Chapter 2
Cotton (*Gossypium hirsutum* L.) Breeding Strategies

Saeed Rauf, Muhammad Shehzad, Jameel M. Al-Khayri, Hafiz Muhammad Imran, and Ijaz Rasool Noorka

Abstract This chapter is focused on the achievements and future prospects of cotton breeding and related biotechnology. Traditional plant breeding has been utilized for the development of pure-line selection for high yielding cotton genotypes in segregating generations through the pedigree method. Selection criteria include boll number plant^{-1}, boll mass, sympodial branches and ginning outturn percentage. Plant breeder efforts have been fruitful in releasing cotton cultivars 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 reached a plateau in the last two decades and the recent increase in yield was due to better cotton husbandry techniques. 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 cultivars 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 palindromic 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 cultivars.

S. Rauf (✉) · M. Shehzad · I. R. Noorka
Department of Plant Breeding and Genetics, College of Agriculture, University of Sargodha, Sargodha, Punjab, Pakistan
e-mail: muhammad.shehzad@uos.edu.pk

J. M. Al-Khayri
Department of Agricultural Biotechnology, King Faisal University, Al-Hassa, Saudi Arabia
e-mail: jkhayri@kfu.edu.sa

H. M. Imran
Central Cotton Research Institute, Multan, Pakistan

J. M. Al-Khayri et al. (eds.), *Advances in Plant Breeding Strategies: Industrial and Food Crops*, https://doi.org/10.1007/978-3-030-23265-8_2

Keywords CRISPR · Genetic gains · Ideotype · Introgression · Transgenic · Wild relatives

2.1 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 is cultivated on an area of 34.74 million ha which was about 3% of the total world arable land (FAO 2014). Among the countries, India, China, the USA and Pakistan are the largest producers of raw cotton. Cotton seed is used by the 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 content and related sesquiterpene aldehydes 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 mt during 2011–2016 which was 12% higher than the average world cotton production during 2001–2010, (65.23 million mt) and 36% higher than the average world cotton production during 1991–2000 (54 million mt). There was an increase of 21% in world cotton production when 1990–2000 and 2001–2010 are compared while an increase of 13% in global cotton production was seen in the present decade. World production grew by 1.5% year^{-1} in the last three decades. The global trade of cotton lint was only USD 21.30 million 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. 2.1.

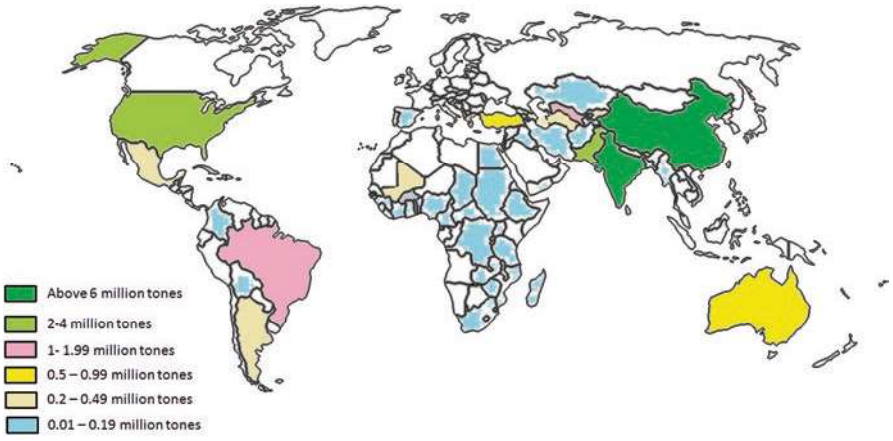

Fig. 2.1 The world map of cotton production. The map prepared as per food and agriculture statistics. (Source: FAO 2016)

Yield gains in cotton have been attributed to traditional plant breeding which developed cotton cultivars having high yield potential, superior lint qualities, tolerance to abiotic and biotic stresses, along with early maturity to convert this crop from a 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 is known to be dependent on the heritability of the traits. Since most economically-relevant traits are polygenic and affected by the environment, the variation among the traits was often masked by the environmental affect. The development of molecular marker systems and whole genome sequences analysis have led to the identification of genes and genomic regions associated with traits of interest by which selecting cotton cultivars is facilitated inbreeding programs (Fig. 2.2).

Cotton is one of the first crops in which transgenic cultivars have been successfully commercialized for large-scale cultivation during the mid-1990s (Zhang 2013). Transgenic cotton provided resistance against the boll worm complex which reduced the pesticide spray by nearly 50% (Traxler and Godoy-Avila 2004; Traxler et al. 2001). 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 the private sector for the research and development of transgenic cotton in various countries (Qiao 2015). Experimentation and evaluation of other transgenes have been carried out with a hope to commercialize them in future for sustainable cotton production. Details are presented in Sect. 2.5. Moreover, the cotton regeneration system has been optimized and several methods for development of transgenic cotton have been devised.

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

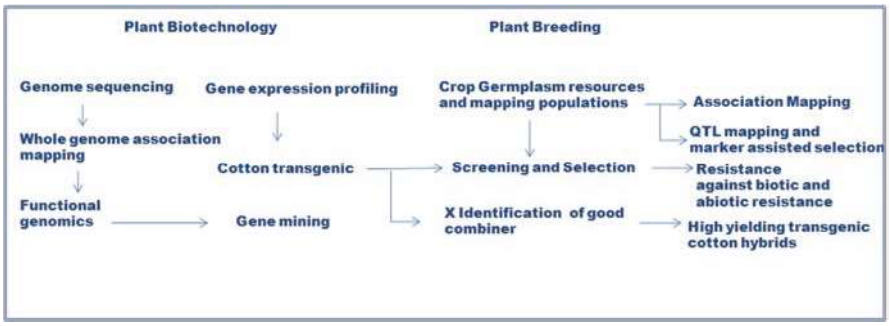

Fig. 2.2 Summary of integrated efforts for the improvement of cotton species through breeding and biotechnology

genes from genome analyses and help to understand the key metabolic pathways of commercial importance such as fiber development and oil content (Arpat et al. 2004).

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

2.2 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 the environment, loss of biodiversity, soil erosion, exhaustion and leaching of soil nutrients, evolution of new pathogens and biotype insects due to various practices of agriculture in cotton zones (Gutierrez et al. 2015). Precision agriculture may be implemented to accurately carry out various cultural activities from sowing to harvesting for continued 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 respond successfully to a particular environment*. Cultivar 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 plays an important role in fully exploiting the benefits of the farm inputs and soil. Good crop stands depend 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.

2.3 Cotton Genetic Resources and Conservation

2.3.1 Cytogenetics and Evolution

Cotton has a narrow genetic base due to a bottleneck which occurred after evolving into allopolyploidy species, domestication of few high yielding genotypes and intense pure-line selection (pedigree selection method) by breeders (Kuraparthy and Bowman 2013; Rauf et al. 2010). The upland *Gossypium* species native to Africa *G. herbaceum* L.-like, carrying the A genome and *G. raimondii* Ulbr.-like species carrying the D genome which is native to South America (Paterson et al. 2012). Parental species intercrossed about 1–2 million year ago, when species carriers of the A genome spread into the Mexican region through transoceanic dispersal (Paterson et al. 2012). Mating between the species lead to the evolution of several tetraploid species, out of which *Gossypium hirsutum* and *G. barbadense* L. are widely cultivated throughout the tropical and subtropical regions of the world. A number of studies using molecular markers or phenotypic based studies have confirmed the presence of low genetic diversity in cotton elite germplasm (Rauf et al. 2010). Therefore, there was a need to broaden genetic diversity within cotton germplasm and to use wild germplasm resources for the improvement of cultivated species (Shaheen et al. 2012). The genus *Gossypium* comprises more than 50 species, out of which 4 are cultivated (Fig. 2.3). These cultivated species have 2 diploid and 2 tetraploid species. Among the 50 species, 7 species are allotetraploid 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 Myanmar. The C and G genomes evolved in Australia, the D native to the Americas and the E genome in the Arabian Peninsula (Wendel 2000).

2.3.2 World Cotton Germplasm Collections

World collections of cotton germplasm exist in eight major sites: India, Brazil, France (CIRAD), the USA, Australia, Uzbekistan, Russia and China. Uzbekistan has the largest germplasm collection of *Gossypium hirsutum* (13,241 accessions) followed by China (7712), India (7633), the USA (6302), Russia (4503), France (2103), Brazil (1660) and Australia (1518 accessions) (Campbell et al. 2010). Uzbekistan (3019 accessions) also holds the world largest collection of *G. barbadense* followed by USA (1584 accessions), Brazil (1509), Russia (1057), China (633), India (534), France (483) and Australia (104 accessions) (Campbell et al. 2010).

