.tif

**B.**

**C.**

**A.**

**Fig. 7** *In-vitro* culture of cotton genotype Coker-312 used for genetic transformation work. (**A**) Callus induction from hypocotyl portion (**B**) Inoculated callus on selective media (**C**) Organogenesis on regeneration media

**8 Transgenic Cotton**

Genetic transformation of cotton is categorized as *Agrobacterium*-mediated transformation, Biolistic transformation, *in planta* pollen tube pathway or pollen tube transformation. Agrobacterium mediated transformation is widely used and reliable method of transformation and is achieved by co-cultivation of explants with agrobacterium cultures. Recombinant DNA plasmid containing genes of interest along with reporter genes was first inserted in TDNA or other plasmids. The recombinant plasmid is than inserted in agrobacterium through various methods including elctroportation. *Agrobacterium tumefacians* have the capacity to transform the plant cells with recombinant T-DNA in cotton cell. Triple genes construct carrying cry1AC, cry2Ab and EPSPS was used to transform T-DNA to produce recombinant plasmid (Naqvi et al. 2017). The sequences were retrieved from the NCBI. The gene sequences were optimized according to upland cotton (Naqvi et al. 2017). 2X 35S promoters and terminator sequences were used to induce expression and terminationfor Cry1Ac gene. The 2x 35S promoter was first cloned in pBlue Script SK-zero using *SwaI* and *BamHI* restriction site while Cry1Ac was cloned in pBlue SK-35S using *BaHI* and *HindIII* restriction site. The terminator was cloned using HindIII and SalI restriction sites. Cry2Ab cassette comprise of figwort mosaic virus promoter, chloroplast signal peptide, partial Cry2Ab (270 bp) and G7 terminator (Naqvi et al. 2017). The cassava mosaic virus promoter (700bp) was used along EPSPS (1.9 kb) and E9 terminator to induce the expression of herbicide resistant gene. Gateway cloning technology was used to construct the cotton transformation vector with synthetic EPSPS gene (accession number KP212901). The plasmid vector pK2GW7,0, an *Agrobacterium* compatible plant expression vector was selected to clone the synthetic EPSPS and NPTII as selection marker gene (Fig.8) The final gene cassette was introduced into *Agrobacterium* strain LBA-4404 (Nazir, 2018 unpublished).

Cotton plasmid.tif

**Fig. 8** *Agrobacterium* compatible plant expressing vector pK2GW7,0 with synthetic EPSPS and NPTII selection marker gene for cotton transformation

A method of transformation and regeneration was patent which involved the co-cultivation of cotton explants (hypocotyls from 8 days old seedling) with agrobacterium for two days on callus initiation media (Murashige and Skoog salts, glucose 30 g L-1, myoinositol 100 mg L-1, nicotinic acid 1mg L-1, pyridoxine HCL 1mg L-1, thiamine HCL 10 mg L-1, magnesium chloride 1.87 g L-1, potassium nitrate 1.90 g L-1 and gelrite 4g L-1) without exogenous plant growth regulators (Strickland 1998). The transformed tissues were screened over the 12.5 – 50 mg L-1kenamycin and 150 mg L-1cefotaxime.The survived cell were then continuously cultured on embryo genic calli inducing media and finally germinated over the shoot inducing media (Strickland 1998). The granular calli was inoculated with *Agrobacterium* culture with recombinant plasmid with gene of interest and screened on 50mgL-1 of kanamycin media. Various stages of callogenesis, regeneration and selection are shown in Fig 7 (Nazir et al., unpublished). The cell suspension culture or embryo axis was also bombarded with highly density particles coated with plasmid using bolistic gun (Finer and McMullen 1990).The recombinant plasmid contained hygromycin resistant gene which was used to screen the transgenic cells over culture media (Finer and McMullen 1990).

