

REVIEW

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Progress and perspective on cotton breeding in Pakistan

SHAHZAD Kashif^{1†}, MUBEEN Iqra^{2†}, ZHANG Meng¹, ZHANG Xuexian¹, WU Jianyong^{1*} and XING Chaozhu^{1*} 

Abstract

Cotton is the prime natural fiber with economic significance globally. Cotton farming and breeding have a long history in Pakistan. The development of high yielding upland cotton (*Gossypium hirsutum*) varieties gradually replaced the cultivation of diploid *Gossypium* species. Climate change along with emergence of new epidemic diseases caused yield loss in recent years. The biotic stress considerably reduced the performance and yield potential of cotton. Suitable breeding strategies are essential to generate useful genetic variations and to identify desired traits. Conventional breeding has remarkably increased cotton yield and fiber quality, which has cultivated the NIAB-78, S-12, MNH-786, and FH-Lalazar like cultivars. However, this phenotypic selection based breeding method has low efficiency to produce stress resilient cotton. The efficiency of traditional breeding has significantly improved by the marker assisted selection technology. Breakthroughs in molecular genetics, bioinformatics analysis, genetic engineering, and genome sequencing have opened new technique routes for cotton breeding. In addition, genetic improvement through quantitative trait loci, transcriptome, and CRISPR/Cas9 mediated genomic editing can provide suitable platform to improve the resistance to stresses induced by bollworms, cotton leaf curl virus, heat, drought, and salt. The approval of transgenic lines harboring triple gene *Cry1Ac* + *Cry2A* + *GTG* are critical for cotton crop. This review has critically discussed the progress and limitations of cotton breeding in Pakistan, and reviewed the utilization of novel genetic variations and selection tools for sustainable cotton production.

Keywords: Cotton yield, Biotic stress, Abiotic stress, Conventional breeding, Molecular marker, Genome selection

Background

Cotton belongs to the genus *Gossypium*, referred as the prime fiber crop, has been grown in tropical, subtropical, and semiarid regions of the world (Tarazi et al. 2020). Fiber as the major product of cotton has been extensively used in textile industry, and required millions of cotton bales yearly (Shahzad et al. 2019a). Utilization of cotton vegetable oil has been increased along with the demand of cotton seed meal for livestock feed (Campbell et al.

2010). Pakistan economy is largely depends on agriculture sector which has contributed 19.2% of the country's Gross Domestic Product, provided 38.5% employment, and the livelihood of almost 70% population depends on agriculture (Pakistan Economic Survey 2020–21). Cotton has an imperative role to boost the agriculture based economic growth of the country. Remarkably, 50% of the total industrial labor along with more than 60% of the total exports comes from cotton crop product chain (Abbas and Waheed 2017). Pakistan is one of the leading countries of cotton production, exportation, and consumption in the world. Cotton is widely cultivated in several regions of Pakistan, in which Punjab and Sindh provinces are the main growing belts, and Punjab is the leading province in terms of the total cultivated area and the total production of cotton bales (Shuli et al. 2018).

[†]Kashif Shahzad and Iqra Mubeen contributed equally

*Correspondence: dr.wujianyong@live.cn; chaozhuxing@126.com

¹ State Key Laboratory of Cotton Biology/Key Laboratory of Biological and Genetic Breeding of Cotton, Ministry of Agriculture and Rural Affairs/Institute of Cotton Research, Chinese Academy of Agricultural Science, Anyang 455000, Henan, China

Full list of author information is available at the end of the article



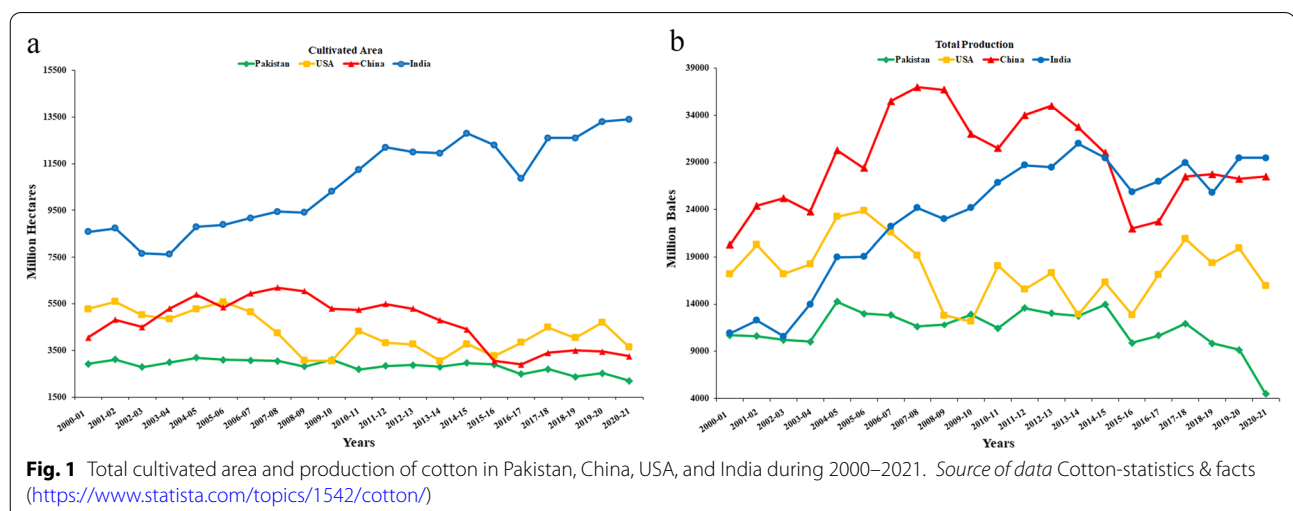
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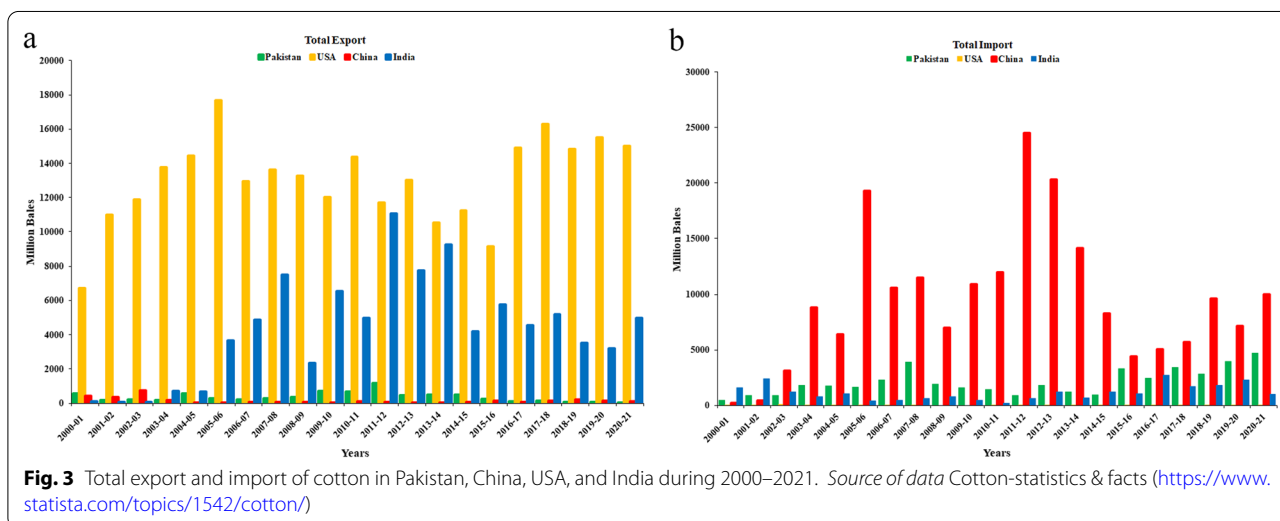
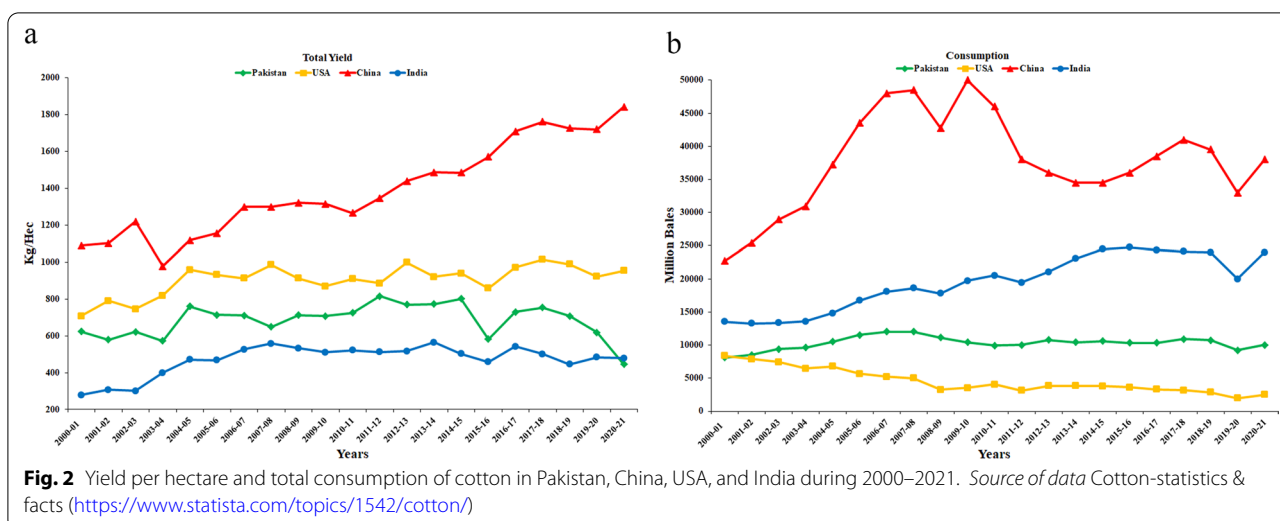
World cotton production has been significantly decreased in the last decade. As expected, sustainable cotton production in Pakistan is limited due to the climate change, unavailability of the quality seed, less genetic diversity, stresses caused by whitefly infestation and cotton leaf curl virus disease, frequent pink bollworm attacks, unstandardized procedure of variety approval, food crops competition, weed crop competition, and instability of output/input prices (Bakhsh et al. 2005; Masood et al. 2011; Ali et al. 2015; Arshad et al. 2018; Ashraf et al. 2018; Shuli et al. 2018; Abbas 2020; Razzaq et al. 2021). Researchers along with breeding community are working hard to reduce recent yield decline of cotton. Nevertheless, breeding programs still encounter problems including the lack of core germplasm resources and the lack of integration of cutting edge molecular research in breeding (such as next-generation sequencing, genome-wide association study, and transgenic breeding), which cause the less efficient cultivation of high quality and resilient cotton in Pakistan. This review focuses on current status of cotton breeding in Pakistan. The history of cotton breeding, recent research progress, and limitation of phenotype based breeding measures are further discussed in this review. Besides, it provides an overview of novel breeding frontiers that are mandatory to sustain the cotton yield in Pakistan.