Fig. 2.3 Ex situ wild germplasm collection at Central Cotton Research Institute, Multan, Pakistan. (**a**) *Gossypium somalense* (Gurke) J.B. Hutch., (**b**) *G. laxum* L.Ll Phillips, (**c**) *G. lobatum* Gentry, (**d**) *G. capitis-viridis* (Harv. & Sand.) Hochr., (**e**) *G. nelsonii* Fryxell, (**f**) *G. longicalyx* J.B. Hutch. & B.J.S. Lee, (**g**) *G. gossypioides* (Ulbr.) Standl., (**h**) *G. anomolum* Wawra & Peyr., (**i**) *G. harkensii* Brandegee, (**j**) *G. arboreum*L. red, (**k**) *G. arboretum.* (Photos by H.M. Imran)

Fig. 2.3 (continued)

Germplasm from various sources has been collected and maintained at the Central Cotton Research Institute, Multan, Pakistan (pers comm, Imran 2018). The collection includes 4243 accessions of *Gossypium hirsutum*, 1025 of *G. arboreum*, 556 of *G. herbaceum* L. and 109 accessions of *G. barbadense*. Several of the classified *Gossypium* species are not maintained in their native sites and cotton germplasm is vulnerable to diseases and insect infestation and many cotton species are at the verge of extinction (Campbell et al. 2010). A survey was carried out in Mexico (2002–2003) 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. The populations of 7 diploid species were collected from door yards, garden plots and as feral plants (Ulloa et al. 2006). In situ preservation of all 11 diploid Mexican species was under threat. Therefore, collaborative efforts are required to preserve these cotton germplasm resources. The conventional method of in situ conservation was not applicable and ex situ 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 and cryopreservation are recommended (Altman et al. 1990).

2.3.3 Utilization of Wild Germplasm

Wild germplasm can be utilized for introgression of cytoplasmic male sterility, diseases and abiotic stress resistance, and to widen the genetic diversity of the cultivated species (Shaheen et al. 2012; Table 2.1). Synthetic species can be reconstituted from donor species i.e. *Gossypium herbaceum* and *G. raimondii.* Linkage drag from wild types i.e. introgression of undesirable alleles along with genes of interest is a major limitation for use of wild species in cotton improvement breeding. The deterioration of yield and quality, a divergent gene regulatory system, chromosome structural differences, hybrid break down and genome assortment without introgression are major limitations in the utilization of wild germplasm for cotton breeding (Diouf et al. 2014; Zhang et al. 2014). Molecular markers were used to reduce the linkage drags in backcross and transgressive breeding populations or development of substitution lines (Wang et al. 2011). Introgression of useful genes such as disease or insect resistance, especially from wild relatives, also introduced non-targeted or undesirable genes (such as photoperiod sensitivity, late maturity, poor fiber, yield traits) which reduce the overall performance of introgressed 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 genes of interest. This could also help reduce the number of backcrosses required to achieve a degree of homozygosity.

Table 2.1 Development of breeding material from wild species of cotton (*Gossypium* spp.)

Interspecific crosses	Breeding material	References
*G. hirsutum*cv. 86-1× *G. armourianum* Kearney	Resistant to sucking pest such as jassid, white fly due to intense hairiness	Junqi et al. (1995)
G. hirsutum × *G. arboreum*	Sterile triploid hybrids which may be amphiploid to transfer cotton leaf curl virus resistance	Tahir and Noor (2011)
G.hirsutum × *G. arboreum*	Eight cultivars were developed after introgression with lint yield. High yielding cotton cv.Shiyuan 321 (Jimian 24) occupied the highest area in Yellow River Valley	Zhenglan et al. (2002)
G. longicalyx × *G. armourianum* ×*G. hirsutum* *G. longicalyx* × *G. herbaceum* × *G. hirsutum*	Trispecies hybrids were resistant to nematodes infestation	Bell and Robinson (2004)
hirsutum Acala 44 × *G. barbadense* Pima S-7	Three QTL CM12, STS1, 314–7 had large effect over *Verticillium* wilt resistance	Bolek et al. (2005)
G. barbadense, G. arboreum and *G. thurberi* Tod.	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	Mehetre (2010)
G. hirsutum × *G. barbadense*	Salt tolerant back cross inbred lines surpassing both parents due to transgressive breeding	Tiwari et al. (2013)
G. *gossypioides*	Resistant to cotton leaf curl virus disease and may be exploited for introgression	Azhar et al. (2013)
G. hirsutum × G. *arboreum* (followed by backcrossing to *G. 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 *G. arborem*	Nazeer et al. (2014)
Synthetic amphiploid *G. hirsutum* × *G. arboreum* (AADDAA)	Amphiploid was resistant to *Verticillium* and drought resistance	Chen et al. (2015a, b)
G. *capitis-viridis* × (*G. hirsutum* × G. *austral* F. Muell.)	Trispecies hybrid was intermediate in canopy characteristics and resistant to insect	Chen et al. (2015a, b)
G. herbaceum (A1) × *G. 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)

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

2.3.4 Colored Cotton

Cotton produces white lint color. However, there is great variability of lint color within tetraploid cottons. Lint is available from various shades of brown color to the light green (Fig. 2.4). Colored cottons provide great potential for the textile industry to produce cloth without use of synthetic dyes and chemicals which can causes allergic reactions to consumers and produce toxic effluent. It is also a major component of organic cotton, i.e. cotton produced with less damage to the environment without the use of synthetic chemicals. However, colored cotton fiber qualities (fiber length, strength, micronaire) are inferior to the white cotton and cotton color is affected by the environment upon maturity. Therefore, there is need for the selec-

Fig. 2.4 Genetic diversity in cotton lint color. (**a**) Light brown, (**b**) Khaki, (**c**) Light green, (**d**) Dark brown, (**e**) White. (Photos by H.M. Imran)

tions of colored cotton with stabilized pigmentation and superior fiber quality for its acceptability in the textile industry.

Colored lint develops as a result of accumulation of natural pigmentation in the fiber after exposure to sunlight. All colors are genetically controlled and have monogenic inheritance. Green colored lint is controlled by the allele Lg (de Carvalho et al. 2014). There were about six alleles (Lc_1–Lc_6) for the brown color (Kohel 1985). Lc_1–Lc_2 produce brown color, Lc_3 produce dark brown color and Lc_4–Lc_6 produces light brown pigmentation in lint (Kohel 1985). Lc_1 was assigned to chromosome 7 and Lc_2 on chromosome 6 (Wang et al. 2014).

2.4 Traditional Cotton Breeding Programs

Traditional breeding based on basic principles and selection methods has been successfully employed in selecting cotton cultivars 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 cultivars resulted in improvement of harvest index per unit area, and switching the cultivars from perennial to annual growth habit and reduction in 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 (Rauf et al. 2010). Several types of cotton ideotypes have been proposed to develop cotton cultivars with high yield potential or sustainable yield under various agro-ecological conditions and biotic and abiotic stresses Table 2.2.

Rapid genetic gain in the seed cotton yield potential of Pakistani cultivars was noted until the 1980s (Fig. 2.5). Yield potential expanded from 600 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, about 1.34 kg ha^{-1} $year^{-1}$. There was also steady increase in the ginning outturn and staple length of the Pakistani cultivars; both traits increased by 0.5% and 0.6% per year, respectively (Figs. 2.5 and 2.6).

Correlation analyses showed that there was high significant ($P \leq 0.05$) and positive relationship between the actual and potential yield, showing that release of high-yielding cultivars had profound effects over actual cotton seed yield in Pakistan (Table 2.3). Correlation between the potential seed cotton yield of cultivars with ginning outturn and staple length was also positive and significant ($P \leq 0.05$) showing that high-yielding cultivars also had superior fiber quality and that the increase in yield potential was not at the expense of fiber quality (Table 2.3). There was no improvement in yield potential later in the 1990s–2005 period. However, there was some increase in actual yield which may be due to better crop management, early

Table 2.2 Various ideotypes of cotton to full fill ambitious breeding objectives of cotton

Breeding objective	Plant ideotypes	References
High yield potential	Compact canopy, high number of boll per meter^2, multiple bolls per fruiting point (boll borne in cluster), medium size boll, good boll opening, earlier anthesis, long reproductive duration, increase photosynthetic rate	Loison et al. (2017a, b) and Sekloka et al. (2008)
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	Karthikeyan et 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	Sekloka et 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 wax, longer root length, greater stem reserve mobilization	Rauf et 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. (2017a, b) and Pauli et al. (2017)

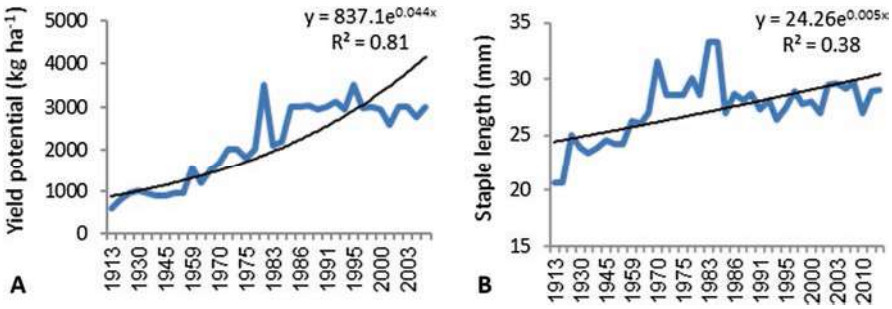

Fig. 2.5 Change in yield potential (**a**) and staple length (**b**) of cotton cultivars release in various periodicals. (Figures were produced by Saeed Rauf from public data published by Pakistan Central Cotton Committee)

sowing and control of insect infestations. Studies have also shown that an increase in lint yield occurred at the expense of seed yield. Estimated genetic gain in lint yield in Cameroon was about 3.3 kg ha^{-1} year^{-1} due to increased ginning outturn. 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 (Loison et al. 2017a, b).