*In planta* methods are devised toavoid the need for the complicated regeneration protocols (kalbande and Patil 2016). *In planta* pollen tube pathway is also a popular method of transformation. The principle of this method is to use the pollen tube formed by the pollen to insert the gene into embryo sac post pollination/fertilization. The steps are such as pollinating the flower, cutting the stylar tissue (10-12 hours post pollination), injecting the vector solution carrying the gene interest. The desired genes are directly inserted in pollen or injected into developing embryo via pollen tube pathways. *In planta* method of transformation in seedling include a vertical cut in 4- day- old seedlings at the junction of cotyledonary leaves to expose the apical meristem (kalbande and Patil 2016). The exposed apical meristem is then treated with *Agrobacterium* transformed with gene of interest. A transformation efficiency of 6.89% was obtained in variety LRK-516. The highest efficiency of transformation was obtained through *Agrobacterium-*mediated transformation as the gene of interest was inserted in the cells of L3 layers which produce germline tissues. Biolistic transformation produces chimeric tissues due to insertion in non-trageted layers.

Transgenic cotton is cultivated on more than 25 million hectare, which is about 70% of the total cotton area around the globe (Anderson and Rajasekaran 2016). 58 various events carrying insect and herbicide resistance with maximum of three genes (two insect resistance+ one herbicide resistance) along with marker genes have been released for general cultivation in various parts of the world. Transgenic cotton adaptation is increasing at rate of about 5% year-1 containing either of *cry* genes or herbicide tolerant genes (Anderson and Rajasekaran 2016). Transgenic cotton is the second major commercial success of *cry* genes (*cry1 Ac, cry2Ab2, cry2Ae, cry1 Ab, cry1A*) along with herbicide resistant genes (*bar,epsps, bxn*and*dmo*) after soybean. Two transgenic varieties NuCOTN33 and NuCOTN35 with trademarkBollgardTM were released for general cultivation in 1996 through a joint venture between the Monsanto and Delta & Pine companies (Traxleret al. 2001). These varieties were also subsequently released in Argentina, China, Australia, South Africa and Mexico. Later on, several new companies introduced the Bt cotton (transgenic cotton transformed by various Cry genes which encode crystal protein δ-endotoxin to kill lepedoptera class of insect) varieties and local varieties in cotton growing countries were incorporated with various *Cry* genes through *Agrobacterium*-mediated transformation (Strickland 1998) or biolisticgenetic transformation (Finer and McMullen 1990), or backcrossed resulting in the wide spread of Bt cotton varieties containing cry genes encoding toxin which provided protection against bollworm complex (Wu et al. 2008).

In comparison to the non-Bt cotton, Bt cotton occupied 85% of the total of cotton cultivation area in USA, 90% in India and Pakistan, 65% China (Anderson and Rajasekaran 2016). Bt cotton had a great success in India which nearly doubled the cotton production due to reduction of yield losses and protection against boll worm. In Pakistan, farmers are still awaiting for the benefits of the Bt cotton due to several factors including weak expression of *cry* genes in local genotypes and their poor adaptation due to adverse climatic conditions, cotton leaf curl virus infestation and sucking pests. Bt technology has reduced the pesticide spray by 40%, reduced yield losses by 20% and farmer profitability was increased by 70%. In future, multiple Bt or Bt like genes will be staked together along with herbicide resistant genes to increase the efficacy, durability against the boll worm complex and to reduce the yield losses due to weeds by various multi or local companies in countries like USA, China, India and Pakistan (Naranjo 2010).The staking of RNAi genes in Btcottonwas used to interfere with the metabolism of juvenile hormone acid methyl transferease in *Helicoverpaarmigera*. The staking of genes increased the efficiacy of the Btcotton against the Bt resistant insects (Ni et al. 2017). *Cry9C* gene and *Cry 2A* or *Cry 1Ac* were pyramided (staked) in Bt cotton to increase the efficacy against the two lepidopetran insect i.e. *Spodopteralitura* and *Heliothisarmigera* (Li et al. 2014).

Gene pyramiding strategy (incorporation of diversified sources of resistance in a single genotype to reduce yield losses) has been adapted to kill insect due to host ability to produce various types of toxins which may delay the buildup of pest resistance (Brévaultet al. 2013). Initial selection exposure over *cry1Ac* increased the survival of *Helicoverpa*over two toxin cotton (Brévaultet al.2013)**.** Some other events of transgenes are under trial or in developmental process with fascinating results (Table 5). However, biotechnological products are put under high watch list and heavy load of formalities which slows down the research from labs to the commercialization success.