Major challenges to sustain the cotton production in Pakistan

Cotton is the most popular cash crops in Pakistan. Hot and dry regions of Bahawalpur, Bahawalnager, Multan, Rahim Yar Khan, D.G. Khan, Khanewal, Rajanpur, Lodhran, and Muzafargar districts are core cotton growing zones in Punjab. In Sindh, cotton is widely cultivated in hot and dry regions of Nawabshah, Sanghar,

Nosheroferoz, and Ghotki districts (Cotictics 2021). Besides economic significance, the total area under cotton cultivation along with the total production has significantly decreased in Pakistan as compared with USA, China, and India (Fig. 1). In Pakistan, the total area of cotton cultivation was 2 700 thousand hectares in 2017–2018, and reduced to 2 200 thousand hectares in 2020–2021 (Fig. 1a), whereas the total production was 11 946 million bales in 2017–2018, and deceased to 4 500 million bales in 2020–2021 (Fig. 1b). The decline of cultivated area is a serious concern especially in Punjab province. Farmers there now prefer to grow maize or sugarcane instead of cotton. Non-availability of incentives to farmers is another main reason of decline in cotton cultivation. Different breeding strategies have considerably improved the index of seed cotton yield per hectare in the world. Still, the output of seed cotton yield per hectare in China and USA is approximately doubled more than Pakistan (Fig. 2a). Unpredictable the weather pattern in addition to frequent pest attacks at critical stages are severally damaging cotton yield in Pakistan. The mean of yield was 753 kg per hectare in 2017 but declined to 445 kg per hectare in 2021. Owing to relatively larger textile industries, Pakistan has increasingly become the major consumer of cotton, and the total consumption has reached nearly 10 000 million cotton bales. China however is the leading cotton consumer in the world followed by India (Fig. 2b). The significant increase in yield is crucial to meet the demand of domestic textile industries. The export of raw cotton was among the leading commodities that has the largest revenue for Pakistan. But, cotton export has been decreasing since 2012 (Fig. 3a). The 40%~50% increase in domestic consumption, higher export of quality fabrics results in lower export of raw cotton. Meanwhile, the import of cotton





bales is now increasing in Pakistan (Fig. 3b). Almost half required consumption demand of extra staple cotton yarn is now fulfilled through importation from Brazil, USA, Tanzania, and Egypt. Thus, policies must be adopted to increase the farmer's profit as well as to fulfill the total domestic demand of cotton bales (Cotictics 2021). Over the years, a rapid increase in the input cost along with non-availability of subsidy has led considerable decrease of cotton planting area. The farmer's community switched to cultivate other crops for higher profit. The higher price along with incentive for cotton can discourage farmers to grow other crops. At the same time, cultivation of cotton in suitable areas of Khyber Pakhtunkhwa and Balochistan should be encouraged to enhance total production. Despite several breeding measures, the

cotton yield is yet far low from the past years (Pakistan Economic Survey 2020–21). The exchange of uncertified cotton seed often deteriorates seed quality. The physically and genetically quality seed is critical to generate appropriate stand establishment during seedling stage (Afzal et al. 2019). However, low quality cotton seeds severely disrupt germination, emergence, and reduce the ability to resist pests. The cultivation area of Bt cotton has been substantially increased after commercial acceptance of Bt cotton in Pakistan. Several Bt cotton lines however became a potential threat in recent years that caused yield loss. Thus, cultivation of unapproved and non-standard Bt lines should be banned at farmer's field (Raania and Zafar 2009). The prolonged heat stress induce physiological, morphological, and biochemical

changes in cotton (Abbas 2020). It influences flowering, pollen fertility, and the number of bolls per plant, and this ultimately reduced the yield potential of cotton. The excessive rain during 2022 has considerably reduced cotton yield in several cotton zones of Punjab and Sindh. Frequent attacks of dusky bug, pink bollworm, and mealy bug deteriorate fiber yield along with fiber quality. Inefficient utilization of fertilizers at critical stages ultimately not only affect various stages of cotton growth but also reduce the final yield harvest in cotton (Ahmad and Raza 2014). Conventional breeding integrated with molecular breeding is therefore key approach to mitigate the challenges posed for sustainable cotton production.

Brief history of cotton breeding in Pakistan

The genus *Gossypium* has identified 50 species which include 45 diploids and 5 allopolyploids. The *G. hirsutum*, *G. barbadense*, *G. mustelinum*, *G. darwinii*, and *G. tomentosum* are allopolyploids. These species belongs to AD genomic group, have different geographic distribution, and commonly known as new world cotton. In contrast, diploid species are comprised of A to G plus K genomic groups, distributed worldwide, and referred as old world cotton. The *G. hirsutum* and *G. barbadense* are commercial tetraploids while *G. herbaceum* and *G. arboreum* are commercially grown diploids. Plant architecture, boll size, seed shape, and fiber characters are the key different traits between tetraploid and diploid species (Wendel and Cronn 2003). History of *G. arboreum* (Known as Desi cotton at locally) cultivation can be traced back to the cultivation in Indus valley of Sindh around 7 000 years ago (Hutchinson 1954; Moulherat et al. 2002). The farmer community of Indus valley had cultivated diploid *G. arboreum* on the marginal land until 1920 in Pakistan. Before the 21 century, the majority of *G. arboreum* cultivars were developed through selection from a single population or a mixture of various cultivars. The Z. Mollisoni, 278-Mollisoni, 39-Mollisoni, 15-Mollisoni, and 119-Sanguineum were the elite approved cultivars with excellent yield and fiber quality before the year 2000. Subsequent varieties developed by selection breeding have not shown significantly increased yield as compared with previously approved cultivars, continuous selection within the narrow genetic background restrains further breeding progress of *G. arboreum* (Yasmin et al. 2008). Gradually, high yielding *G. hirsutum* (Upland cotton) varieties replaced *G. arboreum* in the main cotton cultivation zones. Similarly, diploid cotton *G. herbaceum* was unable to grow well in Pakistan. Most of available diploid species are now mostly utilized to enhance abiotic stress resilient traits in upland cotton genotypes (Ranjan et al. 2012). The introduction of upland cotton in 1818 caused revolution of the cotton production worldwide.