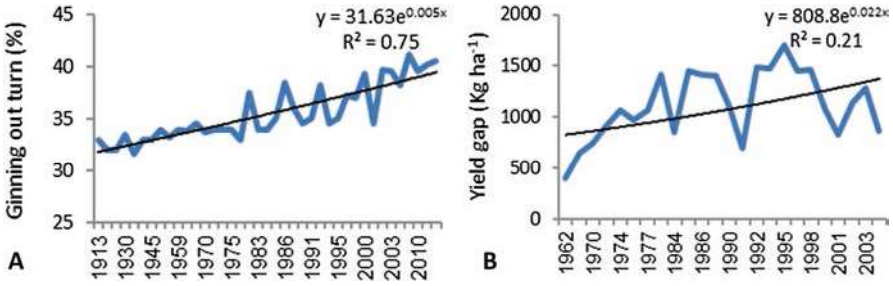

Fig. 2.6 Change in ginning out turn (**a**) and yield gaps (**b**) of cotton cultivars release in various periodicals. (Figures were produced by Saeed Rauf from public data published by Pakistan Central Cotton Committee)

Table 2.3 Correlation coefficients between yield and quality traits

Traits	Yield potential	Actual yield	Yield gap	Ginning out turn (G.O.T.)
Actual yield (kg ha^{-1})	0.72∗			
Yield gap (kg ha^{-1})	0.66∗	−0.06^{ns}		
Ginning out turn (%)	0.76∗	0.61∗	0.22^{ns}	
Staple length (mm)	0.62∗	−0.21^{ns}	−0.02^{ns}	0.47∗

∗Significant at $p \leq 0.05$; *ns* non significant $P \geq 0.05$

Plant breeders also developed 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 hybrid cotton. In India, 40% of cotton cultivation uses hybrid seed, while in Vietnam about 70% of the cotton area is planted by the hybrid seeds. Development of high-yielding hybrids could expand the yield potential of the cultivars. The yield advantage of hybrid cotton in China was more than 20% over the open- pollinated cultivars. Bt hybrid cotton was considered an example of successful integration of traditional cotton breeding with biotechnology, as hybrid Bt cotton was based on the manifestation of heterosis between non-Bt and Bt breeding lines (Dong et al. 2004). Recently several cotton hybrid cultivars with high yield, lint percentage, multiple resistance and wide adaptability have been released for general cultivation in various parts of the world (Table 2.4). New technologies such as molecular markers have been employed to study the genetic purity of hybrids (Dongre et al. 2011). Hybrid cultivars also exploit heterosis for the development of long or extra-longstaple cotton through interspecific hybridization between the *G. hirsutum* × *G. barbadense* species.

Table 2.4 Cotton (*Gossypium* spp.) hybrid performance under various agro-environment conditions

Crosses	Heterosis	References
Hybrid transplantation technology	Transplanting Bt hybrid could help to escape the chilling stress under controlled condition and provided additional 1 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	Blaise (2006)
Hybrid Ji-FRH3018 CMS-3096 × Ref 866	High resistance to bollworm, *Fusarium* wilt and *Verticillium* wilt and high yield and lint quality	Guo et al. (2010)
Cytoplasmic male sterility source from *G.harknenssii*	Negative impact of CMS source over number of bolls per plant and fiber quality traits	Tuteja and Banga (2011)
Cotton hybrids CRI-28, CRI-29, XZM 2 and Jimian18.	Gene differential expression in hybrids when compared to parents that 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 45 °C	Zeng et al. (2012)
G. *hirsutum* × *G. barbadense* chromosome segment introgression lines	Stable heterotic loci (hLP-A4-3) was detected in all 3 years	Guo et al. (2013)
Okra leaf hybrid F1 s, crossed 2 sterile near isolines and 3 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. (2008)
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)

2.5 Genomic Database

The presence of a large number of sequence data sets of cotton has led to creation of an integrated cotton functional data set (Zhu et al. 2017a, b). Cotton functional data set (CottonFGD, https://cottonfgd.org) has been established and allows the easy, quick and user-friendly access to 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. 2017a, b). The comparison of data sets from various upland and sea island cottons identified various polymorphic sites for the development of molecular markers for these traits. These sequences were used for marker-assisted selection (MAS) within F2 populations 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* (*GhD01G2042*), a homeobox-leucine zipper protein was a homologue to the leaf shape gene in *Arabidopsis thaliana*. The draft genome sequence of *G. raimondii* species has been published (Wang et al. 2012) which

showed that the *G.raimondii* genome contains more than 40,976 protein-coding genes. However, the species genome underwent tremendous recombination with more than 40% gene synteny (Wang et al. 2012). Some key genes related to fiber elongation were explored through expressed sequence tags (Arpat et al. 2004). More than 80 genes related to various developmental stages of fiber development were identified (Arpatet al. 2004). Several fiber related genes such as *GhTTG1-GhTTG4*, *GhGa20ox1-3*, *iaaM*, *GbPDF1* and *GhJAZ2* (fiber initiation); *GhSusA1* and *PHYA1* (fiber length); *GbPDF1, E6*, *GhExp1* and *RLK* (fiber strength); *GbTCP*, *WLIM1a*, *PAG1*, *GhCaM7*, *ACO*, *GhHOX3* and *PIP2s* (fiber elongation), have been reviewed and are considered primary genes expressed at various stages of fiber development (Ashraf et al. 2018). The genes were not known to regulate fiber development pathways. *Susy* gene was found to be positively involved while *GhPRP5* was negatively involved in the same fiber elongation pathways. Resistance genes to biotic and abiotic stress along with their putative functions are listed in Tables 2.5 and 2.6, respectively.

Table 2.5 Development of transgenic for protection against various types of biotic stresses

Transgenes	Resistance	References
Hen egg white lysozyme, GAFP4, *FreB* gene (VDAG_06616) *Gastrodia* antifungal proteins	Resistant to *Verticilium* wilt	Wang et al. (2016), Wenfang et al. (2017) and Rehman et al. (2018)
GR79 EPSPS and *N-acetyltransferase* (*GAT*) genes	Resistant to glyphosate	Liang et al. (2017)
cry1Ac	BT toxin induction in *Gossypium hirsutum*	Anayol et al. (2016)
Galanthus nivali agglutinin (GNA) and *Amaranthus caudatusagglutinin*	Genes with anti-aphid function having 75% control over the pest.	Yang et al. (2017)
Pyramiding RNAi and BT technology	Pyramiding RNAi and BT technology was effective to control resistant *Helicoverpa armigera* types	Ni et al. (2017)

Table 2.6 Characterization of various trans-genes under abiotic stresses

Transgenes	Tolerance	References
Isopentenyl transferase 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 abscisic acid	Yu et al. (2012)
Annexin gene, *GhAnn1*	Enhance drought and salt tolerance	Zhang et al. (2015)
PeDREB2a and *HhERF2* from 86 AP2/ERF	Transgenic plants containing PeDREB2a and KcERF showed tolerance to salt and drought stresses	Bo Li et al. (2016)