1. **Improvement of Cotton against Abiotic Stresses**

Cotton yield has been threatened by various abiotic and biotic stresses on lint yield. Heat and drought stress are the major threats for future cotton production due to rapid increase in the CO2 which may increase the day and night temperature by 1–5ºC (Singh et al. 2007). It reduced the boll and flower retention on the plant and caused abscission of 40% bolls (Singh et al. 2007). Moreover, heat stress also had repressing effects over the boll size, number of seed per boll, oil and fiber quality traits (Pettigrew, 2008). High temperature also ameliorated the evapotranspiration losses, which increases the water requirement of the crop.

Breeders have made significant efforts to develop the heat or drought tolerant breeding material (Ur Rahman et al. 2004; Khan et al. 2008; Ullah et al. 2008). The efforts of plant breeders were generally aimed at improving seed cotton yield under targeted environment. However, seed cotton yield per se as selection criteria was complicated due to dependence over wide range of yield component in non-stress conditions and also dependenton the plant resistance under stress conditions. For instance, yield under non-stress condition is product of higher boll number, size of boll, no of fruiting points while under high temperature, yield was product of gametophtic fertility, canopy architecture (foliage position, hairiness), delayed leaf senescence, photosynthesis efficiency, less respiration rate, and harvest index (Kakaniet al. 2005; Jhaet al. 2014). A second approach was to screen the elite and wild germplasm and targeting the physiological or morphological traits which may be introgressed within advanced breeding lines. QTL mapping of the traits related to abiotic stress could further help to speed up the introgression and to decrease the linkage drags in the elite breeding material. Various transgenes have been identified which may further help to enhance the cotton crop tolerance to various abiotic stresses (Table 6). These transgenes were not negatively associated with yield, thus transformation did not induce any yield drags.

1. **Improvement of Cotton against Biotic Stresses**

The cotton plant resistance is conventionally dependenton several morphological traits such as frego bract, nectariless, gossypol glands, red canopy color, leaf trichome, glabrous leaf, okra leaf shape and small leaf area. These morphological traits are linked with insect defense umbrella. However, their utilization in practical plant breeding for creating insect resistant cotton was limited due to their effect on plant morphology and yield. Plant biotechnology has been used to introduce transgenes against various abiotic factors. Bt transgenic cotton containing (*Cry*genes) having resistance to bollworms has gained popularity and replaced the conventional cotton varieties in many parts of the world. Research has been under progress to introduce the transgenic cotton for the diseases and sucking pest resistance. Hen egg white lysozyme, GAFP4, *FreB* gene (VDAG-06616) has been characterized as resistant to verticilium wilt upon introgression in cotton (Table 5). The gene *Galanthus nivali* agglutinin (GNA) and *Amaranthus caudatus* agglutinin was found resistant against aphid infestation (Yang et al. 2017).