It has exceptional yield, early maturity, and moderately good fiber quality. The initial test experiments to promote upland cotton cultivation in Pakistan were taken place in the early eighteen century (Ali 2007). Successful cultivation was at the same time the revolution of textile industry. The demand of raw cotton in textile industry was increased many fold within few years. As a consequence, breeder exploited innovative approaches to improve fiber quality including staple length, strength, and fineness. The 4-F, L.S.S., 289-F, 238-F, 362-F, and AC-134 were the pioneer varieties devolved to empower textile industry. In addition, the exotic germplasm such as Stoneville-213, Deltapine Smooth Leaf, and Coker Wild were introduced, planted at different locations, and crossed to locally well adapted cultivars to incorporate target traits (ur-Rahman et al. 2014). The continuous breeding create varieties with superior characteristics including higher yield, more input responsive as well as tolerance to heat, drought, salt, virus, fungus, and insect pest (Rauf et al. 2019). Subsequently, it not only increased cultivation area of upland cotton but also paved the way to meet domestic fiber demands. The cotton production was further enhanced with the development of Bt (*Bacillus thuringiensis*) cotton in the world. The Bt toxin has increased resistance to major pests in cotton, and has significantly reduced pesticide applications, and has generated high quality cotton. The utilization of transgenic Bt cotton material introduced pest resistant trait into high yielding varieties in Pakistan (Arshad et al. 2018). Several Bt cotton varieties were then approved for the maintenance of economic growth of textile sector. *G. barbadense* (known as Pima or Egyptian cotton) has extra-long staple, but lower yield, longer growing period and susceptible to various diseases (Liu et al. 2015). Egyptian cotton is now grown in less than 5% of total cotton cultivated area worldwide. Unlike upland cotton, breeding programs to promote *G. barbadense* in Pakistan remains major challenge for breeders because the above threshold heat eventually compromise its' yield and fiber quality. This species is now used to integrate alien disease resistance and improve fiber quality in target genotypes (Tiwari et al. 2013).

Cotton breeding methods in Pakistan

Application of appropriate breeding technique is important to create useful variations, and accurate selection strategies is crucial to fix desirable traits in cotton. By conventional breeding, cotton productivity, fiber quality, disease resistance, and stress tolerance have improved dramatically (Bourland and Myers 2015). In Pakistan, selection and hybridization breeding techniques have been extensively applied to develop cotton varieties. X-rays and chemical mutagenesis are used to induce random mutation on limited scale (ur-Rahman et al. 2014;

Razzaq et al. 2021). Beside these breeding measures, heterosis breeding in cotton generates stable and adaptable F_1 hybrids by utilizing the phenomenon of heterosis (Shahzad et al. 2019b). Hybrid cotton seeds are commercially produced by manual emasculation and pollination. Because of the high cost of seed production and limits of male sterility systems, the hybrid cotton production however is still at the low level of utilization in Pakistan. In large-scale breeding programs, hybridization has widely been applied to generate different recombinants, and to identify superior varieties in cotton (Ali et al. 2019). It accomplishes main breeding objectives through normal combination or transgression breeding. In normal combination breeding, the desired traits are introduced into target varieties from the donors. Contrarily the transgressive breeding refers to the selection of progeny with hybrid vigor over inbred parents that outperforms parents in a number of traits (Rieseberg et al. 1999).

Progeny selection has critical significance in cotton breeding. Breeders broadly employed pedigree or mass selection to identify desired plants from heterozygous population. Recurrent selection is another breeding technique sometime applied to accumulate target genes for quantitatively inherited traits. In Pakistan, simple phenotype based selection is extensively used to breed cultivars with target phenotype (Ashraf et al. 2022). Target phenotype can be grouped as qualitative traits and quantitative traits. The qualitative traits are regulated by one or few genes, and is suited to be isolated by simple phenotypic selection. Conversely, quantitative traits are polygenic, are difficult to select, and have low level of heritability (Singh 1985). With limited changes, cotton breeding procedure and progeny selection techniques have remained the same in Pakistan since last century. The brief breeding procedure are listed as follow: contrast inbred parents is screened to generate large number of F_1 crosses via different breeding mating designs. The F_1 seed are then advanced to the F_2 generation without any selection. Mostly, the selection begins in the segregating F_2 generation with criteria in line with target traits. The seeds of individual selected plants are harvested. These seeds are planted to evaluate the performance, and progeny seeds are harvested for further selection (Ashraf et al. 2022). The selection is performed several times until the selected progeny are uniform homozygous line with target trait(s). Eventually, a brief description of the homozygous line is submitted to the Federal Seed Certification and Registration Department (FSC&RD) of Pakistan. The organization conducts National Coordinated Varietal Trials (NCVTs) for two consecutive years to evaluate salient features of varieties. Only varieties with distinct characteristics got approval from FSC&RD.

The approved varieties seed is usually multiplied at government or private seed farms to produce certified seed. Finally, certified seed is distributed to farmers for large scale cultivation (ur-Rahman et al. 2014).

Major cotton breeding organizations of Pakistan

Cotton breeding is the technique to increase cotton yield, enhance fiber quality, and create varieties that are well adapted to the various environment conditions. In Pakistan, many national, provincial, and private organizations have one ultimate target which is to maximize national cotton production to meet the domestic consumption. The Pakistan Central Cotton Committee (PCCC) is an apex national organization with official mandate to provide funds for research, development, and marketing of cotton. Three cotton research institutes along with seven research stations work together to enhance cotton production under the umbrella of PCCC. Multiple pilot research and development projects help to identify bottleneck issues for the sustainable cotton production. The interdisciplinary research at the Central Cotton Research Institute (CCRI) in Multan, Punjab, and the CCRI in Sakrand, Sindh, has made significant contributions to create superior cultivars. Furthermore, CCRI Multan regulates identification, assessment, preservation, maintenance, and distribution of germplasm resources. There are 28 distinct species of diploid and tetraploid cotton that have already been collected and proved to be useful design new cotton breeding programs. The Centre of Excellence in Molecular Biology (CEMB) Lahore, Cotton Research Institute (CRI) Multan, Nuclear Institute of Agriculture and Biology (NIAB), and National Institute for Biotechnology and Genetic Engineering (NIBGE) in Faisalabad are other pioneer institutes that striving hard for revival of cotton production in Pakistan. These organizations have well trained agronomists, breeders, geneticists, cytogeneticists, entomologists, physiologists, and biotechnologists. They are committed to enhance morphological, physiological, and agronomical traits that are eventually important to breed high yielding and resilient cotton varieties. In addition, University of Agriculture Faisalabad (UAF), Mohammad Nawaz Shareef University of Agriculture, Multan (MNSUAM), Islamia University Bahawalpur (IUB), and Bahauddin Zakariya University (BZU), Multan are main government organizations that conduct researches on various topics of cotton. These organizations promote cotton cultivation through organizing various workshops, trainings, and seminars. Along with state organizations, many registered national and international private commercial business companies have launched cotton breeding programs. However, the lack of well-trained breeding staff, inefficient

resources, and lagged breeding facilities have limited their contribution in cotton research and development.