2.6 Mutation Breeding

Mutation breeding programs were initiated to improve cotton in various parts of the world during the 1960s. High fruiting, diseases resistance, early maturity and lower monopodial growth habits were the main targets of these breeding programs (Muthusamy et al. 2005; Iqbal et al. 1994).The mutants were generated with exposure of plant tissues such as seed (30 kR), pollen (10 Gy gamma rays), shoot tip (5–50 Gy gamma rays), ovule (50 Gy gamma rays) or the chemical mutagen 1–5 mM of ethyl methane sulfonate (EMS) (Aslam et al. 2018; Muthusamy and Jayabalan 2011, 2014; Muthusamy et al. 2005; Saeed Iqbal et al. 1994). Lower doses and concentrations generated higher genetic variation for traits such as early flowering, plant height, number of bolls, yield of seed cotton, ginning percentage, seed index, harvest index and fiber traits (Muthusamy and Jayabalan 2011, 2014). Cell lines of cvs. (Acala SJ2, B1654) were developed by exposure of 2.13 µmol to sulfonyl urea. The embryogenic resistant cell had LD_{50} several hundred times greater than unselected lines (Rajeskaran et al. 1996). Regenerated plants were resistant to primsulfuron (0.06–0.21 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 the Nuclear Institute of Agriculture Biology, Faisalabad, Pakistan, led by the late Rana Saeed Iqbal Khan, developed a revolutionary cv., NIAB-78, obtained by irradiating the F_1 cross of (Deltapine × AC-134). The Government of Pakistan awarded him the highest presidential award for the creation of this cultivar and for his services to the cotton community (pers comm, Hafiz M. Hassan Mumtaz 2019). The developed mutant was early maturing and with high yield potential which increased the overall cotton production of Pakistan several fold and was suitable for integration into the double cropping system (wheat-cotton) of Pakistan. It occupied about 90% of the area during early 1980s and total Pakistan cotton production increased from 3 to about 12.8 million bales (www.niab.org.pk). However, the cultivar was abandoned due to its susceptibility to CLCuVD during the mid-1990s (pers comm, Hafiz M. Hassan Mumtaz 2019). A high yielding mutant NIAB-92 was developed by irradiating seed of Stoneville231 with gamma rays at 30 kR from ^{60}CO. It has a semi-hairy, compact canopy with 0–2 monopodial branches as compared to its parental cultivar (Iqbal et al. 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 NIAB-78 with REBA-288. The pollen of REBA-288 were irradiated with 10 Gy 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 cv. NIAB-777 (Aslam et al. 2018). A mutant with high fiber quality (fiber strength = 40.5) was isolated from segregating populations generated after exposure of MD15 to 3.2% v/v ethylmethane sulfonate (Bechere et al. 2013).

2.7 In Vitro 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 confined to only few cultivars i.e. Cocker lines. A Cocker line such as 312 had high regenerable response and a heart-shaped globular embryo cultured on semi-solid media which germinated into plantlets (Trolinder and Goodin 1987). Research efforts were carried out for the optimization of genotype independent protocols for regeneration and genetic transformation.

Cotton regeneration protocols are claimed to be optimized in several cultivars around the globe (Pathi and Tutega 2013). 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 the 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 2013). Explants such as embryo axes 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 meristematic cell of Indian cotton cultivars were regenerated into plantlets (Satyavathi et al. 2002). In vitro regeneration in cotton was shown to have genetically controlled traits and showed moderate heritability when local genotypes were crossed with Cocker lines (Rauf and Rahman 2005). Overdominance to additive type of gene action was involved in the expression of traits such as callus induction, embryogenic callus and germinating embryo Factors affecting cotton response to in vitro culture were reviewed by Ahsan et al. (2014).

Various in vitro techniques may be exploited to widen the genetic diversity within cotton species. Techniques such as in vitro fertilization or embryo rescue could be used to facilitate the wide cross among the cultivated and wild species. Interspecific crosses among species were attempted in the field and fruit boll abscission was prevented by repeated application of plant growth regulators (BAP, NAA) and embryos were rescued and cultured over growth medium for 15 days after pollination (Gill and Bajaj 1984). Cotton embryo culture was improved by the manipulation of components of the culture medium, including nutrients and plant growth regulators (Fuller et al. 2011).

Protoplast fusion of diverse species and genera provide an innovative tool to attempt wide crosses and to transfer the genes of interest from cyto-morphologically incompatible species. Moreover, protoplast fusion could also introduce cytoplasmic genes from donor species (Wang et al. 2007). Theoretically protoplast fusion could be attempted between any species, where protoplast may be successfully isolated and plants may be regenerated from cell culture. Although cotton is a recalcitrant species for in vitro regeneration, reports on the protoplast fusion between various species have been presented in the literature and viable embryos containing asymmetrical protoplast of different species were regenerated. Protoplast fusion starts with the production of in vitro callus culture. The callus of highly regenerable cot-

ton cultivars such as Coker201 is obtained by culturing on MS media (Murashige and Skoog 1962) supplemented with suitable plant growth regulators. The regenerable calli are subcultured on a regular interval. Cell suspension culture is done in growth media devoid of agar and with suitable growth regulator such as BAP 0.1 mg L^{-1} and naphthalene acetic acid 1 mg L^{-1}. Isolation medium for protoplast has been reported as MS salt (Murashige and Skoog 1962), 5 rnM MES (2-(N-morpholino) ethanesulfonic acid), 0.7 M mannitol, 5% (w/v) Cellulysin cellulose and 1% (w/v) Macerase pectinase at a pH of 5.7) (Renfroe et al. 2001). The cells were plasmolyzed before incubation in the isolation medium. They were incubated in isolation media for 5 h at 28 °C and purified by filtration with nylon mesh having 100 mm pores and centrifuged at 125 RCF for 6 min and layered over 20% (w/v) sucrose solution. The protoplast of recipient and donor species is mixed in 2:1. Pigmented lines may be used as markers for identification of hybridity. The protoplast of the species is fused following Evans and Bravo (1983). The fusion is promoted by using 50% polyethylene glycol solution (MW = 6000). Polyethylene glycol fusing solution was eluted with either a glycine buffer followed by a wash with culture media, or by a Tris buffer (Renfroe et al. 2001). The details of the various reports are presented in Table 2.7.

2.8 Transgenic Cotton

Transgenic cotton is cultivated on more than 25 million ha, which is about 70% of the total world cotton area (Anderson and Rajasekaran 2016). Genetic transformation of cotton is categorized as *Agrobacterium*-mediated transformation; biolistic transformation, in planta pollen tube pathway or pollen tube transformation are involved. *Agrobacterium*-mediated transformation is a 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 are first inserted in T-DNA or other plasmids. The recombinant plasmid is then inserted in *Agrobacterium* through various methods including electroporation. *Agrobacterium tumefaciens* have the capacity to transform the plant cells with recombinant T-DNA in cotton cells. Triple genes carrying *Cry1AC*, *Cry2Ab* and *EPSPS*are used to transform T-DNA to produce recombinant plasmid (Naqvi et al. 2017). The sequences are retrieved from the NCBI. The gene sequences are optimized according to upland cotton (Naqvi et al. 2017). The 2X 35S promoters and terminator sequences are used to induce expression and termination for *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 sites. The terminator is cloned using HindIII and SalI restriction sites.*Cry2Ab* cassette comprise figwort mosaic virus promoter, chloroplast signal peptide, partial *Cry2Ab* (270 bp) and G7 terminator (Naqvi et al. 2017). The cassava mosaic virus promoter (700 bp) is used along *EPSPS* (1.9 kb) and E9 terminator to induce the expression of the herbicide resistant gene.

Table 2.7 Protoplast fusion between various species of cotton (*Gossypium* spp.)

Species	Growth condition	Plant morphology	Chromosome no.	References
G. hirsutum (Coker 201) ×*G.klotzschianum* Andersson	Electrofusion Fused protoplast was cultured on KM8P medium supplemented with 2.685 µ*M* α-naphthaleneacetic acid and 0.465 µ*M* kinetic	Plants were different from their parents	71–81	Sun et al. (2004)
G. hirsutum ×*G. bickii* Prokh. and *G. hirsutum* × *G. stockii* Masters	Electrofusion RAPD markers confirmed the hybridity	Hybrids were intermediate and produced viable seed	78	Sun et al. (2005)
G.hirsutum (Coker201) × *G. davidsonii* Kellogg	RAPD (random amplified polymorphic DNA) and SSR were used to confirm hybrids	Regenerated were difficult to transfer into soil and therefore grafted on the root stock	74–84	Sun et al. (2006)
G.hirsutum (YZ-1) × *G. davidsonii*	Asymmetric hybrids were obtained by electrofusion. Both protoplast were treated with chemical and radioactive to retard growth before fusion	Plants were intermediate for many characters and showed recipient like morphology. Simple sequence repeats and amplified polymorphic DNA markers were used to identify hybrids	40–73	Fu et al. (2009)
G .hirsutum L. (cv. Coker 312) × *G. trilobum* (de Candolle) Skovsted	Symmetric electro fusion Molecular markers were used to identify hybrids	Hybrids were different from parents	78	Yu et al. (2012)

A method of transformation and regeneration was patented which involves the cocultivation of cotton explants (hypocotyls from 8-day-old seedlings)with *Agrobacterium* for 2 days on callus-initiation media (Murashige and Skoog salts, glucose 30 g L^{-1}, myo-inositol 100 mg L^{-1}, nicotinic acid 1 mg L^{-1}, pyridoxine-HCL 1 mg L^{-1}, thiamine HCL 10 mg L^{-1}, magnesium chloride 1.87 g L^{-1}, potassium nitrate 1.90 g L^{-1} and gelrite 4 g L^{-1}) without plant growth regulators. The transformed tissues are screened over the 12.5–50 mg L^{-1} kanamycin and 150 mg L^{-1} cefotaxime. Surviving cells are then continuously cultured on embryo genic calli inducing media and finally germinated over the shoot inducing media (Strickland 1998). The cell suspension culture or embryo axis is also bombarded with high

density particles coated with plasmid using a biolistic gun (Finer and McMullen 1990). The recombinant plasmid contains a hygromycin resistant gene which is used to screen the transgenic cells over culture media (Finer and McMullen 1990).