1. **New Emerging Technologies**

Genome editing technique such as CRISPR/Cas (clustered regularly interspaced short palindomic repeats: associated protein) is one of the emerging technologies to knock down the undesirable genes at specific site. This technique is used to edit genome through nuclease guided by the RNA to target a specific site in the genome provided that target site has known sequence. The CRISPR were found to be present in the bacteria, which was used to inactivate viral invasions (Razaet al. 2017). The CRISPR sequences are activated after the invasion by a virus utilizing its associated protein (Cas9). As a result of activation of CRISPR, it deactivates the viral genome and keeps small part of viral genome in its genome as spacer sequence (Razaet al. 2017). The guider RNA and spacer sequence in edited CRISPR/Cas9 system has been expected to be widely utilized for the modification of cotton genome. However, CRISPR/Cas9 genome modification technique requires protospacer adjacent motif. Cas9 proteins induces double stranded break at the target site which can be NHEJ (Non homologous end-joining) or HDR (homologous directed repair) by causing indels (insertion and deletions) in genome. This technique has been attempted to induce resistance against cotton leaf curl virus and verticilum wilt in cotton as replacement of RNAi technology (Iqbalet al. 2017). Optimization of CRISPR/Cas9 technique in cotton is in process (Long et al. 2018). A high CRISPR/Cas activity was observed in targeted genomic sites such as *GhMYB25-like A* and *GhMYB25-like D*. The targeted sites showed 50% editing through sgRNAof the transgenic allotetraploid cotton plants (Li et al. 2017).A mutation efficiency of 47.6–81.8% in two genes i.e. *Cloroplastosalterados 1 (GhCLA1*) and *vacuolar H*+*-pyrophosphatase (GhVP*) was induced through two guide RNAs (Chen et al. 2017).The promoter GHU6 was successfully cloned and provided 6-7 times more expression for sgRNA than AtU6-29 promoter (Long et al. 2018). Multi-site genome editing was done through two sgRNA in single vector which targeted two genes *Discosoma red fluorescent protein2*(*DsRed2*) and *GhCLA1* in cotton. CRISPR/Cas9 successfully targeted both loci and transformation efficiency of 66.7 to 100% was observed (Wang et al. 2018). The albino expression of endogenous gene *GhCLA1* was observed in 75% of the transgenic plants. It is difficult to target both loci in genome A and D controlling single traits due to polyploidy nature of cultivated cotton (Janga et al. 2017). The application of CRISP/Cas9 is severely handicapped due to absence of efficient genetic transformation (Long et al. 2018).The application of CRISPR/Cas9 in cotton genome editing is detailed in a separate chapter in this book.

Genome wide association (GWAS) mapping is also an emerging and an alternative method for detecting QTLs and dissection of quantitative traits in cotton such as plant canopy architecture (Su et al. 2018), agronomic traits i.e. yield and its components (Huang et al. 2017; Gapareet al. 2017), fiber quality traits (Gapareet al. 2017), diseases (Li et al. 2017). GWAS had several advantages over the biparental linkage mapping such as high density mapping covering whole genome, robust, time efficient, cost effective and there was no need of creating mapping populations for QTL mapping (Huang et al. 2017)

1. **Conclusions and Prospects**

Cotton is an important fiber and oilseed crop of the world. It belongs to the genus *Gossypium* which has four cultivated species, two diploid and two tetraploid species. *G. hirsutum* is widely cultivated species for the spinablemedium length fiber in textile industry and occupies 90% of the world cotton primary located in America, Asia and Africa. Traditional plant breeding (based on basic principles and selection methods i.e. Pedigree, bulk and recurrent selection for cotton) led to the substantial increase in fiber yield and quality. However, cotton species especially *hirustum* and *barbadense* are affected by insects and diseases which increases the production cost. Therefore, Bollgard cotton was introduced to reduce the yield losses due to boll worm complex infestation. Introduction of Bt cotton has provided novel method for insect resistance in cotton and reduced yield losses due to lepedopteraninsects.On the other hand, viral diseases continue to challenge the sustainable cotton production in various parts of the world and introgression of resistance against diseases was difficult through traditional plant breeding due rapid emergence of new pathogens. It was hoped that anti-sense and RNAi technology will provide solution to combat cotton disease, howeverhave failedto develop disease resistant cotton. CRISPR/Cas is a new emerging technology that may be applied to correct or modify the genome region associated with susceptibility to indeterminate growth habit, boll losses, diseases and insects. Continues emergence of new viral strains could continue the battle between the pathogen and breeders. Climate changes due to environmental pollution and deposition of green house gases may threaten the key cotton production regions such as indo-Pak subcontinent. It has been observed that heat, salinity, mineral deficiencies and drought stress can pose serious threats to the cotton production. Breeding strategies such as incorporation ofresistant genes for the development of climate resilient cotton crop may be required to combat future climate changes.