Breeding cotton for improved yield and fiber in Pakistan

Pakistan has the leading textile industry in the world. Hence, breeding for superior cultivars is critical to meet domestic cotton demands. Breeders can sometimes increase yield output by unlocking the stagnate potential of the approved cultivars. Yield is the most important economical trait in cotton. Breeder frequently adopts different measures to improve yield-related traits. The number of boll, boll weight, lint percentage, and seed cotton yield are important yield contributing traits (Salahuddin et al. 2010). Yield can be therefore enhanced by improving any individual yield contributing traits. For instance, seed cotton yield is altered by the number of bolls and boll weight (Shahzad et al. 2019a). Traditional breeding is particularly effective to improve cotton production earlier in the twentieth century. The cotton cultivars approved till 1990 were predominantly open type because cultivars with large number of bolls were preferred over boll size to achieve higher yield. For instance, the variety NIAB-78 approved in 1983 has displayed large number of bolls per plant, and is regarded as the most successful variety of all time in Pakistan (Table 1). Another superior variety S-12 approved in 1988 also bears the large number of bolls per plant. Early maturing cotton varieties are desirable because of the shorter growth period which give farmers an opportunity to cultivate wheat in time after cotton. Many breeding strategies have designed to generate early maturing cultivars. It has no adverse effect on harvest index of cotton yield and enabled farmers to grow wheat on scheduled time. The NIAB-78, CIM-240, CIM-443, CIM-506, FH-142, NIAB-Karishma, and S-12 were the main varieties with the potential character of early maturity (Table 1). In particular, the CIM-443 has a growth period of 110~120 days and has performed well in hot temperature. Diverse breeding techniques have been applied to improve boll weight in lateral years. The recently approved cultivars MNH-886 and FH-152 shows higher boll weight compared with earlier approved cultivars (Table 1). In Pakistan, the breeding progress to enhance boll weight is relatively slow than China. Comparative transcripts and metabolic data can offer platform to understand genetic mechanism of targeted traits. So, advanced research can speed breeding for a higher boll weight (Zhang et al. 2016).

The development of Bt cotton has remarkably increased seed cotton yield. Appropriate Bt toxin reduced the lepidopterous pest attack, improved integrated pest

management strategy, and increased cotton yield (Lu et al. 2012). Adoption of Bt varieties is substantially lower in Pakistan as compared with other major cotton-growing countries of the world. The NIBGE, Faisalabad is the first organization to start breeding initiatives for the development of Bt cotton in 2003. Later on, more than 32 Bt cotton varieties have been approved to maximize cotton profitability. But, IR-NIBGE-3701, MNH-886, FH-142, IUB-2013, and FH-Lalazar were the most popular varieties (Table 1). All these cultivars has *CryIAC* toxic gene, has grown in larger scale areas, and has considerably enhanced cotton yield. However, insect pest resistance to the *CryIAC* gene, as well as the circulation of Bt cultivars that have not been carefully bred have harmed the reputation of Bt cotton among farmers in recent years (Ma et al. 2017). However, CEMB, Lahore and NIBGE, Faisalabad have recently launched research programs to generate resilient cotton genotypes that subsequently expressed two (*cryIAC*, *cry2Ab*) or three (*cryIAC*, *cry2Ab*, *cp4-epsps*) genes. Interestingly, several transgenic lines had shown the appropriate expression of target genes in biochemical tests conducted in NCVT test in 2018–2019 (Table 2). Particularly, CKC-3 and Hatf-3 with gene combination *CryIAC* + *Cry2A* + *GTG* can be a step further for the revival of cotton in Pakistan. Cotton is the purest source of natural fiber for the textile industry, and it contains two distinct forms of natural fiber. The short fiber is commonly known as fuzz whereas long fiber is referred as lint. The market value of textile goods is directly influenced by physical properties of fiber quality. The prime physical parameters of quality fiber includes staple length, strength, elongation, fineness, and maturity (Wang et al. 2020). These fiber quality contributing traits directly influenced fiber quality of cotton. Phenotype based selection breeding techniques have certainly improved the fiber quality in Pakistan. The CIM-496, FH-942, MNH-886, IUB-13, and FH-152 not only have increased production potential but also show better fiber quality (Table 1). However, improving yield along with fiber length, strength, and elongation is a difficult challenge in cotton breeding. Because, traditional breeding requires more screening cycles, time, and resources to break negative correlation among fiber quality and yield (Farooq et al. 2018). Using molecular markers that previously known to be linked with fiber quality play an important role to identify candidate genes that are tightly correlated with fiber length, strength, uniformity index, micronaire, maturity, and elongation. Therefore, genetic techniques would be helpful to expedite breeding cycle for a better quality fiber in Pakistan (Liu et al. 2018).

Table 1 Background of major approved upland cotton varieties of Pakistan

Variety Name	Parentage	Breeding organization	Year of release	Potential SCY / (kg·ha ⁻¹)	Potential features
4-F	Selection from American Cotton	CRI, Faisalabad	1914	800	Busy growth, improved fiber quality, large scale cultivation
289-F	Selection from 4-F	CRI, Faisalabad	1921	950	Spreading growth, high yield, high staple length
L.S.S	Bulk selection from 289-F	CRI, Faisalabad	1934	1 000	Spreading growth, medium tall, late maturity
238-F	Selection from 289-F/43	CRI, Faisalabad	1948	1 000	Spreading growth, high yield, improved staple length
Lasani-11	Selection from 181-F	CRI, Faisalabad	1959	1 600	High GOT%, improved fiber quality, lodging resistant
AC-134	148-F × 199-F	CRI, Faisalabad	1959	1 600	Semi spreading growth, High GOT% and fiber strength
362-F	Selection from 289-F	CRI, Faisalabad	1959	1 500	Drought resistant, heat tolerant, high fiber strength
MS-39	Natural hybrid in L-11 field	CRS, Multan	1970	1 650	Spreading growth, high GOT% and staple length
149-F	124-F × Babda	CRS, Multan	1971	2 000	Compact growth, high GOT% and fiber strength
MNH-93	(124-F × Babdal) × (MS-39 × Mex 12)	CRS, Multan	1980	3 500	Spreading growth, medium maturity, popular variety during 1980
NIAB-78	(DPL-16 × AC-134)-F1 mutation	NIAB, Faisalabad	1983	3 500	Higher yield, the most popular variety, wider cultivation in 1987
CIM-70	Coker 8314 × (124-F × Babdal) × Coker 100 WA)	CCRI, Multan	1986	3 100	Heat tolerant, short stature, early maturity
S-12	MNH 93 × 7203-14-4-Arizona	CRS, Multan	1988	3 900	Higher yield potential, early maturity, Highest SCY/ha in 1991
NIAB-86	(DPL-16 × AC-134-F1 30kr) × Stoneville-213	NIAB, Faisalabad	1990	2 800	High GOT% and staple length
CIM-240	CIM-70 × W 1104	CCRI, Multan	1992	3 000	Heat tolerant, early maturity, good boll opening
FH-682	(B-557 × Ala (68)1) × Lankart-57	CRI, Faisalabad	1992	3 200	Early maturity, lodging resistant, spreading growth
MNH-147	431/79 × 283/80	CRS, Multan	1992	3 300	High GOT%, medium maturity, lodging resistant
S-14	H-2102-1/83 × H-2105-1/83	CRS, Multan	1995	3 000	Compact growth, high GOT%, medium maturity
NIAB-Karishma	NIAB-86 × W-83-29-Mex	NIAB, Faisalabad	1996	3 000	Semi spreading, highly heat tolerant, early maturity
MNH-329	K-401/78 × 479-81/83	CRS, Multan	1996	3 000	Spreading growth, bigger boll, high GOT%
CIM-446	CP 15/2 × S-12	CCRI, Multan	1998	3 000	CLCuV and heat tolerant, early maturity
CIM-443	CIM-109 × LRA-5166	CCRI, Multan	1998	3 000	CLCuV and heat tolerant, early maturity
MNH-552	(124-F × Babdal) × LRA -5166	CRS, Multan	2000	2 900	Early maturity, CLCuV tolerant, good boll opening
FH-900	(FH-672 × AET-5) × (B- 557 × LRA-5166)	CRI, Faisalabad	2000	3 000	CLCuV and heat tolerant, semi spreading growth
CIM-482	CIM-229 × CP15/2	CCRI, Multan	2000	3 000	Semi erect, CLCuV tolerant, good boll opening
BH-118	BS-48 × 829-4/90	CRS, Bahawalpur	2000	3 000	CLCuV tolerant, semi compact growth
CIM-506	CIM-360 × CP 15/2	CCRI, Multan	2002	3 000	Early maturity, CLCuV tolerant, high GOT%
NIAB-999	(DPL -16 × AC -134-F1 30kr) × LRA-5166 mutation	NIAB, Faisalabad	2003	3 500	Heat and salt tolerant

Table 1 (continued)