In planta methods were devised to avoid complicated regeneration protocols (Kalbande and Patil 2016). The 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 the embryo sac post pollination/fertilization. The steps are like pollinating the flower, cutting the stylar tissue (10–12 h post pollination) and injecting the vector solution carrying the gene of interest. The desired genes are directly inserted in pollen or injected into the developing embryo via pollen tube pathways. The in planta method of transformation in seedlings includes 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 the gene of interest. A transformation efficiency of 6.89% was obtained in cv. 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 tissue due to insertion in non-targeted layers.

Some 58 transgenic cottons carrying insect and herbicide resistance with a maximum of three genes (two insect resistance+ one herbicide resistance) along with marker genes, which have been released for general cultivation in various parts of the world. Transgenic cotton adaptation is increasing at a 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, after soybean, of *Cry* genes (*Cry1Ac, Cry2Ab2, Cry2Ae, Cry1Ab, Cry1A*) along with herbicide resistant genes (*BAR,EPSPS, BXN, DMO*). Two transgenic cvs. NuCOTN^{33} and NuCOTN^{35} with the trademark Bollgard™ were released for general cultivation in 1996 through a joint venture between the Monsanto and Delta & Pine companies (Traxler et al. 2001). These cultivars were also subsequently released in Argentina, China, Australia, South Africa and Mexico. Later, several new companies introduced the Bt cotton (transgenic cotton transformed by various *Cry* genes which encode crystal protein δ-endotoxin to kill lepidoptera class insects) cultivars and local varieties in cotton growing countries were incorporated with various *Cry* genes through *Agrobacterium*-mediated transformation (Strickland 1998) or biolistic genetic transformation (Finer and McMullen 1990) or backcrossed resulting in the wide spread of Bt cotton cultivars containing *Cry* genes encoding toxin which provides protection against the bollworm complex (Wu et al. 2008).

In comparison to the non-Bt cotton, Bt cotton occupies 85% of the total of cotton cultivation area in the USA, 90% in India and Pakistan and 65% China (Anderson and Rajasekaran 2016). Bt cotton has had 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 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 multinational or local companies in countries like the USA, China, India and Pakistan (Naranjo 2010). The staking of RNAi genes in Bt cottonwas used to interfere with the metabolism of juvenile hormone acid methyl transferease in *Helicoverpa armigera*. The staking of genes increased the efficacy of the Bt cotton 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 insects i.e. *Spodoptera litura* and *Heliothis armigera* (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 insects due to host ability to produce various types of toxins which may delay the buildup of pest resistance (Brévault et al. 2013). Initial selection exposure over *Cry1Ac* increased the survival of *Helicoverpa*over two-toxin cotton (Brévault et al. 2013). Some other events of transgenes are under trial or in developmental process with fascinating results (Table 2.5). However, biotechnological products are put under a high watch list and a heavy load of formalities which slows down the research from laboratories to the commercialization success.

2.9 Improvement of Cotton Against Abiotic Stresses

Cotton yield is 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 CO_2 which may increase the day and night temperatures by 1–5 °C (Singh et al. 2007). Higher temperatures reduce the boll and flower retention of the plant and cause abscission of 40% of the bolls (Singh et al. 2007). Moreover, heat stress also has a repressing effect over boll size, number of seeds per boll, and oil and fiber quality traits (Pettigrew 2008). High temperature also ameliorate the evapotranspiration losses, which increases the water requirements of the crop.

Breeders have made significant efforts to develop heat or drought tolerant breeding material (Khan et al. 2008; Ullah et al. 2008; Ur Rahman et al. 2004). The efforts of plant breeders are generally aimed at improving seed cotton yield under a targeted environment. However, seed cotton yield per se as a selection criteria is complicated due to dependence over wide range of yield components in non-stress conditions and is also dependent on the plant resistance under stress conditions. For instance, yield under non-stress condition is the product of higher boll number, size of boll and number of fruiting points, while under high temperature, yield is the product of gametophytic fertility, canopy architecture (foliage position, hairiness), delayed leaf senescence, photosynthesis efficiency, lower respiration rate and harvest index (Jha et al. 2014; Kakani et al. 2005). A second approach is to screen the

elite and wild germplasm and target 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 2.6). These transgenesare not negatively associated with yield, thus transformation dos not induce any yield drags.

2.10 Improvement of Cotton Against Biotic Stresses

Cotton plant biotic stress resistance is conventionally dependent on several morphological traits such as frego bract, nectriless, gossypol glands, red canopy color, leaf trichome, glabrous leaf, okra leaf shape and small leaf area. These morphological traits are linked tothe insect defense umbrella. However, their utilization in practical plant breeding to create insect resistant cotton is 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 cultivars in many parts of the world. Research is in progress to introduce transgenic cotton for disease and sucking pest resistances. Hen egg white lysozyme, GAFP4, *FreB* gene (VDAG-06616) has been characterized as resistant to *Verticilium* wilt upon introgression in cotton (Table 2.5). The genes *Galanthus nivalis agglutinin* (GNA) and *Amaranthus caudatus agglutinin* (ACA) were found resistant against aphid infestations (Yang et al. 2017).

2.11 New Emerging Technologies

Genome editing techniques such as CRISPR/Cas (clustered regularly interspaced short palindromic repeats: associated protein) is one of the emerging technologies to knock down undesirable genes at a specific site. This technique is used to edit the genome through nuclease guided by the RNA to target a specific site in the genome provided that target site has a known sequence. CRISPR were found to be present in the bacteria which was used to inactivate viral invasions (Aqeel and Raza 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 a small part of the viral genome in its own genome as a spacer sequence (Aqeel and Raza 2017). The guider RNA and spacer sequence in the edited CRISPR/Cas9 system is expected to be widely utilized in the modification of the cotton genome. However, the CRISPR/Cas9 genome modification technique requires a protospacer adjacent motif. Cas9 proteins induce double-stranded breaks at the target site which can be NHEJ (non-homologous end-joining) or HDR

(homologous directed repair) by causing indels (insertion and deletions) in the genome. This technique has been attempted to induce resistance against cotton leaf curl virus and *Verticilium* wilt in cotton as a replacement for RNAi technology (Iqbal et al. 2016). Optimization of the CRISPR/Cas9 technique in cotton is in process (Long et al. 2018). High CRISPR/Cas activity was observed in targeted genomic sites such as*GhMYB25*-likeA and *GhMYB25*-likeD. The targeted sites showed 50% editing through sgRNA of the transgenic allotetraploid cotton plants (Li et al. 2017a, b). A mutation efficiency of 47.6–81.8% in two genes i.e. *Cloroplastos alterados 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 the AtU6-29 promoter (Long et al. 2018). Multi-site genome editing was done through two sgRNA in a single vector which targeted two genes *Discosoma red fluorescent protein2*(*DsRed2*) and *GhCLA1* in cotton. CRISPR/Cas9 successfully targeted both loci and a transformation efficiency of 66.7–100% 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 the polyploidal nature of cultivated cotton (Janga et al. 2017). The application of CRISP/Cas9 is severely handicapped due to the 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 another emerging alternative method for detecting QTL and dissection of quantitative traits in cotton, such as plant canopy architecture (Su et al. 2018), agronomic traits i.e. yield and its components (Gapare et al. 2017; Huang et al. 2017), fiber quality traits (Gapare et al. 2017) and diseases (Li et al. 2017a, b). GWAS has several advantages over the biparental linkage mapping such as high density mapping covering the whole genome, robust, time efficient, cost effective and there was no need to create mapping populations for QTL mapping (Huang et al. 2017).

2.12 Conclusions and Prospects

Cotton is an important world fiber and oilseed crop. It belongs to the genus *Gossypium* which has four cultivated species, two diploid and two tetraploid species. *Gossypium hirsutum* is a widely cultivated species for the spinnable medium length fiber in the textile industry and occupies 90% of the world cotton primarily located in the Americas, Asia and Africa. Traditional plant breeding (based on basic principles and selection methods i.e. pedigree, bulk and recurrent selection for cotton) led to a substantial increase in fiber yield and quality. However, cotton species especially, *G. hirsutum* and *G. barbadense*, are affected by insects and diseases which increase production cost. Therefore, Bollgard cotton was introduced to reduce yield losses due to boll worm complex infestation. Introduction of Bt cotton has provided a novel method for insect resistance in cotton and reduced yield losses

from lepidopteron insects. On the other hand, viral diseases continue to challenge sustainable cotton production in various parts of the world and introgression of resistance against diseases has proved difficult through traditional plant breeding due to rapid emergence of new pathogens. It was hoped that antisense and RNAi technology will provide solutions to combat cotton disease; however, they have failed to 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. Continuous emergence of new viral strains could continue the battle between pathogen and breeder. Climate changes due to environmental pollution and the accumulation of greenhouse gases may threaten key cotton production regions such as the Indo-Pak subcontinent. It has been observed that heat, salinity, mineral deficiencies and drought stress pose serious threats to cotton production. Breeding strategies such as incorporation of resistance genes for the development of climate resilient cotton crops are important to combat future climate changes.