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**Appendix I** Research institutesrelevant to cotton breeding and biotechnology

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| --- | --- | --- |
| Institution | Specialization and research activities | Contact information and website |
| Central cotton research institute, Multan, Pakistan | Varietal development, cotton yield management | http://www.ccri.gov.pk/ |
| Cotton research Institute, Nanjing University China | Cotton genomics and genetics | http://mascotton.njau.edu.cn/ |
| Central Institute for Cotton Research, India | Cotton varietal development, Integrated Pest Management | http://www.cicr.org.in/ |
| Institute of Cotton Research, China | Genetic breeding, germplasm resources, farming cultivation, plant protection, molecular biology | http://www.caas.cn/en |
| Cotton Research Institute, Zimbabwe | Cotton varietal development, Integrated Pest Management | https://www.gfar.net/organizations/cotton-research-institute-1 |
| Australian Cotton Research Institute, Narrabari, USA | Biopesticide, Insecticide Resistance, Cotton Nutrition and Irrigation, Cotton Pathology | https://www.dpi.nsw.gov.au/about-us/research-development/centres/narrabri |
| ACSA International Cotton Institute, USA | Basic education on all aspect of cotton | https://bf.memphis.edu/cotton/index.php/main/instructions |
| Cotton Research Institute, Egypt | Cotton breeding research, production and technology transfer | http://www.arc.sci.eg/InstsLabs/Default.aspx?OrgID=2 |
| International Cotton Advisory Committee, USA | Cotton research and development policies formation | Icac.org |
| Cirad Agriculture Research Institute, France | Cotton germplasm, data bases | https://www.cirad.fr/en |
| Uzbekt Research Institute, Uzbekistan | Cotton germplasm resources | https://en.yellowpages.uz/company/uzbek-scientific-research-institute-of-cotton |
| Nazili Cotton Research Institute, Turkey | Cotton research and development | administrator@nazilli.tagem.gov.tr |

**Appendix II**Cotton genetic resources

|  |  |  |
| --- | --- | --- |
| Cultivar | Important traits | Cultivation location |
| NexGen 5711 B3XF | Bollgard3 XtendFlex Cotton Technology, Smooth leaf, bacterial blight tolerance, and fiber | AMERICOT, USA |
| ST 5517GLTP. | three-gene Bt technology of TwinLink Plus, bacterial blight resistance, and good storm tolerance | Stoneville, USA |
| FM 1953GLTP | * An early/medium maturity Glyphosate Tolerance LibertyLinkTwinLink Plus variety,Bacterial blight resistance, bollworm resistance and fall armyworm. | Bayer, USA |
| PHY 300 W3FE | Early maturing, moderate water stress resistant, superior fiber quality | Phytogen, USA |
| IUB-2013, FH-142, MNH-886 | High yield potential, Cry1A genes, heat resistance, increased boll retention under heat stress | South Punjab, Pakistan |
| Sicot 71 4B3F | High yield, fiber quality, wide regional adaptability, three transgenic trait Monsanto’s Bollgard II and RRFlex transgenic traits, providing both pest resistance and herbicide tolerance | CSIRO, Australia |
| GIZA 86 | High yield extra-long cotton having longest and thinnest fiber | Egypt |
| ICS-105 | High yield and adaptable varieties | Maharashtra, India |
| FM 1944GLB2 | Broadly adapted to all cotton-growing region, Liberty® and glyphosate herbicide tolerant, Lepidopteran resistant | USA |
| Gloria | High yield potential | Turkey |

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| Index Keywords  Vol 4 Ch 21 Cotton |
| Abiotic stress |
| Bollworm complex |
| Bt cotton |
| Colored cotton |
| CRISPR/Cas |
| Crop wild relatives |
| *Cry* genes |
| Cultivar |
| Emasculation |
| *Exsitu* Conservation |
| Fiber length |
| Fiber strength |
| Ginning out turn |
| Hybrid cotton |
| *Inplanta* |
| *Insitu* Conservation |
| Introgression |
| Lint |
| Microniare |
| Organic cotton |
| Pathotypes |
| Pollen tube transformation |
| Regeneration |
| Somatic embryogenesis |
| Stability index |
| Sucking pest |
| Transformation |
| Varieties |
| Viral strains |
| Yield gap |