Variety Name	Parentage	Breeding organization	Year of release	Potential SCY / (kg·ha ⁻¹)	Potential features
NIAB-111	(NIAB-313/12 × CIM-100)-F1 mutation	NIAB, Faisalabad	2004	3 500	CLCuD resistant, heat tolerant
CIM-496	CIM-425 × 755-6/93	CCRI, Multan	2005	3 000	Early maturity, CLCuV and heat tolerant
MNH-786	(S-14 × CIM-448) × (MNH-564 × MNH-516)	CRS, Multan	2006	3 500	High GOT%, Big boll size and weight
BH-167	(VH-53 × BH-142)	CRS, Bahawalpur	2012	3 000	Drought and heat tolerant,
FH-942	(FH-900(S) × CIM-121)	CRI, Faisalabad	2012	3 500	Highly responsive to inputs, early maturity
FH-114	FH-925 × NuCot-N-33B	CRI, Faisalabad	2012	3 500	Medium CLCuV tolerant, early maturity
FH-142	FH-114 × FH-207	CRI, Faisalabad	2013	4 000	CLCuV and heat tolerant, wider adaptability
MNH-886	(FH-207 × MNH-770) × Bollgard-I	CRS, Multan	2013	4 000	Big boll size, CLCuV and heat tolerant
IUB-13	IUB-09 × MNH-789	IUB, Bahawalpur	2015	4 000	CLCuV and heat tolerant, good boll weight
FH-Lalazar	FH-207 × NuCot-N-33B (Bollgard-I)	CRI, Faisalabad	2016	4 500	High Yield, Big boll size, low CLCuV infestation
FH-152	FH-207 × FH-113	CRI, Faisalabad	2018	4 200	Higher boll weight, CLCuV tolerant

SCY seed cotton yield, CRS cotton research station, CRI cotton research institute, CCRI central cotton research institute, NIAB nuclear institute of agriculture and Biology, CLCuV cotton leaf curl virus, CLCuD cotton leaf curl disease, GOT ginning out turn

Table 2 Yield and expression of *Cry* genes in different lines tested in NCVT trial 2018–2019. Source Directorate of Agriculture Research, Pakistan Central Cotton Committee, Multan, Pakistan

Name of Genotypes	Gene technology	Average seed cotton yield (Kg/hac)	ELISA quantification (µg/g)		
			Cry1Ac (C-I)	Cry2A (C-II)	Cry1Ac + Cry2A + GTG(C-III/CKC)
Weal-AG-201(C-II)	<i>Cry1Ac + Cry2A</i>	2 001	3.3	2.73	n/a
Weal-AG-301(CKC)	<i>Cry1Ac + Cry2A + GTG</i>	2 296	3.4	2.62	2.68
Tahafuz-15	<i>Cry1Ac + Cry2A + GTG</i>	2 284	3.8	2.83	2.82
Diamond-2	<i>Cry1Ac + Cry2A</i>	2 292	2.4	2.10	n/a
Suncrop-3	<i>Cry1Ac + Cry2A + GTG</i>	1 927	3.1	2.68	2.63
Tahafuz-12(C-II)	<i>Cry1Ac + Cry2A</i>	2 350	2.80	2.23	n/a
Suncrop(C-II)	<i>Cry1Ac + Cry2A</i>	1 997	2.1	2.18	n/a
TJ-King(C-II)	<i>Cry1Ac + Cry2A</i>	1 843	3.6	2.66	n/a
Rustam-Beej-111(CKC)	<i>Cry1Ac + Cry2A + GTG</i>	1 760	3.0	2.59	2.55
Rustam-Beej-11(C-II)	<i>Cry1Ac + Cry2A</i>	1 854	3.61	2.66	n/a
YBG-2323(CKC)	<i>Cry1Ac + Cry2A + GTG</i>	1 920	3.30	3.30	3.37
YBG-2222(C-II)	<i>Cry1Ac + Cry2A</i>	2 179	2.9	2.92	n/a
Hatf-3(CKC)	<i>Cry1Ac + Cry2A + GTG</i>	1 910	3.2	2.64	2.69
Ghauri-2(CKC)	<i>Cry1Ac + Cry2A + GTG</i>	1 984	3.2	2.56	2.66
Badar-3(C-II)	<i>Cry1Ac + Cry2A</i>	1 830	3.80	2.52	n/a
Badar-4(C-II)	<i>Cry1Ac + Cry2A</i>	1 815	3.50	2.57	n/a
CEMB-Klean-Cotton-6	<i>Cry1Ac + Cry2A + GTG</i>	2 318	3.50	3.50	3.60
CEMB-Klean-Cotton-5	<i>Cry1Ac + Cry2A + GTG</i>	2 353	3.7	2.48	2.68
CEMB-Klean-Cotton-4	<i>Cry1Ac + Cry2A + GTG</i>	2 400	3.7	2.72	2.72
CEMB-Klean-Cotton-3	<i>Cry1Ac + Cry2A + GTG</i>	2 358	2.4	3.49	3.76
Sahara-Klean-5	<i>Cry1Ac + Cry2A + GTG</i>	2 508	3.3	2.52	2.63

Breeding for stress resilient cotton in Pakistan

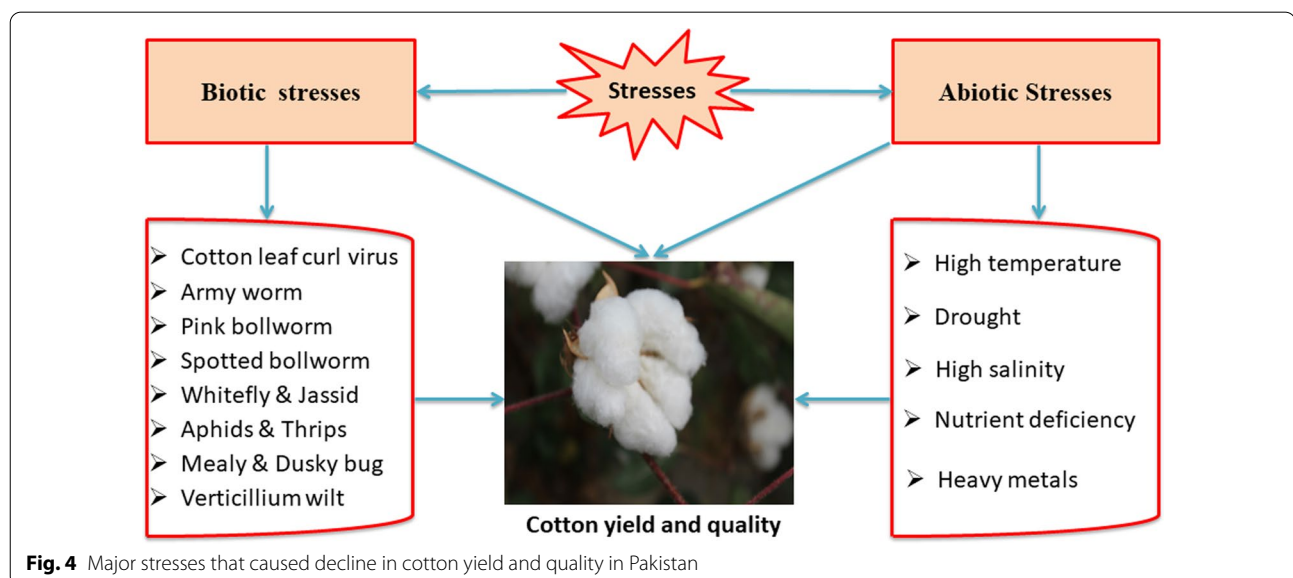
Stress threats sustain cotton production seriously and is frequently divided into biotic and abiotic stress. Pests, viruses, and fungal infections are main examples of biotic stress whereas abiotic stress includes heat, drought, and salinity, etc. The prolonged stress caused morphological, physiological, and biochemical growth changes in plants (Qamer et al. 2021). Cotton is severely prone to many abiotic and biotic stresses in Pakistan that collectively lead to decline in cotton yield and fiber quality (Fig. 4). Recently, high temperature, cotton leaf curl virus, bollworms, and sucking insects have shown adverse influence on overall cotton production and yield per hectare.