Appendices

Appendix I: Research Institutes Relevant to Cotton Breeding and Biotechnology

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

(continued)

Institution	Specialization and research activities	Contact information and website
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 LibertyLink TwinLink Plus variety, bacterial blight resistance, bollworm resistance and fall armyworm	Bayer, USA
PHY 300W3FE	Early maturing, moderate water stress resistant, superior fiber quality	Phytogen, USA
IUB2013, FH142, MNH886	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
GIZA86	High yield extra-long cotton having longest and thinnest fiber	Egypt
ICS105	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

References

Ahsan MZ, Majidano MS, Channa AR et al (2014) Regeneration of cotton (*Gossypium hirsutum* L.) through asexual methods, a review. Am Eurasian J Agric Environ Sci 14(12):1478–1486

Al-Bahrany AM, Al-Khayri JM (2000) Genotype variability in fatty acid composition and chemical characteristics of cotton (*Gossypium hirsutum* L.). Pak J Biol Sci 3:1778–1780

Almeida VCD, Hoffmann LV, Yokomizo GKI et al (2009) In situ and genetic characterization of *Gossypium barbadense* populations from the states of Pará and Amapá, Brazil. Pesq Agropec Bras 44(7):719–725

Altman DW, Fryxell PA, Koch SD, Howell CR (1990) *Gossypium* germplasm conservation augmented by tissue culture techniques for field collecting. Econ Bot 44(1):106–113

Anayol E, Bakhsh A, Karakoç ÖC et al (2016) Towards better insect management strategy: restriction of insecticidal gene expression to biting sites in transgenic cotton. Plant Biotechnol Rep 10(2):83–94

Anderson DM, Rajasekaran K (2016) The global importance of transgenic cotton. In: Ramawat KG, Ahuja MR (eds) Fiber plants, sustainable development and biodiversity. Springer, Cham, pp 17–33

Aqeel M, Raza A (2017) CRISPR/cas9: an emerging revolution in therapeutics. Int J Appl Biol Foren 1:1–4

Arpat A, Waugh M, Sullivan JP et al (2004) Functional genomics of cell elongation in developing cotton fibers. Plant Mol Biol 54(6):911–929

Ashraf J, Zuo D, Wang Q et al (2018) Recent insights into cotton functional genomics: progress and future perspectives. Plant Biotechnol J 16(3):699–713

Aslam M, Haq MA, Bandesha AA, Haidar S (2018) NIAB-777: an early maturing, high yielding and better quality cotton mutant developed through pollen irradiation technique – suitable for high density planting. J Anim Plant Sci 28(2):636–646

Azhar M, Anjum Z, Mansoor S (2013) *Gossypium gossypioides*: a source of resistance against cotton leaf curl disease among D genome diploid cotton species. J Anim Plant Sci 23:1436–1440

Bechere E, Meredith WR, Boykin JC (2013) Registration of mutant population MD 15 M4 *Gossypium hirsutum* L. with enhanced fiber quality. J Plant Regist 7(2):216–219

Bell A, Robinson AF (2004) Development and characteristics of triple species hybrids used to transfer reniform nematode resistance from *Gossypium longicalyx* to *Gossypium hirsutum*. In: Proceedings of beltwide cotton conferences, New Orleans, USA, National Cotton Council of America, pp 422–426. https://naldc.nal.usda.gov/download/12353/PDF

Blaise D (2006) Yield, boll distribution and fibre quality of hybrid cotton (*Gossypium hirsutum* L.) as influenced by organic and modern methods of cultivation. J Agron Crop Sci 192:248–256

Bo Li J, Ni Dong X, Lei Z et al (2016) Simultaneous overexpression of the HhERF2 and PeDREB2a genes enhanced tolerances to salt and drought in transgenic cotton. Protein Pept Lett 23(5):450–458

Bolek Y, El-Zik KM, Pepper AE et al (2005) Mapping of *Verticillium* wilt resistance genes in cotton. Plant Sci 168:1581–1590

Brévault T, Heuberger S, Zhang M et al (2013) Potential shortfall of pyramided transgenic cotton for insect resistance management. Proc Natl Acad Sci 110(15):5806–5811

Campbell BT, Saha S, Percy R, Frelichowski J, Jenkins JN, Park W, Du X (2010) Status of the global cotton germplasm resources. Crop Sci 50(4):1161–1179

Chen D, Wu Y, Zhang X, Li F (2015a) Analysis of [*Gossypium capitis-viridis*×(*G. hirsutum*× *G. australe*)^2] trispecific hybrid and selected characteristics. PLoS One 10(6):e0127023. https://doi.org/10.1371/journal.pone.0127023

Chen Y, Wang Y, Zhao T et al (2015b) A new synthetic amphiploid (AADDAA) between *Gossypium hirsutum* and *G. arboreum* lays the foundation for transferring resistances to *Verticillium* and drought. PLoS One 10(6):e0128981

Chen X, Lu X, Shu N et al (2017) Targeted mutagenesis in cotton (*Gossypium hirsutum* L.) using the CRISPR/Cas9 system. Sci Rep 7:44304. https://doi.org/10.1038/srep44304

Constable GA, Bange MP (2015) The yield potential of cotton (*Gossypium hirsutum* L.). Field Crop Res 182:98–106
de Carvalho LPD, Farias FJC, Lima MMDA, Rodrigues JIDS (2014) Inheritance of different fiber colors in cotton (*Gossypium barbadense* L.). Crop Breed Appl Biotechnol 14(4):256–260
Diouf FBH, Benbouza H, Nacoulima NL et al (2014) Segregation distortions in an interspecific cotton population issued from the [(*Gossypium hirsutum* x *G. raimondii*) ^{2} × *G. sturtianum*] hybrid. Tropicultura 32:73–79
Dong H, Li W, Tang W, Zhang D (2004) Development of hybrid Bt cotton in China – a successful integration of transgenic technology and conventional techniques. Curr Sci 86(6):778–782
Dong HZ, Li WJ, Tang W et al (2005) Increased yield and revenue with a seedling transplanting system for hybrid seed production in Bt cotton. J Agron Crop Sci 191(2):116–124
Dongre AB, Raut MP, Bhandarkar MR, Meshram KJ (2011) Identification and genetic purity testing of cotton F 1 hybrid using molecular markers. Indian J Biotechnol 10:301–306
Evans DA, Bravo JE (1983) Plant protoplast isolation and culture. Int Rev Cytol Suppl 16:33–53
FAO (2014) Food and Agriculture data. Retrieved from http://www.fao.org/faostat/en/#home. Accessed 19 Mar 2016
FAO (2016) Food and Agriculture data. Retrieved from http://www.fao.org/faostat/en/#home. Accessed 20 Feb 2016
Finer JJ, McMullen MD (1990) Transformation of cotton (*Gossypium hirsutum* L.) via particle bombardment. Plant Cell Rep 8(10):586–589
Fu L, Yang X, Zhang X et al (2009) Regeneration and identification of interspecific asymmetric somatic hybrids obtained by donor-recipient fusion in cotton. Chin Sci Bull 54(17):3035–3044
Fuller RJ, Liddiard VM, Hess JR et al (2011) Improving cotton embryo culture by simulating in ovulo nutrient and hormone levels. In Vitro Cell Dev Biol Plant 47(3):410–419
Gairola KC, Nautiyal AR, Dwivedi AK (2011) Effect of temperatures and germination media on seed germination of *Jatropha curcas* Linn. Adv Bioresour 2(2):66–71
Gapare W, Conaty W, Zhu QH et al (2017) Genome-wide association study of yield components and fibre quality traits in a cotton germplasm diversity panel. Euphytica 213(3):66
Gill MS, Bajaj YPS (1984) Interspecific hybridization in the genus *Gossypium* through embryo culture. Euphytica 33(2):305–311
Guo BS, Liu SE, Wang ZX et al (2010) Breeding of high yield, high quality three lines hybrid cotton variety Ji-FRH3018 [J]. J Hebei Agric Sci 7:025
Guo X, Guo Y, Ma J et al (2013) Mapping heterotic loci for yield and agronomic traits using chromosome segment introgression lines in cotton. J Integr Plant Biol 55(8):759–774
Gutierrez AP, Ponti L, Herren HR et al (2015) Deconstructing Indian cotton: weather, yields, and suicides. Environ Sci Eur 27(1):1–17
Huang C, Nie X, Shen C et al (2017) Population structure and genetic basis of the agronomic traits of upland cotton in China revealed by a genome-wide association study using high density SNPs. Plant Biotechnol J 15(11):1374–1386
Iqbal SRM, Chaudhry MB, Aslam M, Bandesha AA (1994) Development of a high yielding cotton mutant, NIAB-92 through the use of induced mutations. Pak J Bot 26:99–104
Iqbal Z, Sattar MN, Shafiq M (2016) CRISPR/Cas9: a tool to circumscribe cotton leaf curl disease. Front Plant Sci 7:475
Janga MR, Campbell LM, Rathore KS (2017) CRISPR/Cas9-mediated targeted mutagenesis in upland cotton (*Gossypium hirsutum* L.). Plant Mol Biol 94(4–5):349–360
Jha UC, Bohra A, Singh NP (2014) Heat stress in crop plants: its nature, impacts and integrated breeding strategies to improve heat tolerance. Plant Breed 133(6):679–701
Jin S, Zhang X, Nie Y et al (2006) Identification of a novel elite genotype for in vitro culture and genetic transformation of cotton. Biol Plant 50(4):519–524
Junqi QS, Yingjun ZBH, Xinlian S (1995) Studies on the interspecific hybrid of *Gossypium hirsutum* Cultivar 86-1× *G. armourianum* and its use in breeding [J]. Acta Agron Sin 5:013
Kakani VG, Reddy KR, Koti S et al (2005) Differences in in vitro pollen germination and pollen tube growth of cotton cultivars in response to high temperature. Ann Bot 96(1):59–67