Biotic stress resilient cotton

Cotton is not resistant to insect pest attack naturally. Almost 145 types of insect pests have already been identified in Pakistan. The widespread use of pesticides not only increases the cost of cotton production, but also eliminates natural competitors which restricts the effect of biological control on pests (Tariq et al. 2007). The chewing and sucking pests is a huge problem to cotton production in Pakistan. The resistant cultivars is still under evaluation and is at developing stage even after introduction of several Bt cultivars (Karar et al. 2020). The severe bollworms invasion throughout growing season caused significant damage to cotton plant. Higher temperature from September to October are the primary reason of the increased pink bollworm in recent years (Khuhro et al. 2015). Even Bt cultivars are susceptible to the pink bollworm attack. Its attack restricts proper boll opening. Additionally, premature boll shedding influenced total yield output and the development of rotten

bolls reduced the lint quality (Mohan et al. 2016). The polyphagous spotted bollworm is another serious pest and its internal feeding affects cotton bolls growth and development (Ahmed et al. 2012). The severe invasion of army bollworm has drastic influence on cotton yield. Unfortunately, the effective control strategies of army worm are still unable to reduce large populations simultaneously. Hence, development of resilient cultivar is only useful strategies for effective control and to design integrated pest management (IPM) programs against bollworms. Among the approved cultivars, CIM-499, CRIS-468, MNH-633, MNH-635, and MNH-886 (Table 1) comparatively performed better against bollworms (Aslam et al. 2004). Meanwhile, resistance breakdown along with emergence of new strains of insect pests remains major challenge for cotton breeders (Abro et al. 2004; Men et al. 2005). In this regard, the targeted genome editing could be vital to develop resilient cotton against bollworms (Boopathi et al. 2015).

Sucking insects can reduce yield about for 30%~50% in cotton. The whitefly, jassid, mealy bug, and dusky bug appeared to be the leading damaging pests which suck the plant sap throughout vegetative and reproductive stages in Bt and non-Bt cotton cultivars (Men et al. 2005; Akhtar et al. 2018). In case of severe infestation, these insects suck essential minerals from plants, disrupt the machinery of photosynthesis, and impair both cotton yield and fiber quality. According to earlier investigations, NIAB-Karishma, NIAB-78, MNH-93, and FH-941 (Table 1) were substantially less vulnerable to sucking insect pest damage (Ghafoor et al. 2011; Majeed et al. 2016). The release of triple genes *CryIAC* + *Cry2A* + *GTG* cultivars would be effective to reduce the damage of



insect pests in future (Table 2). The cotton bugs especially dusky and mealy bugs have become a major threat to quality cotton from last few years. The cotton mealy bug affects the vascular bundles of the cotton, whereas the dusky cotton bug sucks oil sap from the cotton plant (Saeed et al. 2007; Ahmed et al. 2015). The cotton leaf curl disease (CLCuD) is first reported in 1967 in Punjab. It is still the most significant impediment cotton disease in Pakistan (Sattar et al. 2013). Different breeding designs were used to develop CLCuD resilient cultivars. The NIAB-846, NIAB-111, CIM-443, Bt-Cyto-178, Cyto-124, MNH-886, and FH-142 displayed better resistance during first outbreak (Table 1). Meanwhile, emergence of resistant breaking recombinant CLCuD strains (Zhou et al. 1998), inability to produce transgenic cultivars, and lack of modern breeding technology remains major limitation in elimination of CLCuD (Hashmi et al. 2011). Concisely, integration of functional genomic analysis with breeding measures have great potential to produce diseases resilient cotton varieties (Hussain 2015).

Abiotic stress resilient cotton

Global warming is harming sustainable cotton production in the world. The abiotic stresses such as heat, drought, salinity, and nutrient deficiency negatively influence cotton production (Saranga et al. 2009). High temperature that ranges from 40 °C to 45 °C usually prevails during cotton production season in Pakistan. Temperature stress either low or high constraints yield potential of the cotton crop. The chilly weather at sowing stage results in late germination and non-uniform crop establishment (Ahmad et al. 2020). High temperature mediated heat stress is one of the most important reasons contributing to recent decline of yield per hectare in Pakistan. It not only limits nutrients uptake but also reduced seed germination, seedling growth, number of flowering, and total number of bolls per plant (Saleem et al. 2021). Moreover, high temperature stress influenced physiological, metabolic, and biochemical mechanisms of boll formation, lint yield, and fiber quality (Pettigrew 2008). Previous breeding work showed that bulk selection along with pedigree selection is useful to generate heat tolerant varieties. For instance, the NIAB-999, NIAB-111, NIAB-777, CIM-240, CIM-598, and CIM-602 showed better adoptability to heat stress (Table 1). The identification of heat tolerance candidate genes coupled with target genome editing is an important strategy to create heat resilient cotton (Majeed et al. 2021). Cotton's vertical taproot system makes it extremely resistant to drought. Prolonged water deficient however inhibits stomata conductance, nutrient transportation, and photosynthetic linked enzymes activity. Besides this, drought stress induce biosynthesis of reactive oxygen species (ROS) that cause oxidative

damage to cell membranes, proteins, lipids, and nucleic acid in plants (Iqbal et al. 2020). In addition, drought stress significantly reduced cotton quality through functional modification in the structure of roots and leaves (Saleem et al. 2016). The breeding community in Pakistan implemented several traditional and non-traditional strategies to reduce adverse effects of drought on cotton productivity. From different approved cultivars, the CIM-546, CCRI-60, Cyto-177, and BH-167 are recommended in drought areas of Punjab province (Table 1).

Salinity decreases agricultural productivity in dry and semiarid regions around the world (Shahbaz and Ashraf 2013). It disrupts normal morphological, physiological, and biochemical functions of plants. The increased salt concentration not only reduces water uptake but also induces oxidative damage (Rahnama et al. 2010). Cotton is a moderately salt tolerant plant but the level of tolerance differs among genotypes (Akhtar et al. 2010). It is more susceptible to salt stress at the early phases of germination and seedling emergence. High salt concentration inhibits root and shoot development that ultimately changed yield and fiber quality in cotton (Basal 2010). The cultivation of NIAB-78, FH-113, MNH-93, NIAB-999, and CIM-707 showed enhanced salt tolerance (Table 1). But, the development of salt resilient genotypes is yet the main breeding challenge for most of cotton breeders in Pakistan. Availability of essential nutrients is necessary to increase cotton output per hectare. The deficiency of major nutrients impaired cotton growth. In contrast, excessive application of nutrients causes toxic effects. Non-availability of essential nutrients at critical stages along with lower nutrient availability in soil is another reason of lower cotton yield in Pakistan (Sawan et al. 2006). The indeterminate growth pattern consumes higher level of nitrogen, phosphorus, and potassium fertilizer in cotton. The nitrogen supplies influenced yield, maturity, and cotton lint quality (Khan et al. 2017), while phosphorus is essential for shoot development, repetitive expression to the shoot development, flower bud development, and absorption of nitrogen (Sawan et al. 2008). The limited supply of potassium leads to the decline in growth, disease resistance, and seed cotton yield (Oosterhuis et al. 2002). Development of nutrient efficient cultivation is crucial to sustain cotton production and environmental safety. However, breeding for highly input responsive genotypes is still limited in Pakistan.

Major cotton breeding constraints in Pakistan

Despite the global significance of cotton, its production is stagnant since last few decades in Pakistan. Regardless of other problems, the following breeding constraints considerably limited cotton yield and fiber quality.