Kalbande BB, Patil AS (2016) Plant tissue culture independent *Agrobacterium tumefaciens* mediated In-planta transformation strategy for upland cotton (*Gossypium hirsutum*). J Genet Eng Biotechnol 14(1):9–18

Karthikeyan P, Ramya K, Kannan N et al (2015) Genetic analysis in cotton (*Gossypium hirsutum* L.) for mechanical harvesting characters. Proceeding on Proceedings of Future Technologies: Indian Cotton in the Next Decade December 17–19, 2015 at Acharya Nagarjuna University, Guntur - 522 510, India, pp 264–270

Khan AI, Khan IA, Sadaqat HA (2008) Heat tolerance is variable in cotton (*Gossypium hirsutum* L.) and can be exploited for breeding of better yielding cultivars under high temperature regimes. Pak J Bot 40(5):2053–2058

Kohel RJ (1985) Genetic analysis of fiber color variants in cotton. Crop Sci 25:793–797

Kuraparthy V, Bowman DT (2013) Gains in breeding upland cotton for fiber quality. J Cotton Sci 17:157–162

Li L, Zhu Y, Jin S, Zhang X (2014) Pyramiding Bt genes for increasing resistance of cotton to two major lepidopteran pests: *Spodoptera litura* and *Heliothis armigera*. Acta Physiol Plant 36(10):2717–2727

Li C, Unver T, Zhang B (2017a) A high-efficiency CRISPR/Cas9 system for targeted mutagenesis in cotton (*Gossypium hirsutum* L.). Sci Rep 7:43902. https://doi.org/10.1038/srep43902

Li T, Ma X, Li N et al (2017b) Genome-wide association study discovered candidate genes of *Verticillium* wilt resistance in upland cotton (*Gossypium hirsutum* L.). Plant Biotechnol J 15(12):1520–1532

Liang C, Sun B, Meng Z et al (2017) Co-expression of GR79 EPSPS and GAT yields herbicide-resistant cotton with low glyphosate residues. Plant Biotechnol J 15(12):1622–1629

Lian-gen FU (2011) Preliminary report of trial planting of hybrid cotton Tongza411 in Lanxi city and its cultivation technique. Hortic Seed 3:121–129

Liu YD, Yin ZJ, Yu JW et al (2012) Improved salt tolerance and delayed leaf senescence in transgenic cotton expressing the *Agrobacterium* IPT gene. Biol Plant 56(2):237–246

Loison R, Audebert A, Chopart JL et al (2017a) Sixty years of breeding in Cameroon improved fibre but not seed cotton yield. Exp Agric 53(2):202–209

Loison R, Audebert A, Debaeke P et al (2017b) Designing cotton ideotypes for the future: reducing risk of crop failure for low input rainfed conditions in Northern Cameroon. Eur J Agron 90:162–173

Long L, Guo DD, Gao W et al (2018) Optimization of CRISPR/Cas9 genome editing in cotton by improved sgRNA expression. Plant Methods 14:85. https://doi.org/10.1186/s13007-018-0353-0

Mehetre SS (2010) Wild *Gossypium anomalum*:a unique source of fibre fineness and strength. Curr Sci 7:58–71

Mishra R, Wang HY, Yadav NR, Wilkins TA (2003) Development of a highly regenerable elite Acala cotton (*Gossypium hirsutum* cv. Maxxa) – a step towards genotype-independent regeneration. Plant Cell Tissue Organ Cult 73(1):21–35

Murashige T, Skoog F (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plant 15(3):473–497

Muthusamy A, Jayabalan N (2011) In vitro induction of mutation in cotton (*Gossypium hirsutum* L.) and isolation of mutants with improved yield and fiber characters. Acta Physiol Plant 33(5):1793–1801

Muthusamy A, Jayabalan N (2014) Radiation and chemical mutagen induced somaclonal variations through in vitro organogenesis of cotton (*Gossypium hirsutum* L.). Int J Radiat Biol 90(12):1229–1239

Muthusamy A, Vasanth K, Jayabalan N (2005) Induced high yielding mutants in cotton (*Gossypium hirsutum* L.). Mutat Breed News Lett 1:6–8

Naqvi RZ, Asif M, Saeed M et al (2017) Development of a triple gene *Cry1Ac-Cry2Ab-EPSPS*construct and its expression in *Nicotiana benthamiana* for insect resistance and herbicide tolerance in plants. Front Plant Sci 8:55. https://doi.org/10.3389/fpls.2017.00055

Naranjo SE (2010) Impacts of Bt transgenic cotton on integrated pest management. J Agric Food Chem 59(11):5842–5851

Nazeer W, Tipu AL, Ahmad S et al (2014) Evaluation of cotton leaf curl virus resistance in BC1, BC2, and BC3 progenies from an interspecific cross between *Gossypiumarboreum* and *Gossypiumhirsutum*. PLoS One 9(11):e111861

Ni M, Ma W, Wang et al (2017) Next generation transgenic cotton: pyramiding RNAi and Bt counters insect resistance. Plant Biotechnol J 15(9):1204–1213

Pang C, Du X, Ma Z (2006) Evaluation of the introgressed lines and screening for elite germplasm in *Gossypium*. Chin Sci Bull 51(3):304–312

Paterson AH, Wendel JF, Gundlach H et al (2012) Repeated polyploidization of *Gossypium* genomes and the evolution of spinnable cotton fibres. Nature 492(7429):423–427

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

Pauli D, White JW, Andrade-Sanchez P et al (2017) Investigation of the influence of leaf thickness on canopy reflectance and physiological traits in upland and pima cotton populations. Front Plant Sci 8:1405

Pettigrew WT (2008) The effect of higher temperatures on cotton lint yield production and fiber quality. Crop Sci 48(1):278–285

Qiao F (2015) Fifteen years of Bt cotton in China: the economic impact and its dynamics. World Dev 70:177–185

Rajasekaran K, Grula JW, Anderson DM (1996) Selection and characterization of mutant cotton (*Gossypium hirsutum* L.) cell lines resistant to sulfonylurea and imidazolinone herbicides. Plant Sci 119(1–2):115–124

Rauf S, Rahman H (2005) A study of in vitro regeneration in relation to doses of growth regulators in hybrids of upland cotton. Plant Cell Tissue Organ Cult 83(2):209–215

Rauf S, Khan AA, Teixeria daSilva JA, Naveed A (2010) Consequences of plant breeding on genetic diversity. Int J Pl Breed 4(1):1–21

Rauf S, Al-Khayri JM, Zaharieva M et al (2016) Breeding strategies to enhance drought tolerance in crops. In: Al-Khayri JM, Jain SM, Johnson DV (eds) Advances in plant breeding strategies: agronomic, abiotic and biotic stress traits. Springer, Dordrecht, pp 397–445

Rehman L, Su X, Li X et al (2018) FreB is involved in the ferric metabolism and multiple pathogenicity-related traits of *Verticillium dahliae*. Curr Genet 64(3):645–659

Renfroe MH, Hartwig RC, Smith RH (2001) Isolation and fusion of cotton protoplasts. Va J Sci 52:57–65

Roberts RK, English BC, Larson JA et al (2002) Precision farming by cotton producers in six southern states: results from the 2001 southern precision farming survey. University of Tennessee Agricultural Experiment Station, Department of Agricultural Economics, Research Series, 03–02

Saha S, Wu J, Jenkins JN et al (2010) Genetic dissection of chromosome substitution lines of cotton to discover novel *Gossypium barbadense* L. alleles for improvement of agronomic traits. Theor Appl Genet 120(6):1193–1205

Satyavathi VV, Prasad V, Lakshmi BG, Sita GL (2002) High efficiency transformation protocol for three Indian cotton varieties via *Agrobacterium tumefaciens*. Plant Sci 162(2):215–223

Sekloka E, Lancon J, Goze E et al (2008) Breeding new cotton varieties to fit the diversity of cropping conditions in Africa: effect of plant architecture, earliness and effective flowering time on late-planted cotton productivity. Exp Agric 44(2):197–207