Limitation of simple phenotype selection based cotton breeding

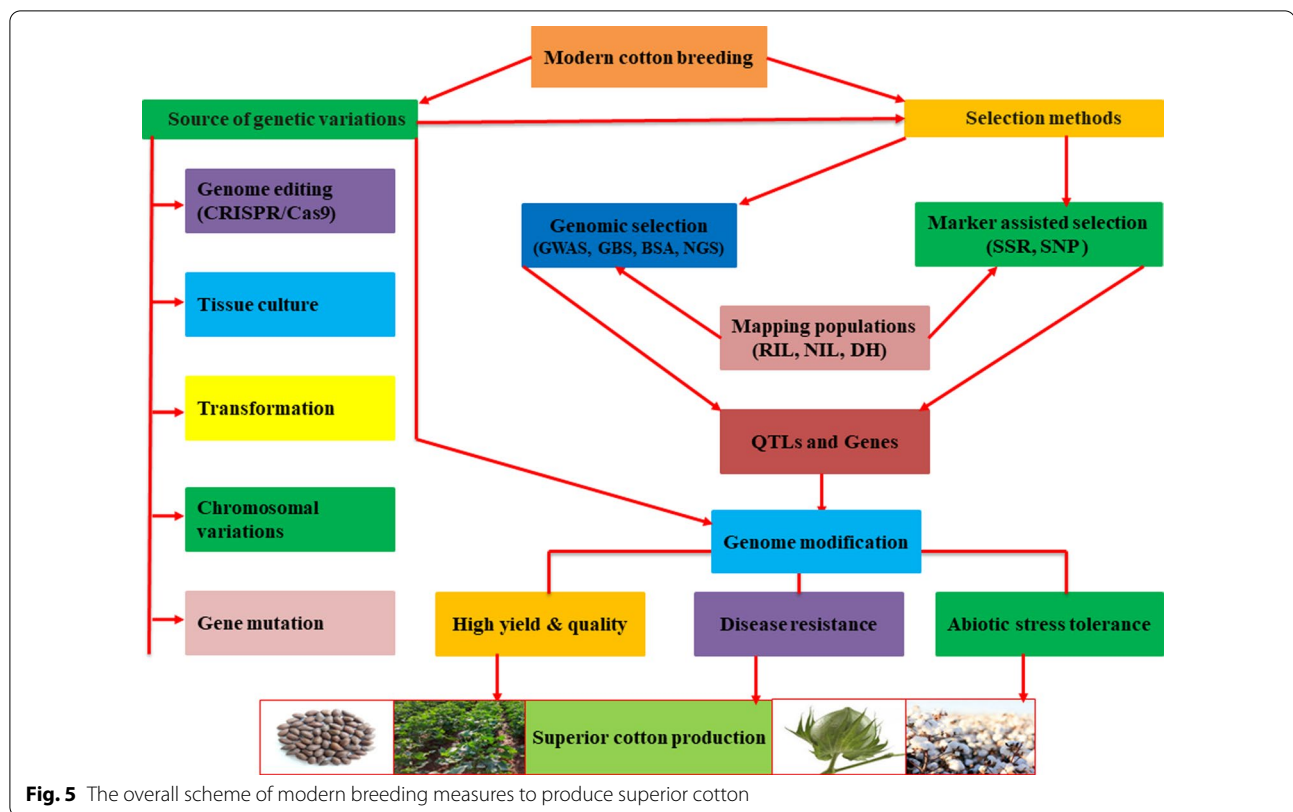
In Pakistan, conventional breeding designs are widely used to produce target genotypes. The parents with higher combining ability are selected through series of screening experiments. A large number of crosses is performed to create useful variation. Sometime, mutagenesis has been applied to induce desirable variation by few breeding teams. The pedigree or bulk selection, backcrossing, and recurrent selection are often applied to select and fix desired traits. Main criteria of progeny selection includes its phenotypic expression for target traits in various environments (Ashraf et al. 2022). Beside remarkable success, the phenotype based cotton breeding has certain limitations. The phenotype based selection is mostly effective for qualitative traits that shows high variation of environmental effects. However, phenotype based selection for quantitative traits such as cotton yield is often not efficient due to the low heritability index (Wricke and Weber 1986). In addition, the phenotypic evaluation is not reliable for most of cotton traits that are vulnerable to environment effects. Some recessive traits may not be visible at the phenotypic level due to recessive gene actions or environmental influences, thus making recessive trait selection even more difficult (Singh 1985). Non-availability of selection methods during early segregation generations is another major bottleneck of phenotype based cotton breeding. This eventually consumes more resources to facilitate several rounds of propagation advancement and selection cycles. Furthermore, around six to ten years are required to restore homozygous characteristics in cotton. These aspects not only decrease breeding efficiency but also deteriorate the genetic constituent of improved cultivars (Singh et al. 2015). The selection of parents is important in cotton breeding. Parents should be genetically superior and physiologically effective to produce elite genotypes. Identification of elite parental materials however remains challenging in phenotype based cotton breeding programs. As parent selection with desirable characters requires general combining ability and special combining ability performance tests in multiple locations which need more screening cycles, resources, and time. Conversely, the estimates of genetic diversity among inbred parents with genetic markers is a powerful approach to identify superior inbred lines (Li et al. 2019). Furthermore, the phenotype based hybridization allows selection of only compatible parents and prevents the introduction of target trait from wild progenitors. Utilization of molecular breeding is therefore critical to improve the efficiency of phenotype based selection breeding.

Lack of core germplasm resources

The genetic diversity among breeding material is vital in any sort of breeding program. It reveals genetic resemblance or difference among various groups of genotypes, strains, and germplasms (Chen et al. 2006). Sometime, genetic variation may or may not be translated at the phenotypic level. An estimation of genetic diversity is therefore useful to characterize plant breeding materials. Previous investigations have shown that *Gossypium* genus has widespread genetic diversity in plant architectures, leaf pubescence, leaf shape, flower color, pollen color, boll size, yield, fiber quality, early maturity, and stress resistance. In Pakistan, most of cotton breeders selected the approved local cultivars to perform crosses, and to develop new cultivars. Some breeders chose closely related cultivars to develop target cultivars. Such breeding practices caused genetic similarity among cultivars that ultimately impede further breeding progress in cotton (Khan et al. 2010). In contrast, the hybridization with genetically diverse parents empowers breeder to generate highly stable and adoptable varieties. Previous studies revealed that cultivated cotton has narrow genetic diversity in Pakistan. This low genetic diversity was the predominant cause of the large scale epidemic of cotton leaf curl virus disease (Rahman et al. 2002; Mumtaz et al. 2010; Ullah et al. 2012). The breeding with high genetic similarity caused decline in cotton yield and the deprived performance against changing environments, diseases, and insect pests (Abbas et al. 2015). These constraints are compelling researchers to evaluate and characterize novel cotton germplasm resources. Because, cotton germplasm resources are important to generate new genetic combination for agronomic, yield, fiber quality as well as resistance to abiotic and biotic stresses (Abdurakhmonov 2007). Interestingly, Pakistan like other major cotton producing countries has long history of cotton germplasm collection, preservation, and maintenance. The CCRI Multan and Pakistan Genetic Resources Institute (PGRI) Islamabad has made considerable efforts to conserve cotton germplasm (ur-Rahman et al. 2014). It is frequently anticipated that the sustainable cotton production mainly depends on utilization of genetic variations from core germplasm. The utilization of germplasm using traditional breeding methods yet remains challenging in cotton. However, exploration of genetic diversity with high-throughput mapping can facilitate identification of core cotton germplasm resources in Pakistan.

Novel breeding frontiers to sustainable cotton production in Pakistan

Several types of molecular techniques have been evolved to improve breeding methodology of agronomic crops. The modern breeding approaches are therefore vital



for the revival of cotton. Figure 5 illustrates various genetic variations and selection tools. Their integration with traditional breeding can facilitate cotton breeding efficiency. In brief, targeted genome editing with CRISPR/Cas platform and targeted phenotype selection through molecular markers could ensure the efficiency of cotton breeding. Therefore, breeders should adopt these techniques to revive quality cotton production in Pakistan.

Marker-assisted breeding

The recent advances in molecular markers have significantly improved the efficiency of conventional plant breeding programs. Different molecular markers are commonly used to develop linkage maps for identification of quantitative trait loci (QTLs). In cotton, markers such as simple sequence repeats (SSRs) (Liu et al. 2000; Li et al. 2019), single nucleotide polymorphism (SNP) (Dilnur et al. 2019; Geng et al. 2021), amplified fragment length polymorphism (AFLP) (Abdalla et al. 2001; Álvarez et al. 2006), and random amplified polymorphic DNA (RAPD) (Iqbal et al. 1997; Lu et al. 2002) proved to be useful to explore genetic diversity, genetic mapping of target traits, identification of nucleotide sequences of candidate genes, and facilitate marker assisted breeding (MAS) (Kalia et al. 2011; Majeed et al. 2019). The QTL

mapping with suitable cotton mapping population made great progress to identify QTLs related with agronomic traits, yield components, fiber quality (Shi et al. 2010; Wang et al. 2015; Keerio et al. 2018), plant architecture (Song et al. 2009), leaf trichomes, leaf pubescence (Ahmed et al. 2020), seed oil, seed gossypol contents (Yu et al. 2012), early maturity (Li et al. 2013), heat tolerance (Jha et al. 2014), drought tolerance (Magwanga et al. 2020), salt tolerance (Sun et al. 2018), and disease resistance (Said et al. 2013). The already identified stable QTL normally prompted error-free MAS of target traits in future. It is well known that MAS is superior than simple phenotype-based selection. Once the association between marker and target trait is developed, MAS facilitates error-free indirect trait selection without target stage of plant. Additionally, MAS reduce the expense, labor, evaluation cycles as well as duration of new cultivar development in cotton (Malik et al. 2014; Singh et al. 2015). The use of molecular markers in cotton breeding is very limited in Pakistan. Most of markers were employed to screen the cultivars against cotton leaf curl virus (Shaheen et al. 2012; Abbas et al. 2015; Rahman et al. 2017), drought (Saleem et al. 2015; Saleem et al. 2018), and to identify yield related QTLs (Iqbal et al. 2017). The integration of conventional tools with identified DNA markers associated with resistance to cotton

leaf curl virus disease was used to breed NIBGE-2 and NIBGE-115 in Pakistan. Similarly, the cotton leaf curl virus disease resilient genotypes CIM-443 and CIM-240 was developed through MAS (Mumtaz et al. 2010). The application of MAS on CRIS-134 cultivar revealed that it has an array of QTLs for drought tolerance (Saleem et al. 2018). Meanwhile, large scale utilization of molecular markers for MAS is yet at the developing phase in Pakistan.