Shaheen T, Tabbasam N, Iqbal MA et al (2012) Cotton genetic resources. A review. Agron Sustain Dev 32:419–432

Shang L, Wang Y, Cai S et al (2016) Partial dominance, overdominance, epistasis and QTL by environment interactions contribute to heterosis in two upland cotton hybrids. G3: Genes Genomes Genet 6(3):499–507

Singh RP, Prasad PV, Sunita K et al (2007) Influence of high temperature and breeding for heat tolerance in cotton: a review. Adv Agron 93:313–385

Strickland SG (1998) U.S. Patent No. 5,846,797. Washington, DC: U.S. Patent and Trademark Office

Su J, Li L, Zhang C et al (2018) Genome-wide association study identified genetic variations and candidate genes for plant architecture component traits in Chinese upland cotton. Theor Appl Genet 131(6):1299–1314

Sun Y, Zhang X, Nie Y et al (2004) Production and characterization of somatic hybrids between upland cotton (*Gossypium hirsutum*) and wild cotton (*G. klotzschianum* Anderss) via electrofusion. Theor Appl Genet 109(3):472–479

Sun Y, Zhang X, Nie Y, Guo X (2005) Production of fertile somatic hybrids of *Gossypium hirsutum*+ *G. bickii* and *G. hirsutum*+ *G. stockii* via protoplast fusion. Plant Cell Tissue Organ Cult 83(3):303–310

Sun Y, Nie Y, Guo X et al (2006) Somatic hybrids between *Gossypium hirsutum* L.(4×) and *G. davidsonii* Kellog (2×) produced by protoplast fusion. Euphytica 151(3):393–400

Taggar GK, Arora R (2017) Insect biotypes and host plant resistance. In: Arora R, Sandhu S (eds) Breeding insect resistant crops for sustainable agriculture. Springer, Singapore, pp 387–421

Tahir MS, Noor UIK (2011) Development of an interspecific hybrid (Triploid) by crossing *Gossypium hirsutum* and *G. arboreum*. Cytologia 76(2):193–199

Tian X, Ruan J-X, Huang J-Q et al (2018) Characterization of gossypol biosynthetic pathway. PNAS 115(23):E5410–E5418. https://doi.org/10.1073/pnas.1805085115

Tiwari RS, Picchioni GA, Steiner RL et al (2013) Genetic variation in salt tolerance at the seedling stage in an interspecific backcross inbred line population of cultivated tetraploid cotton. Euphytica 194:1–11

Tong XH, Daud MK, Zhu SJ (2010) Selection and characterization of a novel glyphosate-tolerant upland cotton (*Gossypium hirsutum* L.) mutant (R1098). Plant Breed 129(2):192–196

Torbett JC, Roberts RK, Larson JA, English BC (2007) Perceived importance of precision farming technologies in improving phosphorus and potassium efficiency in cotton production. Precis Agric 8(3):127–137

Traxler G, Godoy-Avila S (2004) Transgenic cotton in Mexico. AgBio Forum 7(1–2):57–62. http://www.agbioforum.org

Traxler G, Godoy-Avila S, Falck-Zepeda J, Espinoza-Arellano J (2001) Transgenic cotton in Mexico: economic and environmental impacts. Available atfile:///C:/Users/HP/Downloads/Transgenic_Cotton_in_Mexico_Economic_and_Environme.pdf

Trolinder NL, Goodin JR (1987) Somatic embryogenesis and plant regeneration in cotton (*Gossypium hirsutum* L.). Plant Cell Rep 6(3):231–234

Tuteja OP, Banga M (2011) Effects of cytoplasm on heterosis for agronomic traits in upland cotton (*Gossypium hirsutum*). Indian J Agric Sci 81(11):1001–1007

Ullah I, Ashraf M, Zafar Y (2008) Genotypic variation for drought tolerance in cotton (*Gossypium hirsutum* L.): leaf gas exchange and productivity. Flora 203(2):105–115

Ulloa M, Stewart JM, Garcia EA et al (2006) Cotton genetic resources in the western states of Mexico: in situ conservation status and germplasm collection for ex situ preservation. Genet Resour Crop Evol 53(4):653–668

Ur Rahman H, Malik SA, Saleem M (2004) Heat tolerance of upland cotton during the fruiting stage evaluated using cellular membrane thermostability. Field Crop Res 85(2–3):149–158

Wang JE, Sun YQ, Zhu SJ (2007) Advances in cotton protoplast culture and somatic hybridization [J]. Cotton Sci 2:11–21

Wang F, Gong Y, Zhang C et al (2011) Genetic effects of introgression genomic components from Sea Island cotton (*Gossypium barbadense* L.) on fiber related traits in upland cotton (*G. hirsutum* L.). Euphytica 181(1):41–53

Wang K, Wang Z, Li F et al (2012) The draft genome of a diploid cotton *Gossypium raimondii*. Nat Genet 44:1098–1103

Wang L, Liu H, Li X et al (2014) Genetic mapping of fiber color genes on two brown cotton cultivars in Xinjiang. Springerplus 3(1):480

Wang Y, Liang C, Wu S et al (2016) Significant improvement of cotton *Verticillium* wilt resistance by manipulating the expression of *Gastrodia* antifungal proteins. Mol Plant 9(10):1436–1439

Wang P, Zhang J, Sun L et al (2018) High efficient multisites genome editing in allotetraploid cotton (*Gossypium hirsutum*) using CRISPR/Cas9 system. Plant Biotechnol J 16(1):137–150

Wendel JF (2000) Genome evolution in polyploids. Plant Mol Biol 42:225–249. https://doi.org/10.1023/A:1006392424384

Wenfang G, Gangqiang Li, Wand NYC et al (2017) Transgenic cotton against *Verticillium* wilt by over expression of hen egg white lysozyme. SINO-Pak international conference on innovation in cotton breeding & biotechnology, 22–24 November, Multan, Pakistan, pp 4

Wu KM, Lu YH, Feng HQ et al (2008) Suppression of cotton bollworm in multiple crops in China in areas with Bt toxin-containing cotton. Science 321(5896):1676–1678

Wu Y, Chen D, Zhu S et al (2017) A new synthetic hybrid (A_1D_5) between *Gossypium herbaceum* and *G. raimondii* and its morphological, cytogenetic, molecular characterization. PLoS One 12(2):e0169833. https://doi.org/10.1371/journal.pone.0169833

Yang C, Guo W, Li G et al (2017) Transgenic cotton against aphids by overexpression of snowdrop and amaranth lectin. SINO-Pak international conference on innovation in cotton breeding & biotechnology, 22–24 November, Multan, Pakistan

Yu XS, Chu BJ, Liu RE et al (2012) Characteristics of fertile somatic hybrids of *G. hirsutum* L. and *G. trilobum* generated via protoplast fusion. Theor Appl Genet 125(7):1503–1516

Zeng B, Xu X, Zhou S et al (2012) Effects of temperature and light on photosynthetic heterosis of an upland cotton hybrid cultivar. Crop Sci 52(1):282–291

Zhang B (2013) Transgenic cotton: from biotransformation methods to agricultural application. Methods Mol Biol 958:3–15. https://doi.org/10.1007/978-1-62703-212-4_1

Zhang J, Percy RG, McCarty JC (2014) Introgression genetics and breeding between Upland and Pima cotton: a review. Euphytica 198(1):1–12

Zhang F, Li S, Yang S et al (2015) Overexpression of a cotton annexin gene, *GhAnn1*, enhances drought and salt stress tolerance in transgenic cotton. Plant Mol Biol 87:47–67

Zhang J, Wu M, Yu J et al (2016) Breeding potential of introgression lines developed from interspecific crossing between upland cotton (*Gossypium hirsutum*) and *Gossypium barbadense*: heterosis, combining ability and genetic effects. PLoS One 11(1):e0143646

Zhenglan L, Ruqin J, Wennan Z et al (2002) Creation of the technique of interspecific hybridization for breeding in cotton. Sci China Ser C Life Sci 45:331–336

Zhu W, Liu K, Wang XD (2008) Heterosis in yield, fiber quality, and photosynthesis of okra leaf oriented hybrid cotton (*Gossypium hirsutum* L.). Euphytica 164:283. https://doi.org/10.1007/s10681-008-9732-3

Zhu X, Zhang Y, Guo W, Zhang TZ (2011) Relationships between differential gene expression and heterosis in cotton hybrids developed from the foundation parent CRI-12 and its pedigree-derived lines. Plant Sci 180:221–227

Zhu T, Liang C, Meng Z et al (2017a) CottonFGD: an integrated functional genomics database for cotton. BMC Plant Biol 17(1):101

Zhu T, Liang C, Meng Z et al (2017b) Cotton FGD (cotton function genomics database) and two case studies in cotton genomics research. SINO-Pak international conference on innovation in cotton breeding & biotechnology, 22–24 November, Multan, Pakistan