Genomic-assisted breeding

The high throughput sequencing technology has recently been widely used for crop improvement. Major changes have taken place in plant breeding with the advent of molecular genetics, breakthroughs in bioinformatics, genetic engineering, whole genome sequencing, and re-sequencing. Recently, the whole genome of cotton is sequenced and freely available for researchers all over the world (Li et al. 2014; Zhang et al. 2015). Thus, strong knowledge about genetic control of traits can now facilitate the improvement of individual trait rather than a set of traits (Zaidi et al. 2018). The next generation sequencing with its exceptional speed and cost efficiency has been used to understand genomic and transcripts changes in plants. RNA sequence compared with DNA microarray has high accuracy to quantify the differential expression profiles of genes (Wang et al. 2009). Recent studies on transcriptomics, proteomics, metabolomics, DNA methylation, and small RNAs have successfully identified molecular markers and differentially expressed genes (DEG) for particular traits of cotton (Zhang et al. 2013, 2019; Shahzad et al. 2020a). As availability of large scale genomic datasets makes application of molecular techniques quite easy, several key strategies have already been taken into consideration to improve the efficiency of cotton breeding in Pakistan. The preliminary success was achieved with transformation of fiber gene *CpTIP1* into NIAB-846 by using agrobacterium-transformation system (Akhtar et al. 2014). For a particular interest, the application of genome-wide association study (GWAS) has opened new horizon in cotton breeding and genomic research. This method precisely identify an array of SNP closely associated with target traits (Yasir et al. 2019). Furthermore, genomic estimated breeding values empower breeders to make accurate selection of superior genotypes. Genotyping-by-sequencing (GBS) analysis is performed for GWAS and genomic selection because of its relatively low cost and analytical simplicity (Kim et al. 2016). In Pakistan, GWAS is an effective approach to detect allelic variation and identify candidate SNPs tightly correlated with cotton yield, fiber quality, and stress resistance. This help breeders to avoid difficulties of screening as well as enabled them to understand

genetic control of target traits. The specific role of candidate genes can be now quantified with functional genomics research. In this direction, The zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), RNA interference (RNAi), and the CRISPR/Cas system are considered as powerful genome editing tools (Puchta et al. 2013). In Pakistan, agrobacterium-mediated genetic transformation is most frequently applied to generate transgenic cotton line. The CEMB, Lahore and NIBGE, Faisalabad has successfully produced several transgenic cotton lines for insect resistant and herbicide tolerant. In addition, the breeding program was designed to construct stable triple transgenic cultivars. It has been estimated that these new cultivars will produce risk free cotton crop. After the successful implementation of CRISPR/Cas system in other countries, CRISPR/Cas platform, apart from other breeding measures has also been proposed to apply in the cultivation of cotton to control various strains of cotton leaf curl virus in Pakistan (Iqbal et al. 2020). So, application of new emerging genome editing technology has prime importance for accurate development of target genotypes in cotton.

Hybrid cotton breeding

The hybrid breeding utilizes the fixed heterosis to proficiently increase yield and quality of crops. The heterosis is a widespread phenomenon by which hybrid offspring exhibit superior characteristic in growth, biomass, yield, quality, and resistance as compared with inbred parents (Shahzad et al. 2020b). For particular significance, the crossing of genetically superior inbred parents exhibits substantial heterosis in yield and fiber quality traits in cotton (Zhu et al. 2008; Li et al. 2019). In hybrid breeding, the utilization of genetic markers is suggested to select superior inbred parents that display good combining ability, higher yield, and excellent performance in hybrid combinations (Li et al. 2019). Artificial emasculation and pollination have widely been applied to produce hybrid cotton seeds in China and India. Despite the higher cost of seed production, the seed purity of hybrids is not fully ensured with artificial emasculation and pollination system. Comparatively, the application of cytoplasmic male sterility (CMS) system is an ideal platform to produce hybrid cotton seeds (Havey 2004). This system efficiently utilizes heterosis to improve target traits, reduces costs, and ensure seed purity. The CMS-D2 and CMS-D8 are two main CMS lines that are well established to facilitate commercial hybrid cotton seed production in China (Wu et al. 2011; Feng et al. 2021). Considering the importance of hybrid cotton, the commercial use of F_2 hybrids is proposed by many researchers. Because commercialization of elite F_2 hybrids reduced seed production costs, increased yield, improved quality, enhanced

disease resistance, and promotes large scale cultivation of hybrid cotton (Wu et al. 2004). The development of hybrid cotton has practical significance in Pakistan. It can overcome the problems related to seed impurity by producing genetically and physical pure cotton seeds. At the same time, hybrid cotton can be cultivated in wider regions with the advantage of superior yield coupled with improved fiber quality and stress resistance. Other advantages include uniform maturity, higher profitability, and providing employment through labor intensive seed production. To date, H-115, H-151, and H160 are the only cotton hybrids which have been developed in Pakistan, but the poor standard for seed maintenance along with higher cost of seed production further limits the hybrid cotton breeding. Other main limitations include non-availability of methodology for obtaining hybrid cotton seeds for large scale cultivation. Notably, the development of CMS based hybrid breeding systems can provide effective and economic means to produce hybrid seeds. The commercial use of F_2 hybrids is another proposed strategy to improve wider cultivation of hybrids in cotton. Moreover, the ratooning cotton has broad prospects for the permanent maintenance of the male-sterile lines, the utilization of heterosis, and reducing the costs to produce F_1 seeds (Zhang et al. 2020).

Conclusion

Cotton is a vital source of foreign exchange in Pakistan. However, seed cotton yield along with total cultivated area is continuously decreasing in last few years. Climate change and emergence of new diseases significantly influence yield potential of already approved cotton genotypes. Despite several approaches, cotton breeding based on phenotypic selection has certain limitation to acquire desirable traits. Meanwhile, the characterization of available germplasm resources can enhance gene selection pool and at the same time most likely eliminates the bottleneck issues that restricting sustainable cotton yield. Recent progress in genetic analysis, marker development, QTLs mapping, genome sequencing, gene editing, and transformation provides new horizons to cotton breeding. Significant progress has previously been achieved with the development of transgenic line harboring triple genes. In summary, apart from conventional breeding, the application of genome editing tool like CRISPR/Cas can provide efficient platform to understand genetic control of target traits. Thus, it would be a step further strategy to develop pink bollworm, sucking pests, cotton leaf curl virus, and heat resilient cultivars in Pakistan.

Author contributions

Xing CZ gave conceptions for review. Shahad K and Mubeen I collected literature, designed outlines, and wrote initial draft. Shahzad K is a Pakistani

researcher, currently affiliated with Institute of Cotton Research of Chinese Academy of Agricultural Science, China. Zhang M and Zhang XX contributed to the preparation of the final manuscript. Wu JY edited the manuscript for publication. All authors read and approved the final manuscript.

Funding

This work was sponsored by funds from the Zhongyuan Academician Foundation (212101510001) and the General Program of the National Natural Science Foundation of China (31871679).

Availability of data and materials

Not applicable.

Declarations

Consent for publication

All authors have agreed to submit the review article in *Journal of Cotton Research*.

Competing interests

The authors declare that they have no competing interests.

Author details

¹State Key Laboratory of Cotton Biology/Key Laboratory of Biological and Genetic Breeding of Cotton, Ministry of Agriculture and Rural Affairs/Institute of Cotton Research, Chinese Academy of Agricultural Science, Anyang 455000, Henan, China. ²Centre of Agricultural Biochemistry and Biotechnology, University of Agriculture, Faisalabad 38040, Pakistan.

Received: 24 March 2022 Accepted: 21 November 2022

Published online: 21 December 2022

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